Shortening or omitting the dry period in dairy cows

Effects on milk yield, energy balance, metabolic
status, and fertility

Juncai Chen

Shortening or omitting the dry period in dairy cows

**Effects on milk yield, energy balance, metabolic
status, and fertility**

Juncai Chen

Thesis committee

Promotor

Prof. Dr B. Kemp
Professor of Adaptation Physiology
Wageningen University

Co-promotor

Dr A.T.M. van Knegsel
Researcher, Adaptation Physiology Group
Wageningen University

Other members

Prof. Dr L.A. den Hartog, Wageningen University
Prof. Dr H. Sauerwein, University of Bonn, Germany
Prof. Dr G. Opsomer, Ghent University, Belgium
Dr R. Jorritsma, Utrecht University

This research was conducted under the auspices of the Graduate School of
Wageningen Institute of Animal Sciences (WIAS)

Shortening or omitting the dry period in dairy cows

**Effects on milk yield, energy balance, metabolic
status, and fertility**

Juncai Chen

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Thursday 24th of November 2016
at 11 a.m. in the Aula.

Juncai Chen

Shortening or omitting the dry period in dairy cows - Effects on milk yield, energy balance, metabolic status, and fertility

205 pages.

PhD thesis, Wageningen University, Wageningen, NL (2016)

With references, with summary in English

ISBN: 978-94-6257-908-8

DOI: 10.18174/388753

Abstract

During early lactation, dairy cows typically experience negative energy balance (**EB**) caused by the high energy requirement for milk yield, which cannot be met by feed intake. Severity of negative EB has been associated with an increased incidence of metabolic disorders, subfertility, and increased culling rates. Shortening or omitting the dry period (**DP**) and feeding glucogenic diet could possibly improve EB in dairy cows. The objective of this thesis was to study the effects of shortening or omitting the DP on milk yield, EB, metabolism, and fertility over two subsequent lactations in dairy cows fed a lipogenic or glucogenic diet. In the current study, 167 cows were assigned to three DP lengths (0, 30, or 60 days) and two early lactation diets (glucogenic or lipogenic diet), and cows were planned to have same DP length and diet over two subsequent lactations. In the first lactation after DP length and dietary treatments, shortening or omitting the DP improved EB in the early lactation compared with a conventional DP of 60 days. Omitting the DP or feeding a glucogenic diet improved metabolic status in early lactation. Moreover, omitting the DP increased the percentage of cows with normal resumption of ovarian cyclicity. Shortening the DP to 30 d did not influence metabolic status and fertility compared with conventional DP in dairy cows. At onset of the second lactation, cows with a 0-d DP had greater body condition score (**BCS**) than cows with a 60 d DP. In the second lactation, improvement of EB in cows with a 0-d DP was less pronounced than the first lactation, which could be related to the high BCS at onset of lactation and reduced milk yield losses. Shortening or omitting the DP did not influence uterine health status, ovarian activity, and reproductive performance in the second lactation. In second lactation, feeding a glucogenic diet improved metabolic status and shortened the interval from calving to first ovulation compared with a lipogenic diet without affecting EB independent of DP length. Furthermore, shortening or omitting the DP decreased peak yield but did not influence lactation persistency in both lactations. In conclusion, omitting the DP improved metabolic status and resumption of ovarian activity, which was related to an improved EB in early lactation. Shortening the DP for two subsequent lactations could be achieved for most cows with limited milk yield losses. Independent of DP length, glucogenic diet improved EB and metabolic status compared with lipogenic diet in early lactation.

Table of contents

Chapter 1	General introduction	9
Chapter 2	Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation	25
Chapter 3	Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths	53
Chapter 4	Effects of dry period length and dietary energy source on milk yield, energy balance, and metabolic status of dairy cows over two subsequent lactations: effects in the second lactation	83
Chapter 5	Relationships between uterine health and metabolism in dairy cows with different dry period lengths	109
Chapter 6	Effects of dry period length and dietary energy source on lactation curve characteristics over two subsequent lactations	131
Chapter 7	General discussion	159
	Summary	189
	Acknowledgement	195
	About the author	199
	Colophon	205

CHAPTER 1

General introduction

1.1. Introduction

Dairy cows typically experience a negative energy balance (EB) in early lactation resulting from a fast increase in milk production postcalving while feed intake capacity is limited in this period. The severity of negative EB has been related to increased incidence and severity of metabolic disorders, like ketosis and liver fattening (Grummer, 1993), increased incidence of infectious diseases (Collard et al., 2000), reduction in fertility (Staples et al., 1990; Butler, 2003), and increased culling rate (Cook et al., 2001; Seifi et al., 2011). Extensive research efforts have been made to find ways to improve the EB in early lactation in dairy cows. Many studies focused on increasing energy intake via dietary interventions like decreasing the forage to concentrate ratio (Andersen et al., 2002), the addition glucose precursors (Patton et al., 2004) or fats (Beam and Butler, 1997). Moreover, decreasing milking frequency has been proposed to improve EB through reducing milk yield in early lactation (Patton et al., 2006; McNamara et al., 2008). However, increasing dietary energy density is potentially associated with rumen acidosis and decreased dry matter intake (Hayirli and Grummer, 2004). In addition, decreasing the milk frequency to once a day decreases milk yield in both short-term experiments (range: 7 to 40%) and full-lactation studies (range: 22 to 50%) (reviewed by Stelwagen et al., 2013).

The dry period (DP) is a nonlactating period before calving. A DP of 6 to 8 weeks is an established practice to maximize milk yield in the subsequent lactation in most dairy farms (Grummer and Rastani, 2004). In recent years, several studies reported that shortening or omitting the DP improves EB (Rastani et al., 2005) metabolic status (Andersen et al., 2005), and fertility in dairy cows (Watters et al., 2009). However, additional milk yield before calving due to the shortened or omitted DP was not always taken into account. In addition, most studies investigated only consequences of shortening or omitting the DP in the first subsequent lactation after implementation of the DP length treatments. The effects of shortening or omitting the DP on milk yield, EB, metabolic status, and fertility over subsequent lactations are unclear.

This chapter will describe why negative EB and related disorders occur in dairy cows in early lactation. Moreover, the expected effects of DP length on EB, metabolic status, and fertility in dairy cows will be introduced. Lastly, the aim and outline of this thesis will be presented.

1.2 Negative Energy Balance

Over the past decades, milk yield per cow increased dramatically due to the selection for milk production and improved feeding and management systems (Van Knegsel et al., 2005). The modern high-producing dairy cow is able to produce on average 9,000 kg of milk per lactation (CRV, 2015). However, dairy cows usually experience a substantial drop in feed intake during the transition period, which is related to metabolic adaptation (Ingvarlsen and Andersen, 2000), infectious diseases (Johnson, 1998), and numerous changes in feeding and management (Grant and Albright, 2001) during periparturient period. Typically, the maximum feed intake is reached between week 8 and 22 after calving, while the milk yield peaks between week 5 and 7 after calving (Ingvarlsen and Andersen, 2000). As a result, dairy cows can have a severe negative EB in early lactation due to the imbalance between energy expenditure and energy intake in dairy cows.

Energy balance can be estimated from the difference between net energy intake and net energy requirement for maintenance and milk production. In dairy cows, the negative EB usually starts several days before calving, reaches the nadir around 2 weeks after calving (Butler, 2000), and may last until week 14 of lactation (Van Knegsel et al., 2014). The severe negative EB in early lactation predisposes dairy cows to metabolic disorders (Drackley, 1999). During the period of negative EB, body fat and muscle protein are mobilized to compensate for the energy deficit (Van der Drift et al., 2012). The extensive mobilization of body reserves increases substantially the concentration of free fatty acids (FFA) in blood plasma in early lactation. Accordingly, the hepatic uptake of FFA exceeds the capacity of the liver to either oxidize FFA or export them as very low density lipoproteins (Drackley, 1999). This results in increased partial oxidation to ketone bodies and liver triacylglycerol (TAG) accumulation, which could lead to increased incidence of ketosis and fatty liver. Furthermore, it has been indicated that the metabolic stress and metabolic disorders due to negative EB in early lactation are related to a decreased natural antibody concentrations (Van Knegsel et al., 2007a), reduced serum IgM (Lacetera et al., 2005), and depressed immune response (Mallard et al., 1997). This immunosuppression in dairy cows in early lactation could result in increased susceptibility to infectious diseases, such as mastitis (Mallard et al., 1998).

In a number of reviews, negative EB has been identified as the major factor involved in the subfertility or infertility in dairy cows (Lucy, 2001; Butler, 2003; Pryce et al., 2004). Negative EB is related with a lower corpus luteum weight (Vandehaar et al., 1995) and decreased plasma estradiol and progesterone concentrations (Butler, 2000). In addition, negative EB decreases the frequency of luteinizing hormone pulse and decreases the plasma concentration of insulin-like growth factor-1 (**IGF-1**), which both are related to a compromised follicular development in early lactation (Lucy, 2000). Moreover, negative EB in early lactation has been linked to delayed interval from calving to first ovulation (Beam and Butler, 1997) and first estrus after calving (Harrison et al., 1990), a prolonged luteal phase (Opsomer et al., 2000), and a decreased conception rate after first insemination (Reist et al., 2003; Patton et al., 2007).

1.3 Dry Period Length

Dry period is a nonlactating period before calving, which is related to maximal turnover of mammary gland cells and achieving a maximum milk yield in the subsequent lactation (Capuco et al., 1997). In addition, the DP is used to treat cows with subclinical mastitis or high somatic cell count (**SCC**) with antibiotics at drying off (Church et al., 2008). The management strategy of 6 to 8 weeks DP before calving has been widely recommended since the early 1900s. However, recommendations were mostly based on retrospective studies using data from farm records before 1990 (Arnold and Becker, 1936; Wilton et al., 1967; Makuza and McDaniel, 1996), in which cows probably were not assigned randomly to have a short DP and, therefore, not managed for a short DP. Therefore, there may be biases in these retrospective studies due to twinning, abortion, or incorrect breeding dates for cows with a short DP (Grummer and Rastani, 2004). During the recent years, shortening the DP to 30 d or omitting the DP in dairy cows has received much attention. It was reported that shortening or omitting the DP partially shifts milk yield from postcalving to precalving and, therefore, improves EB in the early lactation (Rastani et al., 2005; Van Knegsel et al., 2014). Besides milk yield and EB, shortening or omitting the DP improves metabolic status (Andersen et al., 2005; De Feu et al., 2009) and fertility (Gümen et al., 2005; Watters et al., 2009) in dairy cows.

1.3.1 Effects of Dry Period Length on Milk Yield

Shortening or omitting the DP resulted in less milk yield in the subsequent lactation (Remond et al., 1997; Annen et al., 2004; Rastani et al., 2005). A meta-analysis including 22 studies showed that, across studies, average milk losses were 4.5% (range: -3.2 to 13.2%) in cows with a shortened DP (28-35 d) and 19.1% (range: 9 to 28.9%) in cows with a omitted DP compared with a conventional DP (49-63 d) in the first lactation after the implementation of DP length treatments (Van Knegsel et al., 2013). Although shortening or omitting the DP compromises milk yield in the subsequent lactation, the additional milk produced precalving could, at least partly, compensate for the milk yield losses in the postcalving period. Milk yield losses postcalving after shortening or omitting the DP were completely compensated by additional milk yield precalving in several studies (Annen et al., 2004; Andersen et al., 2005; Rastani et al., 2005), although those studies monitored the cows during a limited period postcalving (range: week 5 to 17 of lactation). In the study of Schlamberger et al. (2010), the 305-d milk yield losses after omitting the DP were reduced from 16 % to 6% when the additional milk yield precalving was taken into account. Additionally, shortening or omitting the DP increased the milk protein percentage in the subsequent lactation compared with a conventional DP (Remond et al., 1997; Andersen et al., 2005; Rastani et al., 2005; De Feu et al., 2009). This implies that the milk yield losses may be further reduced when milk yield is corrected for milk components like protein. Indeed, the milk yield losses decreased from 10.6 to 8.6 kg/d in cows with an omitted DP, and decreased from 4.6 to 3.6 kg/d in cows with an shortened DP after correcting for milk fat and protein content compared with a conventional DP (Van Knegsel et al., 2014).

Little information is available on the effects of shortening or omitting the DP on lactation curve characteristics, for instance peak yield (**PY**), time of peak yield (**PT**), and lactation persistency (**LP**), and these available data are conflicting. Mantovani et al. (2010) reported that omitting the DP resulted in an earlier PT, lower PY, and lower LP in a small study (n = 17) compared with a conventional DP. Atashi et al. (2013) found that a shortened DP (0 to 35 d) was associated with later PT, lower PY, and higher LP in a field study compared with a conventional DP (50 to 60 d). The number of animals used in the study of Mantovani et al. (2010) was relatively low (n = 17) and the field study of Atashi et al. (2013) was not a preplanned experiment, which might bias the results. Therefore, more studies

are needed to verify whether the milk yield losses after shortening or omitting the DP is caused by decreased PY, or decreased LP, or both.

Most studies investigated only the first subsequent lactation after shortening or omitting the DP. To our knowledge, only one controlled study has investigated the effect of omitting the DP on milk yield over 2 subsequent lactations (Remond et al., 1997). They reported that omitting the DP resulted in 22% milk yield losses in the first subsequent lactation, but no milk yield losses in the second subsequent lactation compared with a conventional DP of 60 d. In that study, however, milk yield of the cows was relatively low (around 23 kg/d in first 36 weeks of lactation) and DP omission was achieved for 2 subsequent lactations in only 2 out of 21 cows assigned to the 0 d DP group. This means that most of these cows dried themselves off in the first subsequent lactation after implementation of DP length treatments. For successful application of this management strategy in practice, it is necessary to investigate the effects of shortened or omitted DP on milk yield over multiple lactations in modern high-producing dairy cows. Previous studies reported that shortening or omitting the DP caused less milk yield losses in older cows (parity > 2) compared with young cows (parity = 2) (Annen et al., 2004; Watters et al., 2008; Van Knegsel et al., 2014). Thus, it can be hypothesized that milk yield losses may be less in the second subsequent lactation compared with the first subsequent lactation after omitting or shortening the DP, especially when young cows were used in the first lactation.

1.3.2 Effects of Dry Period Length on Energy Balance and Metabolic Status

Shortening or omitting the DP improves EB in the first subsequent lactation due to the decreased milk yield in early lactation (Rastani et al., 2005; De Feu et al., 2009; Van Knegsel et al., 2014). Shortening or omitting the DP improves EB by 2.9 Mcal/d and 7.7 Mcal/d, respectively, compared with a conventional DP in first 10 weeks after calving (Rastani et al., 2005). De Feu et al. (2009) reported that omitting the DP resulted in an absence of a negative EB in the first 4 weeks of lactation. The improved EB in early lactation is associated with an improved metabolic status, as indicated by decreased plasma FFA and β -hydroxybutyrate (**BHB**) concentrations, decreased liver TAG concentration, and increased plasma insulin, glucose, and IGF-1 concentrations (Andersen et al., 2005; Rastani et al., 2005; De Feu et al., 2009). As mentioned before, the effects of shortening or omitting the DP on metabolic status

in the second subsequent lactation after DP length treatments have not been studied yet.

So far, data on the effect of DP length on expression of hepatic genes involved in carbohydrate or fatty acid metabolism in early lactation in dairy cows are scarce. The altered expression of hepatic genes involved in carbohydrate and fatty acid metabolism play a key role in metabolic adaptation in dairy cows in early lactation (Drackley et al., 2001). It would be interesting to examine the influence of DP length on hepatic gene expression to gain a better insight into the relationship between shortening or omitting the DP and improved metabolic status in the subsequent lactation.

1.3.3 Effects of Dry Period Length on Fertility

Omitting the DP reduced the interval from calving to first ovulation compared with a conventional DP (Gümen et al., 2005; De Feu et al., 2009). Shortening the DP also decreased the interval from calving to first ovulation and reduced the number of anovulatory cows at 70 days in milk (DIM) compared with a conventional DP (Watters et al., 2009). An earlier first ovulation after calving in cows with a shortened or omitted DP has been associated with the improved EB and metabolic status in these cows (Gümen et al., 2005; Watters et al., 2009). Reported effects of shortened or omitted DP on reproductive performance during the subsequent lactation are inconsistent. On the one hand, shortening or omitting the DP were reported to result in an increased first service conception rate and a reduced days open (Gümen et al., 2005; Watters et al., 2009). On the other hand, no effects of DP length on pregnancy rate and days open were found in other studies (Pezeshki et al., 2008; De Feu et al., 2009). Therefore, more studies are needed to clarify the effects of shortening or omitting the DP on fertility in dairy cows. In addition, to our knowledge, no controlled study has determined the consequences of shortening or omitting the DP on interval from calving to first ovulation, days open, and pregnancy rate over 2 subsequent lactations.

Moreover, many cows show irregular ovarian cycles during the pre-service period (Opsomer et al., 2000; Royal et al., 2000). The irregular ovarian cyclicity has been associated with subfertility, for example, more services per conception (Lamming and Darwash, 1998), more days open (Ranasinghe et al., 2011), and lower chance to get pregnant (Gautam et al., 2010). So far, no information is

available on profile of ovarian cyclicity in dairy cows when DP is shortened or omitted. It can be hypothesized that shortening or omitting the DP improves resumption and regularity of ovarian cycles because of the improved EB and metabolic status in early lactation.

1.4 Glucogenic Diet vs. Lipogenic Diet

Besides shortening or omitting the DP, feeding a glucogenic diet, compared with a lipogenic diet, improved EB (van Knegsel et al., 2007c) and metabolic status in early lactation, as indicated by lower plasma FFA and BHB concentrations and greater plasma insulin concentrations (Van Knegsel et al., 2007d). Glucogenic dietary ingredients, like grain, nonfibre carbohydrates, or propylene glycol, can result in the production of propionate in the rumen and glucose in the small intestine, increasing the availability of glucogenic nutrients (Van Knegsel et al., 2005). Lipogenic dietary ingredients, like fat or fiber, can result in the production of acetate and butyrate in the rumen and fat in the small intestine, increasing the availability of lipogenic nutrients (Van Knegsel et al., 2005). In early lactation, the ratio of glucogenic nutrients and lipogenic nutrients is imbalanced because of mobilization of body fat increasing lipogenic nutrients and high milk lactose yield causing a draining of glucogenic nutrients. A low glucogenic/lipogenic ratio in early lactation limits production of citrate to form ATP in the Krebs cycle and respiratory chain reaction (Van Knegsel et al., 2005). Feeding a glucogenic diet restored the balance between glucogenic nutrients and lipogenic nutrients and improved EB and metabolic status mainly through reduction of milk fat output (Van Knegsel et al., 2007b). In addition, feeding a glucogenic diet resulted in a shorter interval from calving to first ovulation compared with a lipogenic diet in multiparous cows (van Knegsel et al., 2007c). Until now, lipogenic and glucogenic diets have been evaluated for cows after a conventional DP of 60 d (van Knegsel et al., 2007c; Van Knegsel et al., 2007d). Shortening or omitting the DP might reduce the requirement for glucogenic nutrients in early lactation because of an improved EB and metabolic status. It can be hypothesized that feeding a glucogenic diet might have less impact on EB and metabolic status in early lactation in dairy cows if cows have a better EB due to the shortened or omitted DP (Van Knegsel et al., 2014). Moreover, the effects of dietary energy source and its interaction with DP length on milk yield, EB, metabolic status, and fertility over 2 subsequent lactations has not been studied yet.

1.5 Aim and Outline of This Thesis

The objective of this thesis is to study effects of shortening or omitting the DP on milk yield, EB, metabolic status, and fertility over 2 subsequent lactations in dairy cows fed a lipogenic or glucogenic diet during early lactation. The effects of 3 DP length (0, 30, and 60 d) and 2 early lactation diets (glucogenic and lipogenic diet) on milk yield, EB, and milk composition in the first subsequent lactation of the current experiment has been reported previously (Van Knegsel et al., 2014). In brief, both shortening or omitting the DP decreased milk yield (32.7 vs. 38.7 vs. 43.3 ± 0.7 kg/d for the 0 vs. 30 vs. 60 d DP, respectively, $P < 0.01$), but improved EB (38 vs. -59 vs. -132 ± 14 kJ/kg^{0.75}·d for the 0 vs. 30 vs. 60 d DP, respectively, $P < 0.01$) in the first 14 weeks of lactation in the first lactation. Chapter 2 and 3 of this thesis focus on metabolism and fertility of dairy cows in the first subsequent lactation after DP length and dietary treatments. In chapter 2, effects of DP length and dietary energy source on plasma metabolites and metabolic hormones, and expression of hepatic genes involved in carbohydrate and fatty acid metabolism are presented. In chapter 3, the ovarian activity, as indicated by milk progesterone profile in the first 100 DIM, in cows with different DP lengths and dietary energy source are presented. Moreover, the relationships between ovarian activity and EB and metabolic status are assessed. Chapter 4 and 5 focus on the second lactation after DP length and dietary treatments. Chapter 4 evaluates the effects of DP length on EB, milk yield, and metabolic status in cows fed a lipogenic or glucogenic diet in the second lactation. In chapter 5, the ovarian activity and reproductive performance were determined in the second lactation after implementation of DP length and dietary treatments in dairy cows. Moreover, the relationships of uterine health status, as indicated by vaginal discharge score, with EB and metabolic status in early lactation are presented. Chapter 6 focuses on shape of the lactation curve in relation with DP length in both lactations. Finally, chapter 7 discusses the long-term effects of shortening or omitting the DP on milk yield, EB, metabolism and fertility, and discusses several factors, e.g. diet, BCS, parity, or uterine health status, that may influence EB and metabolic status in the early lactation, related with DP lengths in dairy cows. In addition, the application of shortening or omitting the DP in practice is discussed.

References

- Andersen, J. B., T. Larsen, M. O. Nielsen, and K. L. Ingvarsten. 2002. Effect of energy density in the diet and milking frequency on hepatic long chain fatty acid oxidation in early lactating dairy cows. *J. Vet. Med. A.* 49:177-183.
- Andersen, J. B., T. G. Madsen, T. Larsen, K. L. Ingvarsten, and M. O. Nielsen. 2005. The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. *J. Dairy Sci.* 88:3530-3541.
- Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. *J. Dairy Sci.* 87:3746-3761.
- Arnold, P. T. and R. B. Becker. 1936. Influence of preceding dry period and of mineral supplement on lactation. *J. Dairy Sci.* 19:257-266.
- Atashi, H., M. J. Zamiri, and M. Dadpasand. 2013. Association between dry period length and lactation performance, lactation curve, calf birth weight, and dystocia in Holstein dairy cows in Iran. *J. Dairy Sci.* 96:3632-3638.
- Beam, S. W. and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. *Biol. Reprod.* 56:133-142.
- Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. *Anim. Reprod. Sci.* 60:449-457.
- Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livest. Prod. Sci.* 83:211-218.
- Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in Holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477-487.
- Church, G. T., L. K. Fox, C. T. Gaskins, D. D. Hancock, and J. M. Gay. 2008. The effect of a shortened dry period on intramammary infections during the subsequent lactation. *J. Dairy Sci.* 91:4219-4225.
- Collard, B. L., P. J. Boettcher, J. C. M. Dekkers, D. Petitclerc, and L. R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. *J. Dairy Sci.* 83:2683-2690.

- Cook, N. B., W. R. Ward, and H. Dobson. 2001. Concentrations of ketones in milk in early lactation, and reproductive performance of dairy cows. *Vet. Rec.* 148:769-772.
- CRV. 2015. Annual report 2015. CRV, Arnhem, the Netherlands.
- De Feu, M. A., A. C. Evans, P. Lonergan, and S. T. Butler. 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status, and postpartum ovarian function in Holstein-Friesian dairy cows. *J. Dairy Sci.* 92:6011-6022.
- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259-2273.
- Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. *J. Dairy Sci.* 84:E100-E112.
- Gümen, A., R. R. Rastani, R. R. Grummer, and M. C. Wiltbank. 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *J. Dairy Sci.* 88:2401-2411.
- Gautam, G., T. Nakao, K. Yamada, and C. Yoshida. 2010. Defining delayed resumption of ovarian activity postpartum and its impact on subsequent reproductive performance in Holstein cows. *Theriogenology* 73:180-189.
- Grant, R. J. and J. L. Albright. 2001. Effect of animal grouping on feeding behavior and intake of dairy cattle. *J. Dairy Sci.* 84:E156-E163.
- Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci.* 76:3882-3896.
- Grummer, R. R. and R. R. Rastani. 2004. Why reevaluate dry period length? *J. Dairy Sci.* 87, Supplement:E77-E85.
- Harrison, R. O., S. P. Ford, J. W. Young, A. J. Conley, and A. E. Freeman. 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. *J. Dairy Sci.* 73:2749-2758.
- Hayirli, A. and R. R. Grummer. 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. *Can. J. Anim. Sci.* 84:337-347.
- Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. *J. Dairy Sci.* 83:1573-1597.

- Johnson, R. W. 1998. Immune and endocrine regulation of food intake in sick animals. *Domest. Anim. Endocrinol.* 15:309-319.
- Lacetera, N., D. Scalia, U. Bernabucci, B. Ronchi, D. Pirazzi, and A. Nardone. 2005. Lymphocyte functions in overconditioned cows around parturition. *J. Dairy Sci.* 88:2010-2016.
- Lamming, G. E. and A. O. Darwash. 1998. The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. *Anim. Reprod. Sci.* 52:175-190.
- Lucy, M. C. 2000. Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in cattle. *J. Dairy Sci.* 83:1635-1647.
- Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *J. Dairy Sci.* 84:1277-1293.
- Makuza, S. M. and B. T. McDaniel. 1996. Effects of days dry, previous days open, and current days open on milk yields of cows in Zimbabwe and North Carolina. *J. Dairy Sci.* 79:702-709.
- Mallard, B. A., L. C. Wagter, M. J. Ireland, and J. C. M. Dekkers. 1997. Effects of growth hormone, insulin-like growth factor-I, and cortisol on periparturient antibody response profiles of dairy cattle. *Vet. Immunol. Immunopathol.* 60:61-76.
- Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. L. Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *J. Dairy Sci.* 81:585-595.
- Mantovani, R., L. Marinelli, L. Bailoni, G. Gabai, and G. Bittante. 2010. Omission of dry period and effects on the subsequent lactation curve and on milk quality around calving in Italian Holstein cows. *Ital. J. Anim. Sci.* 9:101-108.
- McNamara, S., J. J. Murphy, F. P. O'mara, M. Rath, and J. F. Mee. 2008. Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. *Livest. Sci.* 117:70-78.
- Opsomer, G., Y. T. Gröhn, J. Hertl, M. Coryn, H. Deluyker, and A. De Kruif. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology* 53:841-857.

- Patton, J., D. A. Kenny, J. F. Mee, F. P. O'Mara, D. C. Wathes, M. Cook, and J. J. Murphy. 2006. Effect of milking frequency and diet on milk production, energy balance, and reproduction in dairy cows. *J. Dairy Sci.* 89:1478-1487.
- Patton, J., D. A. Kenny, S. McNamara, J. F. Mee, F. P. O'mara, M. G. Diskin, and J. J. Murphy. 2007. Relationships among milk production, energy balance, plasma analytes, and reproduction in Holstein-Friesian cows. *J. Dairy Sci.* 90:649-658.
- Patton, R. S., C. E. Sorenson, and A. R. Hippen. 2004. Effects of dietary glucogenic precursors and fat on feed intake and carbohydrate status of transition dairy cows. *J. Dairy Sci.* 87:2122-2129.
- Pezeshki, A., J. Mehrzad, G. R. Ghorbani, B. De Spiegeleer, R. J. Collier, and C. Burvenich. 2008. The effect of dry period length reduction to 28 days on the performance of multiparous dairy cows in the subsequent lactation. *Can. J. Anim. Sci.* 88:449-456.
- Pryce, J. E., M. D. Royal, P. C. Garnsworthy, and I. L. Mao. 2004. Fertility in the high-producing dairy cow. *Livest. Prod. Sci.* 86:125-135.
- Ranasinghe, R. M. S., T. Nakao, K. Yamada, K. Koike, A. Hayashi, and C. M. B. Dematawewa. 2011. Characteristics of prolonged luteal phase identified by milk progesterone concentrations and its effects on reproductive performance in Holstein cows. *J. Dairy Sci.* 94:116-127.
- Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004-1014.
- Reist, M., D. K. Erdin, D. von Euw, K. M. Tschümperlin, H. Leuenberger, H. M. Hammon, C. Morel, C. Philipona, Y. Zbinden, and N. Künzi. 2003. Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. *Theriogenology* 59:1707-1723.
- Remond, B., J. Rouel, N. Pinson, and S. Jabet. 1997. An attempt to omit the dry period over three consecutive lactations in dairy cows. *Ann. Zootech.* 46:399-408.

- Royal, M., G. E. Mann, and A. P. F. Flint. 2000. Strategies for reversing the trend towards subfertility in dairy cattle. *Vet. J.* 160:53-60.
- Schlamberger, G., S. Wiedemann, E. Viturro, H. H. D. Meyer, and M. Kaske. 2010. Effects of continuous milking during the dry period or once daily milking in the first 4 weeks of lactation on metabolism and productivity of dairy cows. *J. Dairy Sci.* 93:2471-2485.
- Seifi, H. A., S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2011. Metabolic predictors of post-partum disease and culling risk in dairy cattle. *Vet. J.* 188:216-220.
- Staples, C. R., W. W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *J. Dairy Sci.* 73:938-947.
- Stelwagen, K., C. V. C. Phyn, S. R. Davis, J. Guinard-Flament, D. Pomies, J. R. Roche, and J. K. Kay. 2013. Invited review: Reduced milking frequency: Milk production and management implications. *J. Dairy Sci.* 96:3401-3413.
- Van der Drift, S. G. A., M. Houweling, J. T. Schonewille, A. G. M. Tielens, and R. Jorritsma. 2012. Protein and fat mobilization and associations with serum β -hydroxybutyrate concentrations in dairy cows. *J. Dairy Sci.* 95:4911-4920.
- Van Knegsel, A. T. M., H. Van den Brand, J. Dijkstra, S. Tamminga, and B. Kemp. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. *Reprod. Nutr. Dev.* 45:665-688.
- Van Knegsel, A. T. M., G. de Vries Reilingh, S. Meulenberg, H. Van den Brand, J. Dijkstra, B. Kemp, and H. K. Parmentier. 2007a. Natural antibodies related to energy balance in early lactation dairy cows. *J. Dairy Sci.* 90:5490-5498.
- Van Knegsel, A. T. M., H. Van den Brand, J. Dijkstra, and B. Kemp. 2007b. Effects of dietary energy source on energy balance, metabolites and reproduction variables in dairy cows in early lactation. *Theriogenology* 68:S274-S280.
- Van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007c. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. *J. Dairy Sci.* 90:3397-3409.

- Van Kneegsel, A. T. M., H. Van den Brand, E. A. M. Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga, and B. Kemp. 2007d. Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. *J. Dairy Sci.* 90:1477-1485.
- Van Kneegsel, A. T. M., S. G. A. Van der Drift, J. Čermáková, and B. Kemp. 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: A systematic review. *Vet. J.* 198:707-713.
- Van Kneegsel, A. T. M., G. J. Remmelink, S. Jorj Jong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *J. Dairy Sci.* 97:1499-1512.
- Vandehaar, M. J., B. K. Sharma, and R. L. Fogwell. 1995. Effect of dietary energy restriction on the expression of insulin-like growth factor-I in liver and corpus luteum of heifers. *J. Dairy Sci.* 78:832-841.
- Watters, R. D., J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Crump, P. W. Clark, and R. R. Grummer. 2008. Effects of dry period length on milk production and health of dairy cattle. *J. Dairy Sci.* 91:2595-2603.
- Watters, R. D., M. C. Wiltbank, J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Fricke, and R. R. Grummer. 2009. Effect of dry period length on reproduction during the subsequent lactation. *J. Dairy Sci.* 92:3081-3090.
- Wilton, J. W., E. B. Burnside, and J. C. Rennie. 1967. The effects of days dry and days open on the milk and butterfat production of Holstein-Friesian cattle. *Can. J. Anim. Sci.* 47:85-90.

CHAPTER 2

Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation

J. Chen,^{*} J. J. Gross,[†] H. A. van Dorland,[†] G. J. Rummelink,[‡] R. M. Bruckmaier,[†] B. Kemp,^{*} A. T. M. van Knegsel^{*}

^{*}Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [†]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland; [‡]Livestock Research, Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, The Netherlands

2.1 Abstract

In a prior study, we observed that cows with a 0 d dry period (DP) had greater energy balance and lower milk production compared with cows with a 30 or 60 d DP in early lactation. The objective of the current study was to evaluate the influence of DP length on metabolic status and hepatic gene expression in cows fed a lipogenic or glucogenic diet in early lactation. Holstein-Friesian dairy cows ($n = 167$) were assigned randomly to 3×2 factorial design with 3 DP lengths ($n = 56, 55$, and 56 for 0, 30, and 60 d dry, respectively) and 2 early lactation diets ($n = 84$ and 83 for glucogenic and lipogenic diet, respectively). Cows were fed a glucogenic or lipogenic diet from 10 d before the expected calving date and onward. The main ingredient for a glucogenic concentrate was corn and the main ingredients for a lipogenic concentrate were sugar beet pulp, palm kernel, and rumen protected palm oil. Blood was sampled weekly from 95 cows from week 3 precalving to week 8 postcalving. Liver samples were collected from 76 cows in weeks -2 , 2, and 4 relative to calving. Liver samples were analyzed for triacylglycerol concentration and mRNA expression of 12 candidate genes. Precalving, cows with a 0 d DP had greater plasma β -hydroxybutyrate, urea, and insulin concentrations compared with cows with a 30 or 60 d DP. Postcalving, cows with a 0 d DP had lower liver triacylglycerol and plasma free fatty acids concentration (0.20, 0.32, and 0.36 mmol/L for 0, 30, and 60 d DP, respectively), greater plasma glucose, insulin-like growth factor-I, and insulin concentrations (24.38, 14.02, and 11.08 μ IU/mL for 0, 30, and 60 d DP, respectively), and lower hepatic mRNA expression of pyruvate carboxylase, compared with cows with a 30 or 60 d DP. Plasma urea and β -hydroxybutyrate concentrations were greater in cows fed a lipogenic diet compared with cows fed a glucogenic diet. In conclusion, cows with a 0 d DP had an improved metabolic status in early lactation, indicated by lower plasma concentration of free fatty acids, greater plasma concentrations of glucose, insulin-like growth factor-I, and insulin, and lower mRNA expression of pyruvate carboxylase in the liver, compared with cows with a 30 or 60 d DP. Independent of DP length, the glucogenic diet also improved the metabolic status, compared with the lipogenic diet.

Key words: continuous milking, lipogenic diet, glucogenic diet, liver, energy metabolism

2.2 Introduction

A traditional 6 to 8 weeks dry period (**DP**) has been widely recommended and applied for years to ensure maximum milk production during subsequent lactation. However, these recommendations were primarily based on studies using retrospective analysis of milk yield data from farm records before 1990 (Rastani et al., 2005). Milk production of today's cow has increased considerably (Bachman and Schairer, 2003), and cow health and fertility should also be considered when evaluating the optimal DP length (Gümen et al., 2005). In recent years, significant discussions have occurred on shortening or omitting the DP of high-producing dairy cows (Grummer and Rastani, 2004). On the one hand, no or a shortened DP was shown to result in less milk production in subsequent lactation (Rastani et al., 2005; Schlamberger et al., 2010). On the other hand, several studies demonstrated health benefits of no or a shortened DP for dairy cows in early lactation. Omitting the DP increased the plasma concentrations of glucose and insulin and decreased the concentrations of plasma free fatty acids (**FFA**) and β -hydroxybutyrate (**BHB**) (Andersen et al., 2005), suggesting an improved metabolic status for cows with no DP, compared with cows with a traditional DP of 8 weeks. In addition, shortening the DP decreased the concentrations of plasma FFA and BHB in dairy cows in early lactation (Klusmeyer et al., 2009). Moreover, van Knegsel et al. (2014) reported that cows with a 30 d DP and, in particular, cows with a 0 d DP had a substantially improved negative energy balance (**EB**) in early lactation compared with cows with a 60 d DP. Furthermore, a meta-analysis showed that a shortened DP tends to reduce the risk of ketosis in subsequent lactation (van Knegsel et al., 2013).

As reviewed extensively, dietary strategies can improve the metabolic status of dairy cows during early lactation (Butler, 2003; Chagas et al., 2007). Van Knegsel et al. (2007a) showed that a diet high in glucogenic nutrients improved the energy status, indicated by improved EB and decreased plasma BHB and liver triacylglycerol (**TAG**) concentrations, compared with a diet high in lipogenic nutrients. Van Knegsel et al. (2007a), however, tested these diets after a conventional DP length (60 d). Shortening or omitting the DP might reduce the requirement for glucogenic nutrients in early lactation because of the improved EB and metabolic status. Therefore, it can be hypothesized that the beneficial effects

of a glucogenic diet on metabolic status in early lactation are reduced after a shortened or omitted DP compared with a conventional DP.

The liver plays an important role in metabolic adaptation in early lactation. Previous studies showed that hepatic adaptation to new lactation is related to altered expression of genes involved in carbohydrate and fatty acid metabolism (Drackley et al., 2001). Pyruvate carboxylase (*PC*) is involved in carbohydrate metabolism, which was earlier reported to have an altered expression during the transition from precalving to postcalving (Greenfield et al., 2000). The altered expression during the transition from precalving to postcalving was also observed for genes carnitine palmitoyltransferase 1A (*CPT1A*) and carnitine palmitoyltransferase 2 (*CPT2*), which were involved in fatty acid oxidation (Graber et al., 2010). Looor et al. (2005) reported that the expression of gene glycerol-3-phosphate acyltransferase mitochondrial (*GPAM*) involved in liver TAG synthesis decreased from precalving to postcalving. In addition, it can be hypothesized that also the expression of 3-hydroxybutyrate dehydrogenase 2 (*BDH2*) and 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 (*HMGCS2*) change during the transition from precalving to postcalving, because *BDH2* and *HMGCS2* are considered to be involved in the process of ketone body synthesis (Graber et al., 2010). The gene expression of acute phase proteins, serum amyloid-A (*SAA*) and haptoglobin (*Hp*), and insulin-like growth factor-1 (*IGF-1*) also changed during the transition from precalving to postcalving (Radcliff et al., 2003; Gessner et al., 2013). Moreover, although the ruminant liver is not a lipogenic tissue (Khan et al., 2014), Ballard et al. (1972) reported that lipogenic enzyme such as ATP citrate lyase (*ACLY*) could respond to a high carbohydrate diet; hence the expression of *ACLY* and acetyl-CoA-carboxylase (*ACC*) may be expected to be affected by a dietary energy source. To our knowledge, information on the effect of DP length on expression of genes involved in carbohydrate or fatty acid metabolism, and inflammatory processes in dairy cows is scarce. Therefore, the objective of this study was to evaluate the influence of DP length on metabolic status and hepatic gene expression in cows fed a lipogenic or glucogenic diet during early lactation.

2.3 Materials and Methods

2.3.1 Animals and Experimental Design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. Holstein-Friesian dairy cows ($n = 167$) were selected from the Dairy Campus Research dairy herd (WUR Livestock Research, Lelystad, The Netherlands). The experimental design, DP length, and dietary contrasts were described by van Knegsel et al. (2014). In summary, cows were blocked for parity (primiparous or multiparous), expected calving date, milk yield in the previous lactation, and body condition score (**BCS**). Within blocks, each cow was assigned randomly to a 3×2 factorial design with 3 DP lengths (0, 30, or 60 d) and 2 early lactation diets (glucogenic or lipogenic).

During the precalving period, lactating cows were fed a lactation diet supporting 25 kg of milk, and dry cows were fed a dry cow ration. From d 10 before the expected calving date and onward, cows of all treatments were fed 1 kg/d of glucogenic or lipogenic concentrate and increased postcalving stepwise with 0.5 kg/d until the concentrate supply reached 8.5 kg/d. The main ingredient for the glucogenic concentrate was corn and the main ingredients for the lipogenic concentrate were sugar beet pulp, palm kernel, and rumen protected palm oil. Forage was supplied ad libitum and was composed of grass silage, corn silage, wheat straw, and rapeseed meal or soybean meal (51:34:2:13, DM basis). Diets were formulated to be isocaloric (Net energy basis; VEM system; Van Es, 1975) and equal in intestinal digestible protein and degraded protein balance (DVE/OEB system; Tamminga et al., 1994).

Blood samples were collected from 95 ($n = 64$ multiparous and $n = 31$ primiparous) of the 167 cows, and liver biopsies were collected from 76 ($n = 51$ multiparous and $n = 25$ primiparous) of the 167 cows for liver TAG analysis and from 67 ($n = 48$ multiparous and $n = 19$ primiparous) of the 167 cows for gene expression analysis. Table 2.1 shows the distribution of cows per DP length and diet. Cows were housed in a free stall with a slatted floor and cubicles and were milked twice daily (0500 and 1630 h).

2.3.2 Blood Sampling and Analysis

Blood samples were taken weekly from the coccygeal vein from week -3 to 8 relative to calving date at 3 h before the morning feeding. Blood was collected in evacuated tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria) containing NaF for glucose; EDTA for insulin, FFA, BHB, and urea analysis; or heparin for

Table 2.1. Distribution of cows per dry period length (0, 30, 60 d) and diet¹.

Cows, n	0		30		60		Total
	G	L	G	L	G	L	
Cows in experiment	28	28	28	27	28	28	167
- Blood sampling	16	15	17	17	15	15	95
- Liver biopsy for TAG ² analysis	14	12	14	12	12	12	76
- Liver biopsy for gene expression analysis	12	12	11	11	11	10	67

¹G = glucogenic diet; L = lipogenic diet.²TAG = triacylglycerol.

IGF-1. Samples were kept cold on ice for a maximum of 2 h until they were centrifuged at $2,900 \times g$ for 10 min at 4°C. Plasma was decanted, aliquoted, and frozen at -20°C until analysis. Plasma samples were analyzed at a laboratory of the Veterinary Physiology group (Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland). Concentrations of glucose and urea were measured using commercial kits no. 61269 and no. 61974 from BioMérieux (Marcy l'Etoile, France), as previously described (Graber et al., 2012). 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), as previously described (Graber et al., 2012). Insulin-like growth factor-1 and insulin were measured using radioimmunoassay, as previously described (Vicari et al., 2008).

2.3.3 Liver Tissue Sampling and Triacylglycerol Analysis, mRNA Extraction, and Quantitative Real-Time Reverse Transcription-PCR

Liver biopsies were taken in weeks -2, 2, and 4 relative to calving. Before taking the biopsy, the biopsy site was clipped and disinfected. Liver biopsies (approximately 400 mg of wet weight) were obtained through an incision at the location of the greater trochanter at the 11th intercostal space on the right side of the cow. A biopsy was obtained under local anesthesia (7 mL, of lidocaine-HCl 2% with adrenaline, Alfasan Nederland, B.V., Woerden, the Netherlands) and was harvested by moving the biopsy needle (17 gauge $\times$ 200 mm) several times. Tissue samples for TAG determination were kept cold on ice in a 0.9% NaCl solution for a maximum for 2 h and subsequently stored at -20°C until analysis. Tissue samples for the gene expression analysis were collected in a RNA stabilization reagent (RNAlater from

Ambion, Applied Biosystems Business, Austin, TX, USA), kept at 4°C for 24 h, and subsequently stored at –80°C until analysis.

Liver TAG was extracted from liver samples (approximately 150 mg of wet weight) using 0.5 mol/L of KOH in ethanol for 30 min at 70°C, and then 2.5 mol/L of perchloric acid was added to neutralize the mixture. Concentration of liver TAG was determined through enzymatic colorimetric analysis using the Triacylglycerols LiquiColor Mono kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) according to the manufacturer's instructions.

Gene expression in liver tissue was analyzed according to Graber et al. (2012). In short, total RNA was extracted from liver samples (approximately 100 mg wet weight) with pepGOLD TriFast™ (PEQLAB Biotechnologie GmbH, Erlangen, Germany) according to the manufacturer's protocol. Quality of the RNA was determined by measuring absorbance at 260 and 280 nm, followed by gel electrophoresis with ethidium bromide staining. One microgram of total RNA was reverse transcribed with 200 U of Moloney Murine Leukemia Virus Reverse Transcriptase RNase H minus, Point Mutant (Promega Corp., Madison, WI) using random hexamer primers (Invitrogen, Leek, the Netherlands). The obtained cDNA was diluted to a final concentration of 10 ng/μL and stored at –20°C.

Relative gene expression were assessed through real-time quantitative reverse transcription (RT)-PCR using a Rotor-Gene 6000 rotary analyzer (Corbett Research, Sydney, Australia) using the software version 1.7.75. Primers sequences and annealing temperatures for candidate genes were used as previously described: *ACC*, *BDH2*, *CPT1A*, *CPT2*, *GPAM*, *HMGCS2*, and *PC* (Graber et al., 2010), *ACLY* (van Dorland et al., 2009), *IGF-1* (Gross et al., 2011), *SAA*, *Hp*, and tumor necrosis factor-α (*TNF-α*; Zarrin et al., 2014). For each assay, a master mix was prepared that consisted of 0.8 μL of water, 1.0 μL of forward primer (5 pmol), 1.0 μL of reverse primer (5 pmol), 0.2 μL of 50 × SYBR-Green (20 pmol), and 5.0 μL of 2 × SensiMix (1 mM MgCl₂; 2 × SensiMix NoRef DNA Kit). The reverse-transcription PCR was performed using 2 μL of sample volume (containing 20 ng of cDNA) with 8 μL of master mix. The PCR temperature profile consisted of 10 min incubation at 95°C. Cycling conditions consisted of 40 cycles of 15 s at 95°C, optimal annealing temperature for each individual gene for 30 s, and extension at 72°C for 20 s, followed by a melting curve program (60–95°C). All samples were

assayed on the same run to eliminate interassay variation. The mRNA expression of the target genes were calculated relative to the average mRNA expression of two housekeeping genes, *GAPDH* and ubiquitin. Stabilities of *GAPDH* and ubiquitin were assessed across all samples using the geNorm program in the current study (M-value = 0.163; Vandesompele et al., 2002). Primer information of the housekeeping genes are shown in Zarrin et al. (2014). The sizes of the obtained PCR products were verified by gel electrophoresis.

2.3.4 Statistical Analysis

Repeated measures ANOVA (PROC MIXED, SAS version 9.1, SAS Institute Inc., Cary, NC) were performed for data analysis. The cow was considered as the repeated subject. Because preliminary analysis showed that the interaction effect of diet $\times$ week was not significant ($P > 0.05$), it was excluded from the model.

Statistical analyses were performed separately for precalving and postcalving data of plasma metabolites and hormones. Precalving, DP length (0, 30, or 60 d), diet (glucogenic or lipogenic), parity (2, 3, or > 3), week (week -3 to -1 for plasma metabolites and hormones; not included in the liver TAG model), and their interactions were included as fixed effects. Postcalving, DP length (0, 30, or 60 d), diet (glucogenic or lipogenic), parity (2, 3, or > 3), week (week 1 to 8 for plasma metabolites and hormones; weeks 2 and 4 for liver TAG), and their interactions were included as fixed effects. Plasma BHB concentration postcalving were $\log_2$ transformed before analysis to obtain normal distribution of residuals.

The mRNA expression of the target genes normalized by housekeeping genes was $\log_2$ -transformed before analysis. Gene expression (delta cycle threshold, $\log_2$) was analyzed using DP length (0, 30, or 60 d), diet (glucogenic or lipogenic), parity (2, 3, or > 3), and their interaction as fixed effects. To study the effect of time on mRNA expression, DP length (0, 30, or 60 d), diet (glucogenic or lipogenic), parity (2, 3, or > 3), and week (week -2 , 2, and 4) were included as fixed effects.

For plasma metabolites, hormones and mRNA expression, a first-order autoregressive structure [AR(1)] was the best fit and was used to account for within-cow variations. For liver TAG concentration, the compound symmetry structure was the best fit and was used to account for within-cow variations. Model assumptions were evaluated by examining the distribution of residuals. For comparison of DP lengths, P -values are presented after a Bonferroni adjustment. Values are presented

as least squares means with their standard errors of the mean. Differences are regarded as significant if $P < 0.05$, and trends are discussed if $P \leq 0.10$.

2.4 Results

2.4.1 Dry Period Length, Energy Balance, Dry Matter Intake, and Animal Performance

Actual days dry were 4 ± 1 , 30 ± 1 , and 61 ± 1 d (means $\pm$ SEM) for cows with a 0, 30, or 60 d DP, respectively. Results of EB, DMI, milk yield, and body weight were previously reported (van Knegsel et al., 2014). In short, milk production in the last 8 weeks before calving was 13.8 ± 0.5 and 7.7 ± 0.5 kg/d for cows with a 0 or 30 d dry period, respectively. Precalving, DMI was greater for cows with a 0 or 30 d DP compared with cows with a 60 d DP, but EB was greater for cows with a 60 d DP. Postcalving, milk yield was lower (32.7 vs. 38.7 vs. 43.3 ± 0.7 kg/d for the 0 vs. 30 vs. 60 d DP, respectively, $P < 0.01$), whereas EB (38 vs. -59 vs. -132 ± 14 kJ/kg^{0.75}·d for the 0 vs. 30 vs. 60 d DP, respectively, $P < 0.01$), body weight (691 vs. 660 vs. 665 ± 7 kg for 0 vs. 30 vs. 60 d DP, respectively, $P < 0.01$), and BCS (3.0 vs. 2.5 vs. 2.2 ± 0.1 for the 0 vs. 30 vs. 60 d DP, respectively, $P < 0.01$) were greater for cows with a 0 d DP compared with cows with a 30 or 60 d DP. Postcalving, DP length did not affect DMI.

2.4.2 Plasma Metabolites, Hormones, and Liver Triacylglycerol

Precalving, the concentrations of plasma metabolites and hormones are shown in Table 2.2. Precalving, plasma FFA concentration (Figure 2.1 A) were lower ($P < 0.01$) for cows with a 0 d DP compared with cows with a 60 d DP. Plasma BHB (Figure 2.1 B), urea (Figure 2.1 C), and insulin (Figure 2.1 D) concentrations were greater ($P < 0.01$) for cows with a 0 d DP compared with cows with a 30 or 60 d DP. Plasma glucose concentration (Figure 2.1 E) did not differ among DP lengths, but a DP length $\times$ week interaction ($P = 0.03$) showed that plasma glucose concentration increased from week -3 until calving for cows with a 0 d DP but decreased during the same period for cows with a 30 or 60 d DP. Dry period length $\times$ week interaction for plasma IGF-1 concentration (Figure 2.1 F) was found showing a lower decrease in IGF-1 concentration for cows with a 0 d

Table 2.2. Plasma metabolites, hormones, and liver triacylglycerol (TAG) of dairy cows precalving¹ after 0, 30, and 60 d dry period and fed a glucogenic (G) or lipogenic (L) diet (LSM $\pm$ SEM).

	Dry period length (d)				SEM			Diet			SEM			<i>P</i> -value ^{2,3}			
	0	30	60					G	L					DPL	Diet	Week	Parity
Precalving																	
FFA (mmol/L)	0.09 ^b	0.12 ^{ab}	0.14 ^a		0.01	0.12	0.11	0.12	0.11		0.01	0.03	0.72	0.03	0.72	<0.01	0.21
BHB (mmol/L)	0.60 ^a	0.42 ^b	0.42 ^b		0.02	0.49	0.47	0.49	0.47		0.01	<0.01	0.39	<0.01	0.39	0.55	0.94
Glucose (mmol/L)	3.86	3.84	3.77		0.04	3.80	3.85	3.80	3.85		0.03	0.34	0.31	0.34	0.31	0.50	0.16
Urea (mmol/L)	3.60 ^a	2.56 ^b	2.56 ^b		0.13	2.77	3.04	2.77	3.04		0.11	<0.01	0.07	<0.01	0.07	<0.01	0.04
IGF-1 (ng/mL)	161.28	179.10	165.62		6.65	166.05	171.29	166.05	171.29		5.44	0.14	0.50	0.14	0.50	<0.01	<0.01
Insulin (μ IU/mL)	31.18 ^a	19.32 ^b	17.79 ^b		1.73	22.36	23.16	22.36	23.16		1.42	<0.01	0.69	<0.01	0.69	0.08	<0.01
Liver TAG (mg/g of wet weight)	10.49 ^b	14.91 ^a	15.03 ^a		1.33	14.25	12.70	14.25	12.70		1.09	0.03	0.31	0.03	0.31	NM ⁴	0.07

^{a,b}Values within a dry period length in the same row with different superscripts differ ($P < 0.05$).

¹Week -3, -2, and -1 relative to calving.

²The *P*-value of interactions of DPL $\times$ parity, DPL $\times$ diet, and diet $\times$ parity not shown in table.

³DPL = dry period length; W = week.

⁴NM: not included in the model.

Figure 2.1. (A) Plasma FFA (mmol/L); (B) plasma BHB (mmol/L); (C) plasma urea (mmol/L); (D) insulin (μ IU/mL); (E) plasma glucose (mmol/L); (F) plasma IGF-1 (ng/mL) for cows with a 0, 30, or 60 d dry period (DP) during week -3 to 8 relative to calving. Values represent means ($\pm$ SEM) per DP length per week.

Figure 2.2. Liver triacylglycerol (TAG; mg/g of wet weight) for cows with a 0, 30, or 60 d dry period (DP) in weeks -2, 2, and 4 relative to calving. Values represent means ($\pm$ SEM) per DP length per week.

DP compared with cows with a 30 or 60 d DP in early lactation ($P < 0.01$). Liver TAG concentration (Figure 2.2) were lower for cows with a 0 d DP, compared with cows with a 30 or 60 d DP ($P = 0.03$). Young cows (parity = 2) with a 0 d DP had lower liver TAG concentration (9.91 vs. 16.01 vs. 21.71 ± 2.77 mg/g for the 0 vs. 30 vs. 60 d DP, respectively; $P = 0.04$). In older cows, no effects of DP length on liver TAG concentration were found (10.45 vs. 13.67 vs. 11.86 ± 1.15 mg/g for 0 vs. 30 vs. 60 d DP, respectively; $P = 0.66$).

Postcalving, cows with a 0 d DP had lower ($P < 0.01$) plasma FFA and liver TAG concentrations compared with cows with a 30 or 60 d DP (Table 2.3). The DP length did not affect plasma BHB or urea concentrations. Plasma glucose, IGF-1, and insulin concentrations were greater ($P < 0.01$) for cows with a 0 d DP compared with cows with a 30 or 60 d DP. Plasma FFA, urea, insulin, glucose, IGF-1, and liver TAG ($P < 0.01$) concentrations were different over time. Plasma IGF-1 ($P = 0.03$), glucose ($P = 0.04$), and liver TAG ($P = 0.03$) concentrations were affected by DP length $\times$ week interaction. Additionally, plasma glucose ($P = 0.02$) and insulin ($P < 0.01$) concentrations were affected by DP length $\times$ parity interaction.

For both precalving and postcalving period, diet did not affect plasma FFA, insulin, glucose, IGF-1, or liver TAG concentrations. Precalving, plasma urea concentration tended to be lower for cows fed a glucogenic diet compared

Table 2.3. Plasma metabolites, hormones, and liver triacylglycerol (TAG) of dairy cows in early lactation¹ after 0, 30, and 60 d DP and fed a glucogenic (G) or lipogenic (L) diet (LSM $\pm$ SEM).

Postcalving	Dry period length (d)				SEM		Diet		SEM		P-value ^{2,3}				
	0	30	60				G	L			DPL	Diet	Week	Parity	DPL
											$\times$ P				$\times$ W
FFA (mmol/L)	0.20 ^b	0.32 ^a	0.36 ^a	0.02	0.28	0.31	0.02	0.02	<0.01	0.24	<0.01	0.15	0.02	0.01	0.49
BHB (mmol/L) ⁴	0.66	0.62	0.64	0.03	0.62	0.66	0.03	0.03	0.82	0.01	0.13	0.01	0.02	0.01	0.29
Glucose (mmol/L)	3.80 ^a	3.60 ^b	3.54 ^b	0.04	3.64	3.65	0.03	0.03	<0.01	0.90	<0.01	0.02	<0.01	<0.01	0.04
Urea (mmol/L)	2.91	2.81	2.79	0.10	2.62	3.04	0.09	0.09	0.67	<0.01	<0.01	0.27	0.91	0.01	0.68
IGF-1 (ng/mL)	118.26 ^a	84.50 ^b	78.40 ^b	5.33	96.06	91.39	4.30	4.30	<0.01	0.45	<0.01	0.20	<0.01	<0.01	0.03
Insulin (μ IU/mL)	24.38 ^a	14.02 ^b	11.08 ^b	1.21	16.22	16.76	0.99	0.99	<0.01	0.70	<0.01	<0.01	0.03	0.03	0.27
Liver TAG (mg/g of wet weight)	21.44 ^b	46.13 ^a	55.35 ^a	5.38	39.41	42.53	4.40	4.40	<0.01	0.62	<0.01	0.68	0.40	0.01	0.03

^{a,b}Values within a dry period length in the same row with different superscripts differ ($P < 0.05$).

¹Week 1, 2, 3, 4, 5, 6, 7, and 8 relative to calving.

²The P -value of interactions of DPL $\times$ diet, and diet $\times$ parity not shown in table.

³DPL = dry period length; P = parity; W = week.

⁴ P -value based on the binary logarithm of plasma BHB concentration.

with cows fed a lipogenic diet ($P = 0.07$). Postcalving, plasma urea ($P < 0.01$) and BHB ($P = 0.01$) concentrations were lower for cows fed the glucogenic diet compared with cows fed the lipogenic diet. Postcalving, plasma BHB concentration was affected by diet $\times$ parity interaction ($P < 0.01$). In contrast to the lipogenic diet, the glucogenic diet resulted in a lower plasma BHB concentration for young cows (0.50 ± 0.05 mmol/L vs. 0.68 ± 0.05 mmol/L, $P < 0.01$), but not for older cows (0.67 ± 0.04 mmol/L vs. 0.64 ± 0.04 mmol/L, $P = 0.52$).

2.4.3 mRNA Expression of Genes in Liver

The mRNA expression of *CPT1A* and *CPT2* did not differ among DP lengths (Table 2.4). The mRNA expression of *CPT2* was increased from week -2 to 2 for all DP lengths ($P < 0.01$). The mRNA expression of *PC* was lower for cows with a 0 d DP compared with cows with a 30 or 60 d DP in week 4 ($P < 0.01$). No differences in the mRNA expression of *IGF-1* were found among DP lengths. The mRNA expression of *PC* was increased from week -2 to 2 or 4 for all DP lengths ($P < 0.01$). In contrast, mRNA expression of *IGF-1* was decreased from week -2 to 2 or 4 for all DP lengths ($P < 0.01$).

The mRNA expression related to fatty acid and triacylglycerol synthesis (*ACLY*, *GPAM*, and *ACC*) and to ketone body synthesis (*BDH2* and *HMGCS2*) did not differ among the DP lengths. The mRNA expression of *GPAM* was decreased from week -2 to 2 for all DP lengths ($P < 0.01$), whereas the mRNA expression of *ACC* was increased from week -2 to 4 for all DP lengths ($P = 0.03$). The mRNA expression of *ACLY* was decreased from week 2 to 4 for all DP lengths ($P = 0.03$).

The mRNA expression of *SAA*, *Hp*, and *TNF- α* did not differ among the DP lengths. The mRNA expression of *Hp* increased from week -2 to 2 for all DP lengths ($P < 0.01$). The mRNA expression of *SAA* tended to increase from week -2 to 2 for all DP lengths ($P = 0.06$). Diet had no effects on the mRNA expression of *CPT1A*, *CPT2*, *PC*, *IGF-1*, *ACLY*, *ACC*, *GPAM*, *BDH2*, *HMGCS2*, *SAA*, *Hp*, and *TNF- α* for all time points.

Table 2.4. Gene expression (delta cycle threshold, log₂) in liver in dairy cows after 0, 30, and 60 d dry period and fed a glucogenic (G) or lipogenic (L) diet (LSM ± SEM).

Gene ¹	Week	Dry period length (d)			SEM	<i>P</i> -value ²			
		0	30	60		DPL	Diet	Week ³	DPL×D ⁴
Fatty acid oxidation related genes									
<i>CPT1A</i>	−2	15.09	15.35	15.39	0.20	0.48	0.95	0.65	0.87
	2	15.23	15.39	15.49	0.20	0.64	0.78		0.46
	4	15.24	15.59	15.20	0.19	0.32	0.96		0.06
<i>CPT2</i>	−2	16.63	16.51	16.47	0.11	0.55	0.93	<0.01	0.63
	2	16.82	16.75	16.96	0.11	0.38	0.83		0.74
	4	16.69	16.68	16.77	0.09	0.74	0.61		0.53
Gluconeogenesis related gene									
<i>PC</i>	−2	17.15	17.48	17.39	0.21	0.50	0.24	<0.01	0.07
	2	17.87	18.29	18.45	0.21	0.13	0.18		0.73
	4	17.47 ^b	18.62 ^a	18.24 ^a	0.19	<0.01	0.31		0.80
IGF-1 synthesis related gene									
<i>IGF-1</i>	−2	17.59	17.65	17.88	0.20	0.58	0.40	<0.01	0.37
	2	17.13	17.19	17.12	0.20	0.51	0.83		0.58
	4	17.52	17.01	17.10	0.19	0.16	0.57		0.97
Fatty acid synthesis related genes									
<i>ACLY</i>	−2	13.06	13.36	13.37	0.20	0.41	0.33	0.03	0.67
	2	13.13	13.52	13.56	0.19	0.22	0.96		0.95
	4	13.28	13.34	13.12	0.14	0.53	0.73		0.53
<i>ACC</i>	−2	11.02	10.78	10.41	0.19	0.11	0.41	0.03	0.94
	2	10.98	11.04	10.94	0.20	0.94	0.68		0.43
	4	11.20	11.30	11.25	0.23	0.96	0.82		0.34
Triacylglycerol synthesis related gene									
<i>GPAM</i>	−2	17.08	17.26	17.21	0.13	0.54	0.29	<0.01	0.98
	2	16.95	17.02	16.96	0.11	0.91	0.94		0.89
	4	16.98	16.76	16.95	0.12	0.42	0.92		0.84
Ketone body synthesis related genes									
<i>BDH2</i>	−2	17.14	17.02	17.22	0.16	0.72	0.14	0.39	0.53
	2	17.46	17.06	17.16	0.20	0.31	0.97		0.38
	4	17.30	16.91	17.30	0.18	0.22	0.97		0.50
<i>HMGCS2</i>	−2	21.83	21.65	21.81	0.16	0.65	0.84	0.66	0.54
	2	21.81	21.77	22.46	0.19	0.35	0.86		0.72
	4	21.48	21.80	21.65	0.22	0.60	0.82		0.85

^{a,b}Values within a dry period length in the same row with different superscripts differ ($P < 0.05$).

(Continued on the next page)

Table 2.4 (continued).

Gene ¹	Week	Dry period length (d)			SEM	<i>P</i> -value ²			
		0	30	60		DPL	Diet	Week ³	DPL×D ⁴
Immune function related genes									
<i>SAA</i>	−2	16.12	16.12	16.01	0.43	0.98	0.32	0.06	0.57
	2	17.77	17.05	16.59	0.49	0.21	0.17		0.04
	4	17.41	16.16	16.85	0.45	0.17	0.82		0.76
<i>Hp</i>	−2	16.80	16.25	17.09	0.64	0.72	0.68	<0.01	0.61
	2	20.08	18.86	18.53	0.73	0.25	0.17		0.08
	4	18.95	17.48	19.06	0.76	0.32	0.94		0.82
<i>TNF-α</i>	−2	11.25	11.02	10.89	0.21	0.43	0.51	0.40	0.48
	2	11.16	10.99	11.26	0.18	0.58	0.62		0.55
	4	11.07	11.01	10.96	0.15	0.87	0.33		0.67

^{a,b}Values within a dry period length in the same row with different superscripts differ ($P < 0.05$).

¹*ACLY* = ATP citrate lyase; *ACC* = acetyl-CoA-carboxylase; *BDH2* = 3-hydroxybutyrate dehydrogenase 2; *CPT1A* = carnitine palmitoyltransferase 1A; *CPT2* = carnitine palmitoyltransferase 2; *GPAM* = glycerol-3-phosphate acyltransferase mitochondrial; *HMGCS2* = 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2; *Hp* = haptoglobin; *IGF-1* = insulin-like growth factor-I; *PC* = pyruvate carboxylase; *SAA* = serum amyloid-a; *TNF-α* = tumor necrosis factor-α.

²The *P*-value of parity and interaction of DPL × parity and ration × parity not shown in table.

³Week −2, 2, and 4 relative to calving.

⁴DPL = dry period length; D = diet.

2.5 Discussion

In the current study, plasma glucose concentration was greater in cows with a 0 d DP compared with cows with a 30 or 60 d DP in early lactation after calving. Earlier, we showed that cows with a reduced DP (0 or 30 d) had a lower milk yield and milk lactose production compared with cows with a 60 d DP (van Knegsel et al., 2014). Dry period length did not affect DMI in early lactation. Lower milk production and lower lactose production results in a reduced requirement of glucose by the mammary gland (Lemosquet et al., 2009). Herewith, with an equal DMI, omitting the DP seems to result in repartitioning of glucose from milk to body reserves, as indicated by elevated plasma insulin concentration and improved EB (van Knegsel et al., 2014).

In lactating dairy cows, the whole-body glucose flux is primarily derived from hepatic gluconeogenesis. Ruminal propionate is the primary substrate for gluconeogenesis in dairy cows (Drackley et al., 2001). The increased demand for glucose precursors in early lactation, however, cannot be met by ruminal propionate completely because of relatively low DMI. Lower DMI leads to reduced glucose

synthesis from propionate, whereas more endogenous precursors (lactate, glycerol, and amino acids) are used as gluconeogenic substrate (Greenfield et al., 2000). Aschenbach et al. (2010) indicated that increased hepatic mRNA expression of *PC* is critical in gluconeogenesis from endogenous precursors during the transition from precalving to postcalving. In the current study, mRNA expression of *PC* was lower for cows with a 0 d DP in week 4 after calving, compared with cows with a 30 or 60 d DP. The lower mRNA expression of *PC* in cows with a 0 d DP suggests that omitting the DP results in lower rate of gluconeogenesis from endogenous precursors in the liver compared with cows with a 30 or 60 d DP. These results support our hypothesis that reducing the DP decreases the demand for glucose in the mammary gland of dairy cows. In addition, *PC* mRNA expression increased after calving for all DP lengths and was lower for cows with a 0 d DP compared with cows with a 30 or 60 d DP at week 4. This indicates a shorter recovery time in early lactation from elevated hepatic gluconeogenic activity from endogenous precursors in these cows.

In the current study, omitting the DP increased plasma insulin concentration compared with a 30 or 60 d DP in early lactation, which is in line with previous studies (Andersen et al., 2005; De Feu et al., 2009). In early lactation, the elevation of plasma insulin plays a key role in coupling the growth hormone (GH)-IGF axis. It is well known that the liver is the major contributor of plasma IGF-1 (Radcliff et al., 2003). During negative EB, however, hepatic IGF-1 synthesis is reduced because of GH resistance in the liver, which is related to downregulation of the hepatic GH receptor (**GHR**) (Lucy et al., 2001). In the current study, plasma IGF-1 concentration was greater for cows with a 0 d DP compared with cows with a 30 or 60 d DP. Butler et al. (2003) suggested that the greater plasma IGF-1 concentration is associated with an insulin-induced increase of *IGF-1* and *GHR* mRNA expression in the liver. The mRNA expression of *IGF-1* in the liver, however, did not differ among DP lengths in this study, which was unexpected. Grala et al. (2011) also observed no correlation between plasma IGF-1 concentration and hepatic *IGF-1* mRNA expression in the first week after calving. They attributed this to more variable data or post-translational modification.

In early lactation, increased mobilization of adipose tissue is associated with hypoinsulinemia or decreased insulin sensitivity or both (Bell, 1995). High

insulin concentration was reported to decrease responsiveness to GH in adipose tissue by inhibiting GHR expression and, thus, suppressing lipolysis (Butler et al., 2003). The decreased rate of lipolysis results in decreased plasma FFA concentration (Locher et al., 2011). Thus, lower plasma FFA concentration in cows with a 0 d DP, compared with cows with a 30 or 60 d DP reflects decreased fat mobilization, which is in agreement with the greater EB and BCS in these cows, as previously reported (van Knegsel et al., 2014). Schlamberger et al. (2010) also reported that continuous milking (0 d DP) resulted in lower plasma FFA concentration during the first 8 weeks of lactation compared with a 56-d DP.

Free fatty acids can be esterified and stored as TAG in liver. In the current study, the lower plasma FFA concentration may explain the lower liver TAG concentration in cows with a 0 d DP, compared with cows with a 30 or 60 d DP. Bobe et al. (2004) suggested that cows with a moderate (50 to 100 mg/g liver TAG on a wet weight basis) or severe fatty liver (> 100 mg/g liver TAG) were at high risk for metabolic and reproductive problems. The *GPAM* gene encodes the mitochondrial isoform of enzyme glycerol-3-phosphate-acyltransferase. During liver TAG synthesis, GPAM catalyzes the first step of glycerolipid synthesis: addition of fatty acids to glycerol-3-phosphate to form lysophosphatidic acid (Takeuchi and Reue, 2009). In the current study, no differences in mRNA expression of *GPAM* were found among DP lengths, despite the fact that cows with a 0 d DP had lower liver TAG concentration. Looor et al. (2006) suggested that the *GPAM* is not rate limiting for TAG accumulation in the liver.

Although FFA can be used for ketogenesis through mitochondrial oxidation, DP length did not affect plasma BHB concentration in this study. It was suggested that plasma FFA concentration does not determine the extent of β -oxidation and plasma BHB concentration in periparturient dairy cows (van Knegsel et al., 2007b). In line with this concept, Blum et al. (2000) observed that plasma BHB concentration were not correlated with plasma FFA concentration after feeding different fat sources over a 24-h period in week 9 and 19 of lactation in high-yielding dairy cows. On the one hand, plasma BHB could come from the partial β -oxidation of FFA in liver or from conversion of butyrate in rumen (Bergman, 1990). On the other hand, plasma BHB could be used for synthesis of milk fat in the mammary gland (Bauman and Griinari, 2003) or as an alternative energy source for glucose in brain and muscles (Veech, 2004). In the current study, plasma BHB concentration was relatively low

for all DP lengths: on average lower than 1.2 mmol/L, the threshold above which subclinical ketosis is indicated (Duffield et al., 1998).

In the current experiment, DP length did not affect plasma BHB concentration, whereas DP length had an effect on the EB in early lactation (van Knegsel et al., 2014). On the contrary, diet in early lactation did affect plasma BHB concentration. Cows fed lipogenic diet had greater plasma BHB concentration, compared with cows fed glucogenic diet, which is in line with our earlier study (van Knegsel et al., 2007b). This indicates that plasma BHB concentration is more affected by diet composition than DP length in early lactation. In addition, DP length did affect plasma BHB concentration precalving. Cows with a 0 d DP had greater plasma BHB concentration, compared with cows with a 30 or 60 d DP, which is in line with earlier work (Andersen et al., 2005). The greater BHB concentration precalving could be related to greater DMI for cows with a 0 d DP (van Knegsel et al., 2014) or the difference in diet composition, because these cows were fed a lactation diet during the precalving period.

The lack of effect of DP length on plasma BHB might suggest a similar rate of β -oxidation for the different DP lengths in this study. Carnitine palmitoyltransferase 1A and carnitine palmitoyltransferase 2 are enzymes that transport fatty acids into the mitochondria and are converted to ketone bodies through β -oxidation (McGarry and Brown, 1997). In early lactation, the lack of differences in *CPT1A* expression and the expression of genes related to ketogenesis (*BDH2* and *HMGCS2*) may confirm that the similar rate of β -oxidation in the liver among all DP lengths. In contrast, Wiedemann et al. (2013) observed a greater *CPT1A* mRNA expression in early lactation of continuously milked cows compared with cows with a conventional DP. These differences between studies could be explained by the relatively low concentration of FFA for all DP lengths in the current study which may not be in the range of upregulating the *CPT1A* mRNA expression (van Dorland et al., 2009). Additional research is required to confirm this hypothesis. In addition, for all DP lengths, the mRNA expression of *CPT2* increased after calving. Graber et al. (2010) also reported increased mRNA expression of *CPT2* in week 4 postcalving compared with week 3 precalving. Van Dorland et al. (2012) observed that mRNA expression of *CPT2* was greater for cows with greater plasma FFA concentration ($> 500 \mu\text{mol/L}$) than for cows with lower FFA concentration ($< 140 \mu\text{mol/L}$) at d 1 after calving, suggesting that

expression of *CPT2* in liver of dairy cows might be related to the FFA concentration in plasma.

In nonruminants, *ACLY* is believed to be the key enzyme involved in cleavage of cytosolic citrate to generate acetyl-CoA from glucose (Hatzivassiliou et al., 2005). Then acetyl-CoA can be converted to malonyl-CoA, a pivotal precursor for the synthesis of fatty acids, by the *ACC* enzyme (Abu-Elheiga et al., 2001). In ruminants, Ballard et al. (1972) observed increased *ACLY* enzyme activity in response to a high-carbohydrate diet in the liver. In the current study, however, no effects of DP length and diet were observed on mRNA expression of *ACLY* and *ACC* in the liver. Khan et al. (2014) suggested that liver is not a lipogenic tissue in ruminants.

In the current study, expression of mRNAs encoding SAA and Hp proteins increased from late pregnancy to early lactation independent of DP length. The increased gene expression of acute phase proteins after calving has been suggested to be the result of a combination of bacterial contamination and involution of the uterus (Sheldon et al., 2001). Previous studies reported that serum concentrations of SAA and Hp peaked in the first week rather than the second week after calving (Nyman et al., 2008; Chan et al., 2010), which could partially explain the absence of the effects of DP lengths on mRNA expression of *SAA* and *Hp*. Furthermore, elevated mRNA expression of *Hp* in week 4 did not correspond to the findings of Chan et al. (2010), who observed that serum concentrations of Hp and SAA decreased to baseline values in the third week and first week after calving, respectively. In the current study, the elevated mRNA expression of *SAA* and *Hp* in week 4 could be associated with inflammatory diseases such as metritis and mastitis or noninfectious stressors such as tissue injury or psychosocial stressors (Colditz, 2002).

In several studies, hepatic gene expression was investigated in week 1 after calving, because most genes involved in main metabolic and inflammatory processes change greatly at the time of initiation of lactation (Greenfield et al., 2000; Nyman et al., 2008; Wiedemann et al., 2013). In the current study, we investigated gene expression in week 2 after calving, which was based on a previous study in which the EB reached nadir around week 2 after calving (Rastani et al., 2005). Also, the nadirs of EB (van Knegsel et al., 2014), plasma concentrations of glucose, IGF-1,

and insulin occurred around week 2 for cows in the current study, indicating that cows experienced great metabolic challenges during week 2 in lactation.

Precalving, cows with a 0 d DP had not only greater plasma BHB but also lower FFA concentrations compared with cows with a 60 d DP, which is in line with a previous study (Andersen et al., 2005). The lower plasma FFA and liver TAG concentrations may be related to ongoing lactation in late pregnancy in cows with a 0 d DP, because plasma FFA could be absorbed and esterified by the mammary gland (Glasser et al., 2007). The greater plasma BHB and urea concentrations precalving may be caused by greater nutrient absorption from greater feed intake and higher CP content in the diet of cows with a 0 d DP (Andersen et al., 2005). Additionally, greater concentrate intake precalving could explain the greater plasma insulin concentration in cows with a 0 d DP in the current study (Grummer, 1995). Further, the elevation of plasma FFA concentration and the decline of plasma IGF-1 concentration in weeks before calving in our experiment are commonly observed (Fenwick et al., 2008; De Feu et al., 2009; Schlamberger et al., 2010) and can be explained by the decreased feed intake and increased energy requirements during late pregnancy (Ingvarsen and Andersen, 2000).

Contrary to our expectations, no diet effects on plasma metabolites, hormones, liver TAG, or hepatic gene expression were found except for plasma urea and BHB concentrations. Greater plasma BHB and urea concentrations for cows fed a lipogenic diet, compared with cows fed a glucogenic diet, are in line with our earlier study (van Knegsel et al., 2007b). In contrast to this earlier study, the glucogenic diet in the current study did not decrease the mobilization of body fat and was not related to greater plasma insulin concentration and lower plasma FFA concentration. The differences between studies are probably the result of a lower proportion of experimental concentrates used (8.5 vs. 12 kg/d) and smaller dietary contrast (less starch in the glucogenic diet; less fiber and fat in the lipogenic diet) in the current study compared with the previous study. The greater plasma urea concentration in cows fed a lipogenic diet, compared with cows fed a glucogenic diet, is consistent with our previous study (van Knegsel et al., 2007b). This could be related to the use of glucogenic amino acids for gluconeogenesis.

2.6 Conclusions

In the current study, omission of the DP (0 d) but not shortening the DP (30 d) resulted in an improved metabolic status of dairy cows in early lactation compared with a conventional DP (60 d). This improvement was associated with less body fat mobilization because of reduced milk yield, as indicated by decreased plasma FFA and liver TAG concentrations, and increased plasma insulin, glucose, and IGF-1 concentrations. The improved metabolic status in cows with a 0 d DP was confirmed by the lower mRNA expression of *PC* in early lactation. Independent of DP length, a glucogenic diet also improved the metabolic status, as indicated by lower plasma BHB and urea concentrations, compared with a lipogenic diet.

2.7 Acknowledgments

The authors thank the Dutch Dairy Board (PZ, the Netherlands), Product Board Animal Feed (PDV; the Netherlands), and CRV (the Netherlands) for financing the experiment. The first author thanks the Chinese Scholarship Council (CSC) for financial support. Additionally, the authors thank the staff of the Dairy Campus (Lelystad, the Netherlands) and Yolande Zbinden, Claudine Morel, and Olga Wellnitz from the Veterinary Physiology Group of the University of Bern for their technical support and laboratory analyses during the experiment.

References

- Abu-Elheiga, L., M. M. Matzuk, K. A. Abo-Hashema, and S. J. Wakil. 2001. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science* 291:2613-2616.
- Andersen, J. B., T. G. Madsen, T. Larsen, K. L. Ingvarsen, and M. O. Nielsen. 2005. The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. *J. Dairy Sci.* 88:3530-3541.
- Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon, and G. B. Penner. 2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. *IUBMB life* 62:869-877.
- Bachman, K. C. and M. L. Schairer. 2003. Invited review: bovine studies on optimal lengths of dry periods. *J. Dairy Sci.* 86:3027-3037.
- Ballard, F., O. Filsell, and I. Jarrett. 1972. Effects of carbohydrate availability on lipogenesis in sheep. *Biochem. J.* 126:193-200.
- Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. *Annu. Rev. Nutr.* 23:203-227.
- Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73:2804-2819.
- Bergman, E. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* 70:567-590.
- Blum, J., R. Bruckmaier, P. Y. Vacher, and F. Jans. 2000. Twenty-four-hour patterns of hormones and metabolites in week 9 and 19 of lactation in high-yielding dairy cows fed triglycerides and free fatty acids. *J. Vet. Med. A* 47:43-60.
- Bobe, G., J. Young, and D. Beitz. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *J. Dairy Sci.* 87:3105-3124.
- Butler, S. T., A. Marr, S. Pelton, R. Radcliff, M. C. Lucy, and W. Butler. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. *J. Endocrinol.* 176:205-217.

- Butler, W. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livest. Prod. Sci.* 83:211-218.
- Chagas, L., J. Bass, D. Blache, C. Burke, J. Kay, D. Lindsay, M. Lucy, G. Martin, S. Meier, and F. Rhodes. 2007. Invited review: new perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. *J. Dairy Sci.* 90:4022-4032.
- Chan, J. P. W., C. C. Chang, W. L. Hsu, W. B. Liu, and T. H. Chen. 2010. Association of increased serum acute-phase protein concentrations with reproductive performance in dairy cows with postpartum metritis. *Vet. Clin. Path.* 39:72-78.
- Colditz, I. 2002. Effects of the immune system on metabolism: implications for production and disease resistance in livestock. *Livest. Prod. Sci.* 75:257-268.
- De Feu, M., A. Evans, P. Lonergan, and S. Butler. 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status, and postpartum ovarian function in Holstein-Friesian dairy cows. *J. Dairy Sci.* 92:6011-6022.
- Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. *J. Dairy Sci.* 84:E100-E112.
- Duffield, T., D. Sandals, K. Leslie, K. Lissemore, B. McBride, J. Lumsden, P. Dick, and R. Bagg. 1998. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. *J. Dairy Sci.* 81:2866-2873.
- Fenwick, M. A., R. Fitzpatrick, D. A. Kenny, M. G. Diskin, J. Patton, J. J. Murphy, and D. C. Wathes. 2008. Interrelationships between negative energy balance (NEB) and IGF regulation in liver of lactating dairy cows. *Domest. Anim. Endocrinol.* 34:31-44.
- Gümen, A., R. R. Rastani, R. R. Grummer, and M. C. Wiltbank. 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *J. Dairy Sci.* 88:2401-2411.
- Gessner, D., G. Schlegel, J. Keller, F. Schwarz, R. Ringseis, and K. Eder. 2013. Expression of target genes of nuclear factor E2-related factor 2 in the liver of dairy cows in the transition period and at different stages of lactation. *J. Dairy Sci.* 96:1038-1043.

- Glasser, F., M. Doreau, A. Ferlay, and Y. Chilliard. 2007. Technical note: estimation of milk fatty acid yield from milk fat data. *J. Dairy Sci.* 90:2302-2304.
- Graber, M., S. Kohler, T. Kaufmann, M. G. Doherr, R. M. Bruckmaier, and H. A. van Dorland. 2010. A field study on characteristics and diversity of gene expression in the liver of dairy cows during the transition period. *J. Dairy Sci.* 93:5200-5215.
- Graber, M., S. Kohler, A. Muller, K. Burgermeister, T. Kaufmann, R. M. Bruckmaier, and H. A. van Dorland. 2012. Identification of plasma and hepatic parameters related to metabolic robustness in dairy cows. *J. Anim. Physiol. Anim. Nutr. (Berl)* 96:75-84.
- Grala, T., M. Lucy, C. Phyn, A. Sheahan, J. Lee, and J. Roche. 2011. Somatotrophic axis and concentrate supplementation in grazing dairy cows of genetically diverse origin. *J. Dairy Sci.* 94:303-315.
- Greenfield, R., M. Cecava, and S. Donkin. 2000. Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during the transition to lactation. *J. Dairy Sci.* 83:1228-1236.
- Gross, J., H. van Dorland, F. Schwarz, and R. Bruckmaier. 2011. Endocrine changes and liver mRNA abundance of somatotrophic axis and insulin system constituents during negative energy balance at different stages of lactation in dairy cows. *J. Dairy Sci.* 94:3484-3494.
- Grummer, R. and R. Rastani. 2004. Why reevaluate dry period length? *J. Dairy Sci.* 87:E77-E85.
- Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J. Anim. Sci.* 73:2820-2833.
- Hatzivassiliou, G., F. Zhao, D. E. Bauer, C. Andreadis, A. N. Shaw, D. Dhanak, S. R. Hingorani, D. A. Tuveson, and C. B. Thompson. 2005. ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell* 8:311-321.
- Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. *J. Dairy Sci.* 83:1573-1597.
- Khan, M. J., C. B. Jacometo, D. E. Graugnard, M. N. Corrêa, E. Schmitt, F. Cardoso, and J. J. Lóor. 2014. Overfeeding dairy cattle during late-pregnancy alters hepatic PPAR α -regulated pathways including

- hepatokines: impact on metabolism and peripheral insulin sensitivity. *Gene Regul. Syst. Bio.* 8:97.
- Klusmeyer, T. H., A. C. Fitzgerald, A. C. Fabellar, J. M. Ballam, R. A. Cady, and J. L. Vicini. 2009. Effect of recombinant bovine somatotropin and a shortened or no dry period on the performance of lactating dairy cows. *J. Dairy Sci.* 92:5503-5511.
- Lemosquet, S., G. Raggio, G. Lobley, H. Rulquin, J. Guinard-Flament, and H. Lapiere. 2009. Whole-body glucose metabolism and mammary energetic nutrient metabolism in lactating dairy cows receiving digestive infusions of casein and propionic acid. *J. Dairy Sci.* 92:6068-6082.
- Locher, L., N. Meyer, E.-M. Weber, J. Rehage, U. Meyer, S. Dänicke, and K. Huber. 2011. Hormone-sensitive lipase protein expression and extent of phosphorylation in subcutaneous and retroperitoneal adipose tissues in the periparturient dairy cow. *J. Dairy Sci.* 94:4514-4523.
- Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. *Physiol. Genomics* 23:217-226.
- Loor, J. J., H. M. Dann, N. A. J. Guretzky, R. E. Everts, R. Oliveira, C. A. Green, N. B. Litherland, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2006. Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. *Physiol. Genomics* 27:29-41.
- Lucy, M., H. Jiang, and Y. Kobayashi. 2001. Changes in the somatotrophic axis associated with the initiation of lactation. *J. Dairy Sci.* 84:E113-E119.
- McGarry, J. D. and N. F. Brown. 1997. The mitochondrial carnitine palmitoyltransferase system—from concept to molecular analysis. *Eur. J. Biochem.* 244:1-14.
- Nyman, A.-K., U. Emanuelson, K. Holtenius, K. L. Ingvarsen, T. Larsen, and K. Persson Waller. 2008. Metabolites and immune variables associated with somatic cell counts of primiparous dairy cows. *J. Dairy Sci.* 91:2996-3009.
- Radcliff, R., B. McCormack, B. Crooker, and M. Lucy. 2003. Plasma hormones and expression of growth hormone receptor and insulin-like growth factor-I

- p mRNA in hepatic tissue of periparturient dairy cows.
- J. Dairy Sci.*
- 86:3920-3926.
- Rastani, R., R. Grummer, S. Bertics, A. Gümen, M. Wiltbank, D. Mashek, and M. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004-1014.
- Schlamberger, G., S. Wiedemann, E. Viturro, H. Meyer, and M. Kaske. 2010. Effects of continuous milking during the dry period or once daily milking in the first 4 weeks of lactation on metabolism and productivity of dairy cows. *J. Dairy Sci.* 93:2471-2485.
- Sheldon, I., D. Noakes, A. Rycroft, and H. Dobson. 2001. Acute phase protein responses to uterine bacterial contamination in cattle after calving. *Vet. Rec.* 148:172-175.
- Takeuchi, K. and K. Reue. 2009. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. *Am. J. Physiol. Endocrinol Metab.* 296:E1195-E1209.
- Tamminga, S., W. M. Van Straalen, A. P. Subnel, R. G. Meijer, A. Steg, C. J. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. *Livest. Prod. Sci.* 40:139-155.
- Van Dorland, H., H. Sadri, I. Morel, and R. Bruckmaier. 2012. Coordinated gene expression in adipose tissue and liver differs between cows with high or low NEFA concentrations in early lactation. *J. Anim. Physiol. Anim. Nutr.* 96:137-147.
- Van Dorland, H. A., S. Richter, I. Morel, M. G. Doherr, N. Castro, and R. M. Bruckmaier. 2009. Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. *J. Dairy Sci.* 92:1924-1940.
- Van Es, A. 1975. Feed evaluation for dairy cows. *Livest. Prod. Sci.* 2:95-107.
- Van Knegsel, A., H. Van den Brand, J. Dijkstra, W. Van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007a. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. *J. Dairy Sci.* 90:3397-3409.
- Van Knegsel, A., H. Van den Brand, E. Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga, and B. Kemp. 2007b. Dietary energy source in

- dairy cows in early lactation: metabolites and metabolic hormones. *J. Dairy Sci.* 90:1477-1485.
- van Kneegsel, A., S. G. van der Drift, J. Čermáková, and B. Kemp. 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: A systematic review. *Vet. J.* 198:707-713.
- Van Kneegsel, A. T., G. J. Remmelink, S. Jorjong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *J. Dairy Sci.* 97:1499-1512.
- Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3:research0034.0031–0034.0011.
- Veech, R. L. 2004. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot. Essent. Fatty Acids* 70:309-319.
- Vicari, T., J. J. Van den Borne, W. J. Gerrits, Y. Zbinden, and J. W. Blum. 2008. Postprandial blood hormone and metabolite concentrations influenced by feeding frequency and feeding level in veal calves. *Domest. Anim. Endocrinol.* 34:74-88.
- Wiedemann, S., G. Sigl, C. Schmautz, M. Kaske, E. Viturro, and H. H. Meyer. 2013. Omission of dry period or milking once daily affects metabolic status and is reflected by mRNA levels of enzymes in liver and muscle of dairy cows. *Livest. Sci.* 154:193-203.
- Zarrin, M., O. Wellnitz, H. van Dorland, and R. Bruckmaier. 2014. Induced hyperketonemia affects the mammary immune response during lipopolysaccharide challenge in dairy cows. *J. Dairy Sci.* 97:330-339.

CHAPTER 3

Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths

J. Chen,* N. M. Soede,* H. A. Van Dorland, § G. J. Remmelink, ‡ R. M. Bruckmaier, § B. Kemp,* A. T. M. van Knegsel*

*Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands; ‡Livestock Research, Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, the Netherlands; §Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland.

3.1 Abstract

The objectives of the current study were to evaluate the effects of dry period (DP) length on ovarian activity in cows fed a lipogenic or a glucogenic diet within 100 d in milk (DIM), and to determine relationships between ovarian activity and energy balance and metabolic status in early lactation. Holstein-Friesian dairy cows ($n = 167$) were randomly assigned to 1 of 3 DP lengths (0, 30, or 60 d) and 1 of 2 diets in early lactation (glucogenic or lipogenic diet) resulting in a 3×2 factorial design. Cows were monitored for body condition score (BCS), milk yield, dry matter intake (DMI), and energy balance from calving to week 8 postcalving, and blood was sampled weekly from 95 cows from calving to week 8 postcalving. Milk samples were collected three times a week until 100 DIM postcalving for determination of progesterone concentration. At least two succeeding milk samples with progesterone concentration ≥ 2 ng/mL or greater were used to indicate the occurrence of luteal activity. Normal resumption of ovarian cyclicity was defined as the onset of luteal activity (OLA) occurring at 45 DIM or less, followed by regular ovarian cycles of 18 to 24 d in length. Within 100 DIM postcalving, cows with a 0 d DP had greater incidence of normal resumption of ovarian cyclicity (53.2%; 25 out of 47 cows) compared with cows with a 60 d DP (26.0%; 13 out of 50 cows, $P = 0.02$). Independent of DP length or diet, cows with OLA at less than 21 DIM had greater BCS during weeks 1 and 2 ($P = 0.01$) and weeks 1 through 8 ($P = 0.01$) postcalving compared with cows with OLA at greater than 30 DIM. Cows with first ovarian cycle of medium length (18 to 24 d) had greater energy balance ($P = 0.03$), plasma concentrations of insulin ($P = 0.03$), glucose ($P = 0.04$), and insulin-like growth factor 1 ($P = 0.04$) than cows with long ovarian cycle lengths (> 24 d), but had lower plasma β -hydroxybutyrate ($P < 0.01$) and liver triacylglycerol ($P = 0.02$) concentrations compared with cows with short ovarian cycle lengths (< 18 d) during weeks 1 through 8 postcalving. Diet did not affect the measured ovarian activity variables within 100 DIM. In conclusion, omitting the DP (0 d) increased the incidence of normal resumption of ovarian cyclicity in dairy cows within 100 DIM compared with a conventional DP (60 d). Short (< 18 d) or long (> 24 d) ovarian cycles during the first ovarian cycle postcalving were associated with severe negative energy balance and poor metabolic status in early lactation.

Keywords: continuous milking, progesterone, ovulation, diet, metabolic status

3.2 Introduction

During early lactation, dairy cows experience a negative energy balance (**EB**) because feed intake is inadequate to meet nutrient demands for milk production. Selection for greater milk production in recent years amplifies the differences between feed intake potential and milk production potential, which has led to a heightened negative EB during early lactation in dairy cows (Diskin et al., 2006). During the period of negative EB, body reserves are mobilized to compensate for the energy imbalance which results in increased plasma free fatty acid (**FFA**), β -hydroxybutyrate (**BHB**), and liver triacylglycerol (**TAG**) concentrations, whereas plasma glucose, insulin and insulin-like growth factor-1 (**IGF-1**) concentrations are decreased (Chen et al., 2015). The negative EB-induced metabolic changes during early lactation are associated with impaired fertility, such as a prolonged interval from calving to first ovulation and first estrus, and decreased pregnancy rates (Beam and Butler, 1998; Patton et al., 2007). Impaired fertility in dairy cows in negative EB appears to be related to effects of alterations in metabolites and metabolic hormones on reproductive tissues. For example, insulin and IGF-1 stimulated the proliferation of bovine granulosa cells *in vitro* (Spicer et al., 1993), and are related to increased follicle diameter and estradiol concentration *in vivo* (Simpson et al., 1994). Further, elevated concentrations of FFA and BHB compromised oocyte development and embryo quality in dairy cows *in vitro* (Leroy et al., 2006; Van Hoeck et al., 2011).

A dry period (**DP**) length of 6 to 8 weeks has been applied on dairy farms since the beginning of the last century (Arnold and Becker, 1936). Nowadays, the primary purpose of the DP is to maximise milk yield in subsequent lactation (Van Knegsel et al., 2013). In addition, the onset of the DP is used to treat cows with subclinical mastitis with antibiotics (Bradley and Green, 2000). Shortening or omitting the DP, however, allows dairy cows to produce additional milk precalving which at least partly compensates for the loss of milk produced postcalving (Annen et al., 2004; Andersen et al., 2005). Grummer and Rastani (2004) suggested that shortening or omitting the DP could reduce the physiological changes in late lactation by necessitating fewer diet changes and regrouping of cows. Furthermore, shortening and, in particular, omitting the DP resulted in a significant improvement in EB (Rastani et al., 2005; Van Knegsel et al., 2014) and improved metabolic status (Chen et al., 2015) during early lactation. It can be hypothesised that the

improved EB and metabolic status after shortening or omitting the DP improves fertility in dairy cows.

Indeed, Watters et al. (2009) found that shortening the DP led to an earlier onset of first ovulation and reduced the number of anovulatory cows at 70 d in milk (**DIM**). However, Santschi et al. (2011) reported that shortening the DP did not influence pregnancy rate, days open, or services per conception. To our knowledge, only two studies are available concerning the effect of omitting the DP on reproductive performance in dairy cows. Both found that omitting the DP reduced the interval from calving to first ovulation (Gümen et al., 2005; De Feu et al., 2009).

In an earlier study, we found that a glucogenic diet, compared with a lipogenic diet, tended to shorten the interval from calving to onset of luteal activity (**OLA**) in multiparous cows after a conventional DP (60 d) (Van Knegsel et al., 2007c). The earlier OLA for cows fed a glucogenic diet was related to an improved metabolic status, as indicated by lower plasma FFA and BHB, and greater insulin concentrations (Van Knegsel et al., 2007b). The interaction between dietary energy source and DP length on fertility variables, however, has not yet been studied. It can be hypothesized that the beneficial effects of a glucogenic diet on fertility could be decreased after a shortened or omitted DP because these cows have an improved metabolic status in early lactation.

The primary objective of this study was to evaluate the effects of DP length on ovarian activity in cows fed a lipogenic or glucogenic diet within 100 DIM. A further objective was to determine relationships between ovarian activity and EB and metabolic status in early lactation.

3.3 Materials and Methods

3.3.1 Animals and Experimental Design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. Holstein-Friesian dairy cows (N = 167) were selected from the Dairy Campus Research dairy herd (WUR Livestock Research, Lelystad, The Netherlands). The experimental design, cow management, and diet composition have been reported previously (Van Knegsel et al., 2014). In summary, dairy cows were blocked by parity, expected calving date, milk yield in previous lactation, and body condition score (**BCS**). Within blocks, each cow was

assigned randomly to a 3×2 factorial design with 1 of 3 DP lengths (0, 30, or 60 d) and 1 of 2 early lactation diets (glucogenic or lipogenic). Cows with a 30 or 60 d DP were fed a far-off diet beginning at 7 d before drying off, and were milked once daily from 4 d before drying off. Cows with a 30 or 60 d DP were treated with an intramammary antibiotic at drying off (Supermastidol, Virbac Animal Health, Barneveld, the Netherlands). Cows with a 0 d DP were milked twice daily until calving. Before calving, dry cows were fed a dry cow diet, while lactating cows were fed a lactation diet supporting 25 kg of milk. All cows were fed 1 kg/d of glucogenic or lipogenic concentrate from 10 d before the expected calving date. Postcalving, the experimental concentrate supply was increased in increments of 0.5 kg/d until a level of 8.5 kg/d was reached. Details on diet composition were presented earlier (Van Knegsel et al., 2014). In short, the main ingredient of the glucogenic concentrate was corn and the main ingredients of the lipogenic concentrate were sugar beet pulp, palm kernel, and rumen-protected palm oil. Forage was offered ad libitum throughout the study, comprising grass silage, corn silage, wheat straw, and rapeseed meal or soybean meal (51:34:2:13, dry matter basis). Diets were formulated to be isocaloric (net energy basis; VEM system (Van Es, 1975) and equal in intestinal-digestible protein and degraded protein balance (DVE/OEB system; Tamminga et al., 1994). Cows were housed in a free stall with a slatted floor and cubicles, and were milked twice daily (at 0500 and 1630 h).

3.3.2 Body Condition and Energy Balance

Milk yield and feed intake for each cow (Insentec BV, Marknesse, the Netherlands) were recorded daily and averaged per week. Body condition was scored (on a 1–5 scale) every month. Energy balance was calculated according to the VEM system (Van Es, 1975; CVB, 2005) as the difference between VEM supplied with feed and VEM required for maintenance and milk production. The VEM required for animal maintenance is $42.4 \text{ VEM/kg}^{0.75} \cdot \text{d}$ ($1000 \text{ VEM} = 6.9 \text{ MJ NE}$) and the VEM required for milk production is 443 VEM/kg of fat and protein-corrected milk (Van Es, 1975). A correction factor to scale requirements to an average cow was applied in calculating the maintenance and milk energy requirements as described by Van Es (Van Es, 1975).

3.3.3 Milk Sampling and Progesterone Assay

Milk samples were collected three times a week on Monday, Wednesday, and Friday during morning milking until 100 DIM and stored at -20°C until analysis for concentration of progesterone (**P4**). Milk P4 concentration was measured by enzyme immunoassay (Ridgeway Science Ltd., Gloucestershire, UK) as described by Roelofs et al. (Roelofs et al., 2006). The intraassay and interassay coefficients of variation were 9.7% and 12.5%, respectively. Cows treated for cysts (Fertagyl, Intervet Inc., Millsboro, DE, USA; $n = 20$) were included in the analysis until the time of treatment.

3.3.4 Definitions of Normal and Abnormal Resumption of Ovarian Cyclicity

Two or more succeeding milk samples with P4 concentration of 2 ng/mL or greater were used to indicate the occurrence of luteal activity. Ovarian cycle length was defined as the interval between occurrence of luteal activity in one ovarian cycle and occurrence of luteal activity in the next ovarian cycle. On the basis of the P4 profile, the resumption of ovarian cyclicity for each cow within 100 DIM postcalving was classified into 1 of 4 categories according to Pushpakumara et al. (2003):

(1) Normal resumption of ovarian cyclicity: OLA occurring at 45 DIM or less, followed by regular ovarian cycles of 18 to 24 d in length (Figure 1A).

(2) Abnormal resumption, type I: OLA occurring at more than 45 DIM postcalving (delayed first ovulation or anovulation, Figure 1B).

(3) Abnormal resumption, type II: OLA occurring at 45 DIM or less, but is followed by one or more ovarian cycles with delayed luteolysis for 20 d or more (prolonged luteal phase, Figure 1C).

(4) Abnormal resumption, type III: absence of luteal activity for more than 14 d between two ovarian cycles (cessation of cyclicity, Figure 1D).

Cows that experienced more than one abnormal resumption type (17 cows) were classified according to the first type to occur postcalving.

Figure 3.1. Examples of milk progesterone profiles: (A) normal resumption of ovarian cyclicity; (B) abnormal resumption, type I: delayed first ovulation or anovulation; (C) abnormal resumption, type II: prolonged luteal phase; (D) abnormal resumption, type III: cessation of cyclicity.

3.3.5 Blood and Liver Tissue Sampling and Analysis

Blood and liver tissue sampling and analysis have been reported previously (Chen et al., 2015). In short, blood samples were taken weekly from the coccygeal vein from calving through week 8 and liver biopsies were taken in weeks 2 and 4 postcalving. 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). Insulin-like growth factor-I and insulin were measured using radioimmunoassay, as previously described (Vicari et al., 2008). Concentration of liver TAG was determined through enzymatic colorimetric analysis using the Triacylglycerides LiquiColor Mono kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany), according to the manufacturer's instructions.

3.3.6 Statistical Analysis

The number of cows per treatment for cows with OLA, complete first ovarian cycle, complete second ovarian cycle, complete P4 profile, blood sample, and liver biopsy within 100 DIM is shown in Table 3.1. First, the GLIMMIX procedure of SAS (Version 9.2; SAS Institute, Inc., Cary, NC, USA) was used to analyze resumption of ovarian cyclicity. Preliminary analysis showed that the effect of parity (2, 3, or > 3) on resumption of ovarian cyclicity was not significant ($P > 0.05$) and was therefore excluded from the model. Fixed effects in the model (model 1) were DP length (0, 30, or 60 d), diet (glucogenic or lipogenic), and DP length $\times$ diet. Twenty-six cows were excluded from this analysis because of incomplete P4 profile with 100 DIM for different reasons: (1) Treatment for cysts ($n = 8$ for cows with a 0 d DP, $n = 6$ for cows with a 30 d DP, and $n = 6$ for cows with a 60 d DP); (2) Nonreproductive reasons ($n = 1$ for cows with a 0 d DP and $n = 5$ for cows with a 30 d DP).

Second, the MIXED procedure of SAS was used to analyze the d to OLA, luteal phase length, cycle length, and maximal and mean P4 concentrations for the first and second ovarian cycles postcalving, separately. Preliminary analysis showed that the interaction effects of diet $\times$ parity and DP length $\times$ parity were not significant ($P > 0.05$), and were therefore excluded from the model. Fixed effects in the model (model 2) were DP length (0, 30, or 60 d), diet (glucogenic or lipogenic), parity (2, 3, or > 3), and DP length $\times$ diet.

Third, cows were classified on the basis of characteristics of their ovarian cycles to analyze relationships between luteal activity and EB and metabolic status in early lactation. The first classification was on the basis of days to OLA (< 21, 21 to 30, or > 30 DIM). The second classification was on the basis of the length of the first ovarian cycle (< 18, 18 to 24, or > 24 d). Subsequently, the MIXED procedure of SAS was used to analyze difference in BCS, milk yield, DMI, plasma metabolites, hormones, and liver TAG concentration among classification on the basis of days to OLA or on the basis of the length of first ovarian cycle. Data were analyzed for weeks 1 and 2 and weeks 1 through 8 separately. Fixed effects in the model (model 3) were classified on the basis of days to OLA or classified on the basis of the length of the first ovarian cycle, DP length, diet, parity (2, 3, or > 3), and time (not included in the models of liver TAG concentration and BCS during weeks 1 and 2). The cow was considered to be the repeated subject. Model assumptions were evaluated by examining the distribution of residuals. Plasma BHB concentration postcalving was natural logarithm-transformed before analysis to obtain normal distribution of residuals. For multiple comparisons, *P* values are presented after a Bonferroni adjustment. Values are presented as the least square mean with their standard errors of the mean. Differences are regarded as significant if *P* < 0.05, and trends are discussed if *P* ≤ 0.10.

3.4 Results

3.4.1 Milk Production, Energy Balance, and Body Condition Score Precalving

Results of milk production, energy balance, and BCS precalving were previously reported (Van Knegsel et al., 2014). In short, total milk yields in the last eight weeks precalving was 429 ± 30 kg for cows with a 30 d DP and 774 ± 27 kg for cows with a 0 d DP. During last 8 weeks before calving, cows with a 0 d DP had greater DMI (18.1 vs. 15.5 vs. 14.6 ± 0.2 kg/d for the 0 vs. 30 vs. 60 d DP, respectively; *P* < 0.01), whereas cows with a 60 d DP had greater EB (76 vs. 75 vs. 160 ± 8 kJ/kg^{0.75}·d for the 0 vs. 30 vs. 60 d DP, respectively; *P* < 0.01). Precalving, DP length did not affect BCS (3.1 vs. 3.0 vs. 3.0 ± 0.1 for the 0 vs. 30 vs. 60 d DP, respectively; *P* = 0.84) and body weight (732 vs. 716 vs. 726 ± 8 kg for the 0 vs. 30 vs. 60 d DP, respectively; *P* = 0.34).

Table 3.1. Distribution of cows per dry period length and diet¹.

Cows, n	Dry period length (d)						Total
	0		30		60		
	G	L	G	L	G	L	
Cows in experiment	28	28	28	27	28	28	167
- Cows with OLA ²	26	28	26	24	27	26	157
- Cows with complete first ovarian cycle	24	25	24	21	24	25	143
- Cows with complete second ovarian cycle	20	23	17	17	17	19	113
- Cows with complete P4 profile within 100 DIM	22	25	22	22	24	26	141
- Cows subjected to blood sampling	16	15	17	17	15	15	95
- Cows underwent liver biopsy	14	12	14	12	12	12	76

¹G = glucogenic diet; L = lipogenic diet.²OLA = onset of luteal activity.

3.4.2 Effects of Dry Period Length and Diet on Ovarian Activity

Within 100 DIM postcalving, cows with a 0 d DP had greater ($P = 0.02$) incidence of normal resumption of ovarian cyclicity (53.2%; 25 out of 47 cows) compared with cows with a 60 d DP (26.0%; 13 out of 50 cows, Table 3.2). Cows with a 30 d DP tended to have greater ($P = 0.09$) incidence of normal resumption of ovarian cyclicity (47.7%; 21 out of 44 cows) compared with cows with a 60 d DP (26.0%; 13 out of 50 cows). Diet did not affect incidence of normal or abnormal resumption of ovarian cyclicity.

In the first ovarian cycle postcalving, cows with a 0 d DP tended to have an earlier OLA compared with cows with a 60 d DP (23.1 vs. 28.9 DIM, $P = 0.07$, Table 3.3). Interaction of DP length $\times$ diet was present ($P < 0.01$) for maximal P4 concentration. Cows fed a lipogenic diet had greater maximal P4 concentrations compared with cows fed a glucogenic diet (13.79 vs. 10.68 ng/mL, $P = 0.04$) after a 30 d DP, whereas cows fed a lipogenic diet tended to have lower maximal P4 concentrations compared with cows fed a glucogenic diet (10.51 vs. 13.39 ng/mL, $P = 0.06$) after a 60 d DP. No differences were found between glucogenic and lipogenic diets regarding maximal P4 concentrations (12.24 vs. 11.60 ng/mL, $P = 0.66$) after a 0 d DP.

In the second ovarian cycle postcalving, DP length and the diet did not affect d to luteal activity, luteal phase length, cycle length, or P4 concentrations. Additionally, some characteristics of the ovarian cycles were affected by parity.

Table 3.2. Incidence of normal or abnormal resumption of ovarian cyclicity during the preconception period for cows after 0, 30, and 60 d dry period and fed a glucogenic (G) or lipogenic (L) diet within 100 days in milk.

Variable	Dry period length (d)				Diet		P-value	
	0	30	60		G	L	DPL ¹	Diet
								DPL × diet
Normal resumption of ovarian cyclicity (%)	53.2 (25/47) ^a	47.7 (21/44) ^{ab}	26.0 (13/50) ^b	42.4 (31/73)	41.2 (28/68)		0.02	0.90
Abnormal resumption of ovarian cyclicity:								
Type I: delayed first ovulation or anovulation (%)	2.1 (1/47)	18.2 (8/44)	16.0 (8/50)	9.6 (7/73)	14.7 (10/68)		0.11	0.36
Type II: prolonged luteal phase (%)	44.7 (21/47)	34.1 (15/44)	50.0 (25/50)	46.6 (34/73)	39.7 (27/68)		0.23	0.23
Type III: cessation of cyclicity (%)	0.0 (0/47)	0.0 (0/44)	8.0 (4/50)	1.4 (1/73)	4.4 (3/68)		0.99	0.99

^{a,b} Values within dry period length in the same row with different superscripts differ ($P < 0.05$).
¹DPL = dry period length.

Mean P4 concentrations of young cows (parity = 2) were 8.56 ng/mL and 10.45 ng/mL in the first and second ovarian cycles compared with 6.26 ng/mL ($P < 0.01$) and 7.60 ng/mL ($P < 0.01$) in the first and second ovarian cycles of older cows (parity > 2), respectively. Maximal P4 concentrations of young cows was 14.67 ng/mL in the first ovarian cycle and 17.26 ng/mL in the first and second ovarian cycle compared with 10.64 ng/mL ($P < 0.01$) and 13.42 ng/mL ($P < 0.01$) in the first and second ovarian cycles of older cows (parity > 2), respectively. Diet did not affect d to luteal activity, luteal phase length, cycle length, or P4 concentration in the first or second ovarian cycles postcalving.

3.4.3. Relationship between Metabolism and Days to Onset of Luteal Activity

During weeks 1 and 2 postcalving, cows with OLA at less than 21 DIM had greater BCS ($P = 0.01$) compared with cows with OLA at greater than 30 DIM (Table 3.4). Cows with OLA at less than 21 DIM had lower liver TAG concentration ($P = 0.03$) compared with cows with OLA between 21 and 30 DIM. Moreover, cows with OLA at less than 21 DIM had greater DMI ($P = 0.04$) compared with cows with OLA between 21 and 30 DIM.

During weeks 1 through 8 postcalving, cows with OLA at less than 21 DIM had greater BCS ($P = 0.01$) compared with cows with OLA at greater than 30 DIM and had lower liver TAG concentration ($P = 0.04$) compared with cows with OLA between 21 and 30 DIM.

During both weeks 1 and 2 and weeks 1 through 8 postcalving, BCS, energy balance, plasma concentrations of glucose, IGF-1, and insulin were greater, but milk yields, plasma concentration of FFA, and liver TAG concentrations were lower for cows with a 0 d DP compared with cows with a 30 or 60 d DP.

3.4.4 Relationship between Metabolism and Length of First Ovarian Cycle

During weeks 1 and 2 postcalving, cows with medium ovarian cycle lengths (18 to 24 d) had lower plasma BHB concentrations ($P = 0.01$) and greater plasma glucose concentrations ($P = 0.04$) compared with cows with long ovarian cycle lengths (> 24 d, Table 3.5). Cows with medium ovarian cycle lengths (18 to 24 d) had lower liver TAG concentration ($P = 0.02$) compared with cows with short ovarian cycle lengths.

Table 3.3. Characteristics of ovarian cycles of dairy cows after 0, 30, and 60 d dry period and fed a glucogenic (G) or lipogenic (L) diet (LSM ± SEM).

Variable	Dry period length (d)				SEM		Diet		SEM		P-value			
	0	30	60				G	L			DPL ¹	Diet	Parity	DPL × diet
First ovarian cycle														
Days to luteal activity (d)	23.1	28.2	28.9	2.0	27.7	25.8	1.6	0.07	0.39	0.32	0.96			
Luteal phase length (d)	17.5	18.2	19.9	1.4	17.8	19.4	1.2	0.49	0.35	0.30	0.46			
Cycle length (d)	24.3	25.5	28.2	1.6	25.3	26.7	1.3	0.20	0.44	0.60	0.55			
P4, max ² (ng/mL)	11.93	12.12	11.94	0.73	11.97	12.02	0.60	0.98	0.96	<0.01	<0.01			
P4,mean ³ (ng/mL)	6.79	7.28	7.02	0.44	7.10	6.96	0.37	0.74	0.78	<0.01	0.06			
Second ovarian cycle														
Days to luteal activity (d)	46.8	48.7	50.4	2.0	48.1	49.1	1.6	0.41	0.66	0.90	0.76			
Luteal phase length (d)	18.1	17.3	18.2	1.1	17.1	18.6	0.9	0.83	0.26	0.02	0.08			
Cycle length (d)	24.9	25.8	27.2	1.2	25.6	25.8	1.2	0.33	0.91	0.07	0.09			
P4, max ² (ng/mL)	15.33	14.55	14.28	0.90	14.54	14.90	0.73	0.67	0.72	<0.01	0.29			
P4,mean ³ (ng/mL)	9.17	8.51	8.05	0.54	8.64	8.51	0.44	0.32	0.84	<0.01	0.31			

¹DPL = dry period length.
²Maximal P4 concentration.
³Mean P4 concentration.

During weeks 1 through 8 postcalving, cows with medium ovarian cycle lengths had greater EB ($P = 0.04$), plasma concentrations of glucose ($P = 0.04$), IGF-1 ($P = 0.04$) and insulin ($P = 0.03$), but lower plasma BHB ($P < 0.01$) concentration compared with cows with long ovarian cycle lengths. In addition, cows with medium ovarian cycle lengths had lower plasma BHB concentrations ($P < 0.01$) and lower liver TAG concentrations ($P = 0.02$) compared with cows with short ovarian cycle lengths.

During both weeks 1 and 2 and weeks 1 through 8 postcalving, BCS, energy balance, plasma concentrations of glucose, IGF-1, and insulin were greater, but milk yields, plasma concentrations of FFA, and liver TAG concentrations were lower for cows with a 0 d DP compared with cows with a 30 or 60 d DP.

3.5 Discussion

In the current study, omitting the DP increased the incidence of normal resumption of ovarian cyclicity within 100 DIM, defined as OLA occurring within 45 DIM and followed by regular ovarian cycles of 18 to 24 d in length, compared with a conventional DP in dairy cows. In previous studies, cows with normal resumption of ovarian cyclicity were reported to have lower number of services per conception (Lamming and Darwash, 1998), fewer days open (Ranasinghe et al., 2011), and were more likely to become pregnant (Gautam et al., 2010) compared with cows with abnormal resumption of ovarian cyclicity. Together, these findings suggest that omitting the DP has the potential to improve fertility in dairy cows.

The overall incidence of cows with normal resumption of ovarian cyclicity was 41.8% (59 out of 141 cows) in the current study, which is similar to 44.5% (59 out of 110 cows) reported by Shrestha et al. (2005), but lower than 53.5% (239 out of 448 cows) reported by Opsomer et al. (1998) and 67% (27 out of 40 cows) reported by Pushpakumara et al. (2003). In the current study, abnormal resumption of cyclicity mostly consisted of a prolonged luteal phase (43.4%; 61 out of 141 cows), an incidence that was higher than that reported in previous studies (15%–35.2%) (Opsomer et al., 1998; Pushpakumara et al., 2003; Shrestha et al., 2004b). The prolonged luteal phase is 1 of the 2 most common ovarian disturbances in dairy cows (Shrestha et al., 2004a), and is associated with reduced pregnancy rate and extended days open (Ranasinghe et al., 2011). A high milk yield is one of the major risk factors for a prolonged luteal phase in high-producing dairy cows (Kafi et al., 2012).

Table 3.4. Milk yield, dry matter intake, energy balance, metabolites and hormones during early lactation for dairy cows categorised according to days to onset of luteal activity (OLA) (LSM $\pm$ SEM).

Variable	Days to OLA				Dry period length (d)				SEM				P-value			
	<21 d	21 to 30 d	> 30 d	SEM	0	30	50	60	SEM	OLA	DPL ¹	Diet	Parity	Time ²		
Cows, n	66	52	39		54	50	53									
Weeks 1 and 2																
BCS ^{3,4}	2.7 ^a	2.7 ^{ab}	2.4 ^b	0.1	2.8 ^a	2.7 ^a		2.3 ^b	0.1	0.01	<0.01	0.84	0.04	NM ⁵		
Milk yield (kg/d)	28.7	29.3	28.8	0.8	24.2 ^c	29.1 ^b		33.5 ^a	0.7	0.80	<0.01	0.46	<0.01	<0.01		
DMI (kg/d)	17.5 ^a	16.4 ^b	17.3 ^{ab}	0.3	17.0	17.3		17.0	0.3	0.04	0.79	0.61	0.61	<0.01		
EB ⁶ (kJ/kg ^{0.75} ·d)	-132	-149	-124	29	-62 ^a	-120 ^{ab}		-224 ^b	29	0.84	<0.01	0.23	<0.01	<0.01		
FFA (mmol/L)	0.41	0.45	0.47	0.06	0.34 ^b	0.43 ^{ab}		0.56 ^a	0.05	0.72	0.01	0.50	0.13	0.09		
BHB ⁷ (mmol/L)	0.64	0.66	0.63	0.04	0.67	0.58		0.68	0.04	0.77	0.16	0.51	0.24	0.36		
Glucose (mmol/L)	3.56	3.51	3.59	0.08	3.82 ^a	3.49 ^b		3.35 ^b	0.07	0.78	<0.01	0.03	0.01	<0.01		
Urea (mmol/L)	2.70	2.58	2.63	0.15	2.79	2.60		2.53	0.14	0.83	0.40	0.06	0.80	<0.01		
IGF-I (ng/mL)	87.45	78.33	82.23	8.24	109.55 ^a	78.62 ^b		59.84 ^b	7.89	0.67	<0.01	0.85	<0.01	0.80		
Insulin (μ U/mL)	15.13	13.99	11.96	2.01	21.34 ^a	11.80 ^b		7.95 ^b	1.93	0.57	<0.01	0.30	0.08	0.03		
Liver TAG ⁸ (mg/g of wet weight)	34.6 ^b	56.9 ^a	53.4 ^{ab}	6.5	28.1 ^b	53.4 ^a		63.4 ^a	6.3	0.03	<0.01	0.91	0.37	NM		

^{a,b} Values within days to OLA and within dry period length with different superscripts differ ($P < 0.05$).

¹DPL = dry period length.

²Week relative to calving (for DMI, milk yield, energy balance, and concentration of metabolites and hormones) or month relative to calving (for BCS).

³Body condition score on a scale of 1 to 5.

⁴Body condition was scored during the first month relative to calving.

⁵NM: Not included in the model. (Continued on the next page)

Table 3.4. (continued).

Variable	Days to OLA			SEM	Dry period length (d)			SEM	P-value				
	< 21 d	21 to 30 d	> 30 d		0	30	60		OLA	DPL ¹	Diet	Parity	Time ²
Weeks 1 through 8													
BCS ³	2.7 ^a	2.6 ^{ab}	2.4 ^b	0.1	2.9 ^a	2.5 ^b	2.2 ^c	0.1	0.01	<0.01	0.84	0.02	0.01
Milk yield (kg/d)	37.4	38.4	38.5	0.8	32.8 ^c	38.6 ^b	42.9 ^a	0.8	0.49	<0.01	0.75	<0.01	<0.01
DMI (kg/d)	22.2	21.6	22.3	0.3	21.7	22.1	22.1	0.3	0.09	0.36	0.86	0.56	<0.01
EB ⁶ (kJ/kg ^{0.75} ·d)	−109	−118	−113	20	−28 ^a	−110 ^b	−190 ^c	20	0.87	<0.01	0.08	<0.01	<0.01
FFA (mmol/L)	0.28	0.28	0.32	0.02	0.22 ^b	0.30 ^{ab}	0.36 ^a	0.03	0.60	<0.01	0.29	0.06	<0.01
BHB ⁷ (mmol/L)	0.68	0.62	0.61	0.04	0.65	0.61	0.65	0.04	0.30	0.74	0.02	0.02	0.14
Glucose (mmol/L)	3.69	3.61	3.68	0.05	3.79 ^a	3.63 ^{ab}	3.56 ^b	0.04	0.41	<0.01	0.14	<0.01	<0.01
Urea (mmol/L)	2.83	2.84	2.86	0.11	2.99	2.78	2.76	0.10	0.97	0.21	<0.01	0.65	<0.01
IGF-I (ng/mL)	97.37	95.01	94.50	5.92	116.46 ^a	90.60 ^b	79.81 ^b	5.66	0.93	<0.01	0.73	<0.01	<0.01
Insulin (μIU/mL)	18.40	16.98	15.03	1.39	24.19 ^a	14.80 ^b	11.42 ^b	1.32	0.27	<0.01	0.61	0.01	<0.01
Liver TAG ⁸ (mg/g of wet weight)	30.0 ^b	47.2 ^a	47.9 ^{ab}	5.5	24.0 ^b	48.8 ^a	53.4 ^a	5.7	0.04	<0.01	0.94	0.58	<0.01

⁶Energy balance; calculated utilising the Dutch net energy evaluation (NEM) system (Van Es, 1975).⁷P values based on the natural logarithm of plasma BHB concentration.⁸Concentration of liver triacylglycerol was analyzed based on two measurements (wk 2 and 4).

Table 3.5. Milk yield, dry matter intake, energy balance, metabolites and hormones during early lactation for dairy cows categorised according to length of first ovarian cycle (LSM $\pm$ SEM).

Variable	Ovarian cycle length				Dry period length (d)				SEM				P-value			
	Short (< 18 d)		Medium (18 to 24 d)		Long (> 24 d)		SEM		0		30		60		SEM	
	23	59	61	49	45	49	49	49	0	30	45	49	0	30	45	49
Cows, n	23	59	61	49	45	49	49	49								
Weeks 1 and 2																
BCS ^{3,4}	2.5	2.6	2.7	0.1	3.0 ^a	2.6 ^a	2.2 ^b	0.1	0.51	0.83	0.06	NM ⁵	<0.01	0.83	0.06	NM ⁵
Milk yield (kg/d)	29.2	28.4	29.1	0.9	24.3 ^c	29.0 ^b	33.4 ^a	0.8	0.68	<0.01	0.44	<0.01	<0.01	0.44	<0.01	<0.01
DMI (kg/d)	17.2	17.3	16.7	0.4	17.1	17.2	16.9	0.4	0.31	0.84	0.49	0.43	<0.01	0.84	0.49	0.43
EB ⁶ (kJ/kg ^{0.75} ·d)	-153	-110	-152	33	-68 ^a	-111 ^a	-236 ^b	31	0.47	<0.01	0.36	<0.01	<0.01	0.36	<0.01	<0.01
FFA (mmol/L)	0.51	0.37	0.47	0.06	0.34 ^b	0.43 ^{ab}	0.57 ^a	0.06	0.25	0.01	0.53	0.27	0.11	0.01	0.53	0.27
BHB ⁷ (mmol/L)	0.62 ^{ab}	0.56 ^b	0.72 ^a	0.05	0.68 ^{ab}	0.55 ^b	0.68 ^a	0.04	0.01	0.03	0.78	0.40	0.34	0.03	0.78	0.40
Glucose (mmol/L)	3.64 ^{ab}	3.62 ^a	3.40 ^b	0.09	3.80 ^a	3.48 ^b	3.38 ^b	0.07	0.04	<0.01	0.02	<0.01	<0.01	<0.01	0.02	<0.01
Urea (mmol/L)	2.75	2.44	2.79	0.17	2.86	2.59	2.53	0.15	0.17	0.24	0.10	0.67	0.01	0.24	0.10	0.67
IGF-I (ng/mL)	81.81	94.26	70.76	8.90	112.01 ^a	75.27 ^b	59.55 ^b	8.34	0.08	<0.01	0.56	<0.01	0.91	<0.01	0.56	<0.01
Insulin (μ U/mL)	13.04	14.98	13.30	2.30	22.38 ^a	11.10 ^b	7.84 ^b	2.12	0.77	<0.01	0.49	0.15	0.04	<0.01	0.49	0.15
Liver TAG ⁸ (mg/g of wet weight)	64.0 ^a	35.2 ^b	51.0 ^{ab}	7.0	31.9 ^b	49.4 ^{ab}	68.9 ^a	6.4	0.02	<0.01	0.56	0.30	NM	<0.01	0.56	0.30

^{a,b} Values within ovarian cycle length and within dry period length with different superscripts differ ($P < 0.05$).

¹DPL = dry period length.

²Week relative to calving (for DMI, milk yield, energy balance, concentration of metabolites and hormones) or month relative to calving (for BCS).

³Body condition score on a scale of 1 to 5.

⁴Body condition score was scored during the first month relative to calving. (Continued on the next page)

Table 3.5. (continued).

Variable	Ovarian cycle length			Dry period length (d)			SEM			P-value			
	Short (< 18 d)	Medium (18 to 24 d)	Long (> 24d)	0	30	60				Ovarian cycle	DPL ¹	Diet	Parity Time ²
Weeks 1 through 8													
BCS ³	2.5	2.6	2.6	0.1	3.0 ^a	2.5 ^b	2.2 ^c	0.1	0.81	<0.01	0.74	0.01	0.01
Milk yield (kg/d)	39.1	36.8	38.4	0.9	32.9 ^c	38.3 ^b	43.1 ^a	0.8	0.14	<0.01	0.94	<0.01	<0.01
DMI (kg/d)	22.4	22.2	21.6	0.3	21.8	22.1	22.2	0.3	0.09	0.50	0.88	0.74	<0.01
EB ⁶ (kJ/kg ^{0.75} ·d)	-132 ^{ab}	-73 ^a	-134 ^b	23	-36 ^a	-105 ^b	-199 ^c	21	0.04	<0.01	0.14	<0.01	<0.01
FFA (mmol/L)	0.34	0.25	0.31	0.03	0.22 ^b	0.30 ^{ab}	0.37 ^a	0.03	0.06	<0.01	0.36	0.16	<0.01
BHB ⁷ (mmol/L)	0.66 ^a	0.56 ^b	0.70 ^a	0.04	0.67	0.59	0.66	0.04	<0.01	0.16	0.06	0.06	0.16
Glucose (mmol/L)	3.60 ^{ab}	3.73 ^a	3.59 ^b	0.05	3.76 ^a	3.62 ^{ab}	3.54 ^b	0.05	0.04	<0.01	0.21	<0.01	<0.01
Urea (mmol/L)	3.02	2.70	2.95	0.11	3.06	2.81	2.80	0.11	0.09	0.12	<0.01	0.91	<0.01
IGF-I (ng/mL)	95.37 ^{ab}	104.09 ^a	85.84 ^b	6.27	117.24 ^a	87.62 ^b	80.45 ^b	5.86	0.04	<0.01	0.91	<0.01	<0.01
Insulin (μIU/mL)	15.19 ^{ab}	19.75 ^a	15.23 ^b	1.54	24.62 ^a	14.58 ^b	10.97 ^b	1.44	0.03	<0.01	0.29	0.03	<0.01
Liver TAG ⁸ (mg/g of wet weight)	57.1 ^a	30.6 ^b	41.1 ^{ab}	6.0	27.3 ^b	45.5 ^{ab}	55.9 ^a	5.5	0.02	0.01	0.32	0.55	<0.01

⁵NM. Not included in the model.⁶Energy balance; calculated utilising the Dutch net energy evaluation (NEM) system (Van Es, 1975).⁷P values based on the natural logarithm of plasma BHB concentration.⁸Concentration of liver triacylglycerol was analyzed based on two measurements (wk 2 and 4).

In the current study, cows had higher milk yields (average milk production: 9921 kg in 305 d) compared with those previous studies, which could explain the greater occurrence of prolonged luteal phase. Furthermore, Opsomer et al. (2000) suggested that uterine infections, such as metritis, could compromise prostaglandin $F_{2\alpha}$ (**PGF_{2α}**) production and thereby delay luteolysis, which results in a prolonged luteal phase. A meta-analysis showed that shortening or omitting the DP does not alter the incidence of metritis (Van Kneegsel et al., 2013). This could partly explain the lack of differences among DP lengths for incidence of cows with prolonged luteal phase in the current study, a finding that is in agreement with a previous study (Gümen et al., 2005).

Another common ovarian disturbance is delayed first ovulation or anovulation in dairy cows. The effects of delayed first ovulation or anovulation has been widely reported to be associated with a prolonged interval from calving to first artificial insemination, more days open, and a reduced pregnancy rate in previous studies (Lamming and Darwash, 1998; Shrestha et al., 2004b; Gautam et al., 2010). In the current study, 12.1% of all cows took longer than 45 DIM to have OLA, but cows with a 0 d DP seemed less likely to have delayed first ovulation or anovulation (2.1%; 1 out of 47 cows) compared with cows with a 30 d (18.2%; 8 out of 44 cows) or 60 d (16.0%; 8 out of 50 cows) DP. However, no statistical difference was detected after Bonferroni adjustment of *P*-values. Further research with a larger number of cows is needed to confirm the effects of DP length on the incidence of delayed first ovulation. Nevertheless, there was a tendency of shorter intervals from calving to first ovulation postcalving, as indicated by a tendency for reduced d to OLA, for cows with a 0 d DP compared with cows with a 30 or 60 d DP. This result was in accordance with previous studies that demonstrated a reduced interval from calving to first ovulation after an omitted DP in cows without a DP (Gümen et al., 2005; De Feu et al., 2009). Several studies have observed positive effects of an earlier first ovulation after calving on fertility in dairy cows (Lucy et al., 1992; Darwash et al., 1997; Galvão et al., 2010). Cows with early first ovulation had more ovulation cycles before first artificial insemination (Butler and Smith, 1989) and higher first service pregnancy rates compared with cows with late or no ovulation (Galvão et al., 2010). Lucy et al. (1992) reported that cows having first ovulations before 42 DIM required fewer services per conception compared with cows having first ovulations after 42 DIM. However, Opsomer et

al. (2000) observed that an early first ovulation increased the risk for prolonged luteal cycles before service. In the current study, cows with a 0 d DP had earlier first ovulations, but these cows did not show greater incidence of prolonged luteal phase or longer luteal phase length during the first or second ovarian cycles compared with cows with a 30 or 60 d DP.

Reducing DP length could reduce the interval from calving to first ovulation (Gümen et al., 2005; De Feu et al., 2009; Watters et al., 2009), but the effects of reducing DP length on reproductive performance during the subsequent lactation are contradictory. In some studies a reduced DP length resulted in an increased first service conception rate and reduced days open (Gümen et al., 2005; Watters et al., 2009), but other studies did not report effects of DP length on first service conception rate, days open, or pregnancy rate (Pezeshki et al., 2008; De Feu et al., 2009). The reason for this discrepancy could be because reproductive performance is not only related to the resumption of ovarian cyclicity, but is also related to reproductive management (e.g., heat detection), milk production level, energy balance, climate, and so forth (Lucy, 2001).

In a previous study, we showed that a glucogenic diet could result in a shorter interval from calving to first ovulation compared with a lipogenic diet in multiparous cows (Van Knegsel et al., 2007c). In addition, Garnsworthy et al. (2008) reported that starch intake (glucogenic nutrients) was related positively to the number of small ovarian follicles in dairy cows present at around 60 DIM. In contrast to these findings, dietary energy source did not affect timing of OLA in the current study. This discrepancy could be due to the smaller contrast between diets in the current study. Firstly, the amount of experimental concentrates used was less in the current study (8.5 kg/d) compared with the previous study (12 kg/d) (Van Knegsel et al., 2007c). Secondly, in the current study, less fibre and fat was used in the lipogenic diet, and less starch was used in the glucogenic diet in compared with the previous study (Van Knegsel et al., 2007c).

Earlier we reported that, in the current experiment, omitting the DP improved EB in early lactation because of a reduction in milk production (Van Knegsel et al., 2014). In addition, under the statistical model used in the current study, omitting the DP improved EB and metabolic status, as indicated by increased concentrations of plasma insulin, IGF-1, and glucose and decreased concentrations of FFA and liver TAG. It can be hypothesized that the beneficial effects of omitting

the DP on resumption of ovarian cyclicity is related to the improved metabolic status. Therefore, cows were regrouped on the basis of the characteristics of the ovarian cycle to analyze relationships between ovarian activity and EB and metabolic status.

In the current study, independent of DP length or dietary treatments, the average liver TAG concentration was lower and BCS was greater during weeks 1 and 2 and 1 through 8 in cows with OLA at less than 21 DIM compared with cows with OLA between 21 and 30 DIM or at greater than 30 DIM postcalving. Similarly, Rukkwamsuk et al. (1999) reported that postcalving liver TAG concentration was correlated positively with the interval from calving to first ovulation. Shrestha et al. (2005) reported that cows with delayed first ovulation had lower BCS compared with cows with normal resumption of ovarian cyclicity and proposed that delayed ovulations in cows with elevated liver TAG concentrations or with poor BCS were caused by severe negative EB during early lactation. The negative EB compromises ovarian follicular development by suppressing plasma IGF-1 concentration and pituitary luteinizing hormone pulsatility (Lucy, 2000), which may lead to a prolonged interval from calving to first ovulation (Shrestha et al., 2004a). In the current study, however, EB did not differ among cows with OLA at less than 21 DIM, between 21 and 30 DIM, and at greater than 30 DIM during weeks 1 and 2 and 1 through 8. De Vries and Veerkamp (2000) observed that total energy deficit or nadir of EB could only explain 3 to 4% of the variation in interval from calving to first ovulation. Lucy (2001) suggested that other factors may also contribute to prolonged intervals from calving to first ovulation. For instance, prolonged intervals from calving to first ovulation have been related to uterine infection (Opsomer et al., 2000), mastitis (Huszenicza et al., 2005), and lameness (Petersson et al., 2006).

In the current study, the length of the first ovarian cycle was associated with EB and metabolic status. Cows with medium ovarian cycle lengths (18 to 24 d) were characterised by a better EB and better metabolic status during weeks 1 and 2 and 1 through 8 postcalving, compared with cows with long ovarian cycle lengths (> 24 d). Negative EB is associated with decreased plasma insulin, glucose and IGF-1 concentrations and increased plasma FFA, BHB, and liver TAG concentrations (Van Knegsel et al., 2005), which was also apparent in the current study. In the current study, long ovarian cycles were caused primarily by prolonged

luteal phases. Consistent with our results, Hommeida et al. (2005) reported that cows with prolonged luteal phases had more severe negative EB, which was reflected by greater BCS loss, compared with cows with normal resumption of ovarian cyclicity. The mechanisms behind prolonged ovarian cycle are not fully clear. Nevertheless, several studies reported that a prolonged luteal phase was associated with uterine infection, which compromises secretion and release of $\text{PGF}_{2\alpha}$ (Opsomer et al., 2000; Shrestha et al., 2004b; Ranasinghe et al., 2011). Lipopolysaccharide, the main pathogenic ligand of *Escherichia coli*, is increased postcalving in cows with uterine infections (Mateus et al., 2003). Lipopolysaccharide stimulates the secretion of prostaglandins and particularly prostaglandin E_2 (PGE_2) (Herath et al., 2006). The ratio of PGE_2 to $\text{PGF}_{2\alpha}$ decides the fate of the corpus luteum, with persistence if PGE_2 dominates or luteolysis if $\text{PGF}_{2\alpha}$ dominates (Kaneko and Kawakami, 2009). In addition, negative EB in early lactation was found to be associated with compromised innate immune function in dairy cows (Van Knegsel et al., 2007a). In a previous study, it was reported that BHB decreased the ability of neutrophils to form neutrophil extracellular traps and the bactericidal effect against *Escherichia coli* in bovine (Grinberg et al., 2008). On the basis of these findings, it is likely that uterine infections, which is associated with severe negative EB and poor metabolic status, is one of the factors causing prolonged luteal phase in dairy cows in the current study.

Insulin and IGF-1 are critical for proliferation of bovine granulosa cell and FSH-stimulated estrogen production from granulosa cells *in vitro* (Spicer et al., 1993). Zollers et al. (1993) suggested that reduced concentration of estradiol before ovulation may result in decreased synthesis of P4 receptors and lead to a loss of uterine P4 dominance. This loss might lead to an early synthesis of oxytocin receptors and therefore increase uterine responsiveness to oxytocin, which initiates the positive feedback loop between oxytocin and $\text{PGF}_{2\alpha}$ resulting in premature luteolysis (Zollers et al., 1993; Mann and Lamming, 2000). This premature luteolysis leads to a short ovarian cycle length. However, plasma concentrations of insulin and IGF-1 are not significantly different between cows with short ovarian cycle lengths (< 18 d) and cows with medium ovarian cycle lengths in the current study. This was probably due to low sample numbers in short ovarian cycle length group. But we found that cows with short ovarian cycle lengths had greater plasma BHB concentrations and liver TAG concentrations, which are indicative of negative

energy balance. These results imply that short ovarian cycle length is associated with severe negative EB and poor metabolic status.

Most of the differences among cows with short, medium, or long ovarian cycle lengths were found during weeks 1 through 8 rather than weeks 1 and 2 postcalving, although EB and metabolites involved in energy metabolism changed significantly during weeks 1 and 2 postcalving (Chen et al., 2015). The first reason for this could be that the first ovarian cycle on average started almost 4 weeks postcalving and EB and metabolic status may have a significant influence on the length of the ovarian cycle after 4 weeks postcalving. The second reason could be that more frequently repeated measures in the analysis during weeks 1 through 8 compared with weeks 1 and 2 increased the sensitivity of analyses.

3.6 Conclusions

The results of this study show that omitting the DP (0 d) improved fertility, as indicated by an increased incidence of normal resumption of ovarian cyclicity and tendency for a reduced interval from calving to OLA within 100 DIM, compared with a conventional DP (60 d) in dairy cows. In addition, a glucogenic diet, compared with a lipogenic diet, did not affect the incidence of resumption of ovarian cyclicity within 100 DIM postcalving. Prolonged intervals from calving to OLA were associated with a poor BCS and increased liver TAG concentration during weeks 1 and 2 and 1 through 8 postcalving. Short (<18 d) or long (>24 d) first ovarian cycles length were associated with severe negative EB and poor metabolic status, as reflected by increased liver TAG, plasma BHB and FFA concentrations, and decreased plasma insulin, IGF-1 and insulin concentrations during weeks 1 through 8 postcalving.

3.7 Acknowledgements

The authors thank the Dutch Dairy Board (PZ, the Netherlands), Product Board Animal Feed (PDV, the Netherlands), and CRV (the Netherlands) for financing the experiment. The first author would like to thank the Chinese Scholarship Council (CSC) for financial support. Additionally, the authors thank Bas Jonkers, Kristina Simon, Ger de Vries Reilingh, the staff of the Dairy Campus (Lelystad, the Netherlands), and the Veterinary Physiology Group of the

Chapter 3

University of Bern (Switzerland) for their technical support and laboratory analyses during the experiment.

References

- Andersen, J. B., T. G. Madsen, T. Larsen, K. L. Ingvarsten, and M. O. Nielsen. 2005. The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. *J. Dairy Sci.* 88:3530-3541.
- Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. *J. Dairy Sci.* 87:3746-3761.
- Arnold, P. and R. Becker. 1936. Influence of preceding dry period and of mineral supplement on lactation. *J. Dairy Sci.* 19:257-266.
- Centraal Veevoederbureau (CVB). 2005. Gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. CVB, Lelystad, the Netherlands.
- Beam, S. and W. Butler. 1998. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *J. Reprod. Fertil. Suppl.* 54:411-424.
- Bradley, A. and M. Green. 2000. A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. *J. Dairy Sci.* 83:1957-1965.
- Butler, W. and R. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767-783.
- Chen, J., J. Gross, H. van Dorland, G. Remmelink, R. Bruckmaier, B. Kemp, and A. van Knegsel. 2015. Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation. *J. Dairy Sci.* 98:1033-1045.
- Darwash, A., G. Lamming, and J. Woolliams. 1997. Estimation of genetic variation in the interval from calving to postpartum ovulation of dairy cows. *J. Dairy Sci.* 80:1227-1234.
- De Feu, M., A. Evans, P. Lonergan, and S. Butler. 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status, and postpartum ovarian function in Holstein-Friesian dairy cows. *J. Dairy Sci.* 92:6011-6022.

- De Vries, M. and R. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. *J. Dairy Sci.* 83:62-69.
- Diskin, M., J. Murphy, and J. Sreenan. 2006. Embryo survival in dairy cows managed under pastoral conditions. *Anim. Reprod. Sci.* 96:297-311.
- Gümen, A., R. R. Rastani, R. R. Grummer, and M. C. Wiltbank. 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *J. Dairy Sci.* 88:2401-2411.
- Galvão, K., M. Frajblat, W. Butler, S. Brittin, C. Guard, and R. Gilbert. 2010. Effect of early postpartum ovulation on fertility in dairy cows. *Reprod. Domest. Anim.* 45:e207-e211.
- Garnsworthy, P., A. Lock, G. Mann, K. Sinclair, and R. Webb. 2008. Nutrition, metabolism, and fertility in dairy cows: 1. Dietary energy source and ovarian function. *J. Dairy Sci.* 91:3814-3823.
- Gautam, G., T. Nakao, K. Yamada, and C. Yoshida. 2010. Defining delayed resumption of ovarian activity postpartum and its impact on subsequent reproductive performance in Holstein cows. *Theriogenology* 73:180-189.
- Grinberg, N., S. Elazar, I. Rosenshine, and N. Shpigel. 2008. β -Hydroxybutyrate abrogates formation of bovine neutrophil extracellular traps and bactericidal activity against mammary pathogenic *Escherichia coli*. *Infect. Immun.* 76:2802-2807.
- Grummer, R. and R. Rastani. 2004. Why reevaluate dry period length? *J. Dairy Sci.* 87:E77-E85.
- Herath, S., D. Fischer, D. Werling, E. Williams, S. Lilly, H. Dobson, C. Bryant, and I. Sheldon. 2006. Expression and function of Toll-like receptor 4 in the endometrial cells of the uterus. *Endocrinology* 147:562-570.
- Hommeida, A., T. Nakao, and H. Kubota. 2005. Onset and duration of luteal activity postpartum and their effect on first insemination conception rate in lactating dairy cows. *J. Vet. Med. Sci.* 67:1031.
- Huszenicza, G., S. János, M. Kulcsar, P. Korodi, J. Reiczigel, L. Katai, A. Peters, and F. De Rensis. 2005. Effects of clinical mastitis on ovarian function in postpartum dairy cows. *Reprod. Domest. Anim.* 40:199-204.
- Kafi, M., A. Mirzaei, A. Tamadon, and M. Saeb. 2012. Factors affecting the occurrence of postpartum prolonged luteal activity in clinically healthy high-producing dairy cows. *Theriogenology* 77:421-429.

- Kaneko, K. and S. Kawakami. 2009. The roles of PGF_{2α} and PGE₂ in regression of the corpus luteum after intrauterine infusion of *Arcanobacterium pyogenes* in cows. *Theriogenology* 71:858-863.
- Lamming, G. and A. Darwash. 1998. The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. *Anim. Reprod. Sci.* 52:175-190.
- Leroy, J., T. Vanholder, G. Opsomer, A. Van Soom, and A. Kruif. 2006. The in vitro development of bovine oocytes after maturation in glucose and β - Hydroxybutyrate concentrations associated with negative energy balance in dairy cows. *Reprod. Domest. Anim.* 41:119-123.
- Lucy, M., C. Staples, W. Thatcher, P. Erickson, R. Cleale, J. Firkins, J. Clark, M. Murphy, and B. Brodie. 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Anim. Prod.* 54:323-331.
- Lucy, M. 2000. Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in cattle. *J. Dairy Sci.* 83:1635-1647.
- Lucy, M. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *J. Dairy Sci.* 84:1277-1293.
- Mann, G. and G. Lamming. 2000. The role of sub-optimal preovulatory oestradiol secretion in the aetiology of premature luteolysis during the short oestrous cycle in the cow. *Anim. Reprod. Sci.* 64:171-180.
- Mateus, L., L. Lopes da Costa, P. Diniz, and A. Ziecik. 2003. Relationship between endotoxin and prostaglandin (PGE₂ and PGFM) concentrations and ovarian function in dairy cows with puerperal endometritis. *Anim. Reprod. Sci.* 76:143-154.
- Opsomer, G., M. Coryn, H. Deluyker, and A. Kruif. 1998. An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. *Reprod. Domest. Anim.* 33:193-204.
- Opsomer, G., Y. Gröhn, J. Hertl, M. Coryn, H. Deluyker, and A. De Kruif. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology* 53:841-857.
- Patton, J., D. Kenny, S. McNamara, J. Mee, F. O'mara, M. Diskin, and J. Murphy. 2007. Relationships among milk production, energy balance, plasma

- analytes, and reproduction in Holstein-Friesian cows. *J. Dairy Sci.* 90:649-658.
- Petersson, K., E. Strandberg, H. Gustafsson, and B. Berglund. 2006. Environmental effects on progesterone profile measures of dairy cow fertility. *Anim. Reprod. Sci.* 91:201-214.
- Pezeshki, A., J. Mehrzad, G. Ghorbani, B. De Spiegeleer, R. Collier, and C. Burvenich. 2008. The effect of dry period length reduction to 28 days on the performance of multiparous dairy cows in the subsequent lactation. *Can. J. Anim. Sci.* 88:449-456.
- Pushpakumara, P., N. Gardner, C. Reynolds, D. Beever, and D. Wathes. 2003. Relationships between transition period diet, metabolic parameters and fertility in lactating dairy cows. *Theriogenology* 60:1165-1185.
- Ranasinghe, R., T. Nakao, K. Yamada, K. Koike, A. Hayashi, and C. Dematawewa. 2011. Characteristics of prolonged luteal phase identified by milk progesterone concentrations and its effects on reproductive performance in Holstein cows. *J. Dairy Sci.* 94:116-127.
- Rastani, R., R. Grummer, S. Bertics, A. Gümen, M. Wiltbank, D. Mashek, and M. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004-1014.
- Roelofs, J., F. Van Eerdenburg, W. Hazeleger, N. Soede, and B. Kemp. 2006. Relationship between progesterone concentrations in milk and blood and time of ovulation in dairy cattle. *Anim. Reprod. Sci.* 91:337-343.
- Rukkwamsuk, T., T. Wensing, and T. A. Kruip. 1999. Relationship between triacylglycerol concentration in the liver and first ovulation in postpartum dairy cows. *Theriogenology* 51:1133-1142.
- Santschi, D., D. Lefebvre, R. Cue, C. Girard, and D. Pellerin. 2011. Incidence of metabolic disorders and reproductive performance following a short (35-d) or conventional (60-d) dry period management in commercial Holstein herds. *J. Dairy Sci.* 94:3322-3330.
- Shrestha, H., T. Nakao, T. Higaki, T. Suzuki, and M. Akita. 2004a. Resumption of postpartum ovarian cyclicity in high-producing Holstein cows. *Theriogenology* 61:637-649.

- Shrestha, H., T. Nakao, T. Suzuki, T. Higaki, and M. Akita. 2004b. Effects of abnormal ovarian cycles during pre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. *Theriogenology* 61:1559-1571.
- Shrestha, H., T. Nakao, T. Suzuki, M. Akita, and T. Higaki. 2005. Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan. *Theriogenology* 64:855-866.
- Simpson, R., C. Chase, L. Spicer, R. Vernon, A. Hammond, and D. Rae. 1994. Effect of exogenous insulin on plasma and follicular insulin-like growth factor I, insulin-like growth factor binding protein activity, follicular oestradiol and progesterone, and follicular growth in superovulated Angus and Brahman cows. *J. Reprod. Fertil.* 102:483-492.
- Spicer, L., E. Alpizar, and S. Echternkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. *J. Anim. Sci.* 71:1232-1241.
- Tamminga, S., W. M. Van Straalen, A. P. Subnel, R. G. Meijer, A. Steg, C. J. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. *Livest. Prod. Sci.* 40:139-155.
- Van Es, A. 1975. Feed evaluation for dairy cows. *Livest Prod Sci* 2:95-107.
- Van Hoeck, V., R. Sturmey, P. Bermejo-Alvarez, D. Rizados, A. Gutierrez-Adan, H. Leese, P. E. Bols, and J. L. Leroy. 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. *PLoS One* 6:e23183.
- Van Knegsel, A., H. Van den Brand, J. Dijkstra, S. Tamminga, and B. Kemp. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. *Reprod. Nutr. Dev.* 45:665-688.
- Van Knegsel, A., G. de Vries Reilingh, S. Meulenberg, H. Van den Brand, J. Dijkstra, B. Kemp, and H. Parmentier. 2007a. Natural antibodies related to energy balance in early lactation dairy cows. *J. Dairy Sci.* 90:5490-5498.

- Van Knegsel, A., H. Van den Brand, J. Dijkstra, and B. Kemp. 2007b. Effects of dietary energy source on energy balance, metabolites and reproduction variables in dairy cows in early lactation. *Theriogenology* 68:S274-S280.
- Van Knegsel, A., H. Van den Brand, J. Dijkstra, W. Van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007c. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. *J. Dairy Sci.* 90:3397-3409.
- Van Knegsel, A., S. Van der Drift, J. Čermáková, and B. Kemp. 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: A systematic review. *Vet. J.* 198:707-713.
- Van Knegsel, A. T., G. J. Remmelink, S. Jorjong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *J. Dairy Sci.* 97:1499-1512.
- Vicari, T., J. J. Van den Borne, W. J. Gerrits, Y. Zbinden, and J. W. Blum. 2008. Postprandial blood hormone and metabolite concentrations influenced by feeding frequency and feeding level in veal calves. *Domest. Anim. Endocrinol.* 34:74-88.
- Watters, R. D., M. C. Wiltbank, J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Fricke, and R. R. Grummer. 2009. Effect of dry period length on reproduction during the subsequent lactation. *J. Dairy Sci.* 92:3081-3090.
- Zollers, W., H. Garverick, M. Smith, R. Moffatt, B. Salfen, and R. Youngquist. 1993. Concentrations of progesterone and oxytocin receptors in endometrium of postpartum cows expected to have a short or normal oestrous cycle. *J. Reprod. Fertil.* 97:329-337.

CHAPTER 4

Effects of dry period length and dietary energy source on milk yield, energy balance, and metabolic status of dairy cows over two subsequent lactations: effects in the second lactation

J. Chen,^{*} G. J. Remmelink,[‡] J. J. Gross,[†] R. M. Bruckmaier[†] B. Kemp,^{*} A. T. M. van Knegsel^{*}

^{*}Adaptation Physiology Group, Department of Animal Science, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands;

[‡]Livestock Research, Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, The Netherlands; [†]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland.

4.1 Abstract

The objective of the current study was to evaluate the effect of dry period (DP) length on milk yield, energy balance (EB), and metabolic status in cows fed a lipogenic or glucogenic diet in the second subsequent lactation after implementation of DP length and dietary treatments. Holstein-Friesian dairy cows ($n = 167$) were assigned randomly to 1 of 3 DP lengths (0, 30, or 60 d) and 1 of 2 early lactation diets (glucogenic or lipogenic) for 2 subsequent lactations. Results of the first subsequent lactation were reported previously. In the second subsequent lactation, 19 cows in the 0 d DP group were assigned to a new group (0→67 d DP) because these cows had a milk yield of < 4 kg/d at least 30 d before expected calving date and were dried off. Milk yield was recorded and EB was calculated from week -8 to 9 relative to calving. Blood samples were taken weekly from week -3 to 8 relative to calving. Liver samples were taken in weeks -2, 2 and 4 relative to calving. At the onset of lactation, cows with a 0 or 0→67 d DP had greater body condition score (BCS) than cows with a 60 d DP. During the first 9 weeks, cows with a 0 or 30 d DP produced 5.0 and 4.3 kg less milk per day, respectively, but had similar EB compared with cows with a 60 d DP. Cows with a 0 or 30 d produced additional milk precalving, which could compensate milk yield losses in the first 9 weeks postcalving. Cows with a 0 d DP did not have milk yield losses or improve EB as much as in the first lactation. Cows with a 0 d DP had greater plasma insulin and insulin-like growth factor-1 (IGF-1) and lower liver triacylglycerol concentrations than cows with other DP lengths. Cows with a 0→67 d DP had lower EB, and greater plasma free fatty acids (FFA) and β -hydroxybutyrate (BHB) concentrations than cows with other DP lengths. Feeding a glucogenic diet increased plasma glucose, IGF-1, and insulin concentrations, and decreased plasma FFA, BHB, and urea concentrations compared with a lipogenic diet, independent of DP length. In conclusion, omitting the DP or feeding a glucogenic diet improved metabolic status in early lactation of the second lactation after implementation of DP length and dietary treatments, although effects of omitting the DP were less pronounced than the first lactation. The less pronounced improvement of EB in the second lactation was related to the high BCS at the onset of lactation and reduced milk yield losses in cows with a 0 d DP.

Key words: continuous milking, energy metabolism, long-term effect

4.2 Introduction

The management strategy of drying off dairy cows at 6 to 8 weeks before calving has been generally accepted since the beginning of last century, although the milk yield of modern dairy cows in late lactation has increased considerably (Bachman and Schairer, 2003). During the past few years, shortening or omitting the dry period (**DP**) in high milk-producing dairy cows has received considerable attention. It was reported that shortening or omitting the DP improved energy balance (**EB**) in the subsequent lactation because of reduced milk yield in early lactation (Van Knegsel et al., 2014). This improved EB is related to an improved metabolic status in early lactation, indicated by decreased plasma concentrations of free fatty acids (**FFA**) (De Feu et al., 2009), β -hydroxybutyrate (**BHB**) (Andersen et al., 2005), and liver triacylglycerol (**TAG**) (Chen et al., 2015a), and increased plasma concentrations of insulin (Andersen et al., 2005), glucose (Rastani et al., 2005), and insulin-like growth factor-1 (**IGF-1**) (De Feu et al., 2009). In addition, days from calving to first ovulation and days from calving to pregnancy were reduced after shortening or omitting the DP (Rastani et al., 2005; Watters et al., 2009; Chen et al., 2015b).

Most studies investigated only consequences of shortening or omitting the DP for the lactation immediately following the DP length treatment (the first subsequent lactation). The effect of shortening or omitting the DP on milk yield, EB, metabolic status, and fertility over 2 subsequent lactations is still unclear. To our knowledge, only one controlled study has investigated the effect of omitting the DP on milk yield 2 subsequent lactations (Rémond et al., 1997). Those authors found that omitting the DP over two consecutive lactations was difficult to implement on all cows because of the challenge in maintaining milk secretion in late lactation. In that study, however, milk yield was relatively low and the authors discussed that omitting the DP is a possible management strategy for cows with persistent and high milk yield. Therefore, it can be hypothesized that omitting the DP over 2 subsequent lactations is possible in cows with persistent and high milk yield, and could improve EB and metabolic status in early lactation of the second subsequent lactation, as in the first lactation after implementation of DP length treatments.

In the first lactation after DP length treatment, feeding a glucogenic diet in early lactation improved EB further by a reduction in milk fat content (van Knegsel

et al., 2014), and decreased plasma BHB concentration independent of DP length (Chen et al., 2015a). However, the effects of dietary energy source and its interaction with DP length on milk yield, EB, and metabolic status over 2 subsequent lactations have not been studied. We can hypothesized that feeding a glucogenic diet in early lactation of the second lactation also improves EB and metabolic status compared with a lipogenic diet as found in the first lactation.

In the current study, cows were followed for 2 subsequent lactations with same DP management strategy and dietary strategy in each lactation. Results of the first lactation after implementation of DP and dietary treatments on EB and milk yield (van Knegsel et al., 2014), metabolites and metabolic hormones (Chen et al., 2015a), and fertility (Chen et al., 2015b) were reported previously. The objective of this study was to evaluate the influence of DP length on milk yield, EB, and metabolic status in cows fed a lipogenic or glucogenic diet during early lactation in the second lactation after start of DP length and dietary treatments.

4.3 Materials and Methods

4.3.1 Animals and Experimental Design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. A total of 167 Holstein-Friesian dairy cows (60 primiparous and 107 multiparous) were selected from the Dairy Campus Research dairy herd (WUR Livestock Research, Lelystad, the Netherlands) for this experiment. The experimental design, DP lengths, and dietary contrasts have been reported previously (van Knegsel et al., 2014). Briefly, dairy cows were blocked by parity, expected calving date, milk yield in previous lactation, and BCS. Within blocks, cows were assigned at random to 1 of 3 DP lengths (0, 30, or 60 d) and 1 of 2 early lactation diets (glucogenic or lipogenic). The drying-off protocol for cows with the 30 d and 60 d DP consisted of a transition to the far-off ration at d 7 before drying-off, and milking once daily at d 4 before drying-off cows. Cows with a 30 d or 60 d DP were treated with an intramammary antibiotic at drying off (Supermastidol, Virbac Animal Health, Barneveld, the Netherlands). Before calving, lactating cows were fed a lactation diet supporting 25 kg of milk. All cows were fed 1 kg/d of glucogenic or lipogenic concentrate from 10 d before the expected calving date. From calving until 100 DIM, the supply of concentrates increased stepwise by

0.5 kg/d until it reached 8.5 kg/d. After 100 DIM, all cows were fed the standard lactation concentrate. The main ingredient for the glucogenic concentrate was corn and the main ingredients for the lipogenic concentrate were sugar beet pulp, palm kernel, and rumen protected palm oil. Forage was offered ad libitum throughout the study and was composed of grass silage, corn silage, wheat straw, and rapeseed meal or soybean meal (51:34:2:13, DM basis). Diets were formulated to be isocaloric [net energy basis; Dutch net energy evaluation (**VEM**) system; Van Es, 1975] and equal in intestinal digestible protein and degraded protein balance (**DVE/OEB**) system (Tamminga et al., 1994). Cows were housed in a freestall with a slatted floor and cubicles, and were milked twice daily (0500 and 1630 h).

We planned for cows to have the same DP length and diet over 2 subsequent lactations. Results of the first lactation after implementation of DP and dietary treatments on EB and milk yield (van Knegsel et al., 2014), metabolites and metabolic hormones (Chen et al., 2015a), and fertility (Chen et al., 2015b) were reported previously. After the first lactation, 18 cows were excluded due to nonpregnancy (8 cows with a 0 d DP, 8 cows with a 30 d DP, 2 cows with a 60 d DP), 8 cows were excluded due to leg problems (3 cows with a 0 d DP, 3 cows with a 30 d DP, 2 cows with a 60 d DP), and 3 cows with a 0 d DP were excluded due to udder problems, 8 cows were excluded due to other reasons such as abortion, and pneumonia (3 cows with a 0 d DP, 3 cows with a 30 d DP, 2 cows with a 60 d DP). Therefore, the second lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

4.3.2 Body Condition Score, Milk Yield, and Energy Balance

Body condition was scored (1–5 scale) monthly. Body weight was recorded after each milking and averaged per week from week –8 to 9 relative to calving. Milk yield and feed intake were recorded daily (Insentec BV, Marknesse, the Netherlands) per cow and averaged per week from week –8 to 9 relative to calving. Energy balance was calculated per week according to the VEM system (Van Es, 1975; CVB, 2005) as the difference between VEM supplied with feed

and VEM required for maintenance and milk yield. The VEM required for animal maintenance is $42.4 \text{ VEM/kg}^{0.75} \cdot \text{d}$ (1,000 VEM = 6.9 MJ of net energy) and the VEM required for milk yield is 443 VEM/kg of fat- and protein-corrected milk (**FPCM**) (Van Es, 1975). A correction factor to scale requirements to an average cow was applied in calculating the maintenance and milk energy requirements as described by Van Es (1975).

4.3.3 Blood and Liver Tissue Sampling and Analysis

Blood and liver tissue sampling and analysis have been reported previously (Chen et al., 2015a). Briefly, blood samples were taken weekly from wk -3 to 8 relative to calving and liver biopsies were taken in wk -2, 2, and 4 relative to calving. Blood samples were kept on ice for a maximum of 2 h until they were centrifuged at $2,900 \times g$ for 10 min at 4°C and subsequently stored at -20°C until analysis. Plasma and liver samples were analyzed for indicators of metabolic status. Concentrations of glucose and urea were measured using commercial kits no. 61269 and no. 61974 from bioMérieux (Marcy l'Etoile, France), as previously described (Graber et al., 2012). 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, as previously described (Graber et al., 2012). Insulin-like growth factor-I and insulin were measured using radioimmunoassay, as previously described (Vicari et al., 2008). Liver tissue samples were kept on ice in a 0.9% NaCl solution for up to 2 h and subsequently stored at -20°C until analysis. Liver tissue sample with a wet weight of approximately 150 mg was homogenized for 15 s and TAG was extracted from liver sample using 0.5 mol/L KOH in ethanol for 30 min at 70°C, and then 2.5 mol/L perchloric acid was added to neutralize the mixture. The concentration of liver TAG was determined through enzymatic colorimetric analysis using the Triacylglycerols LiquiColor Mono kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) according to the manufacturer's instructions.

4.3.4 Statistical analysis

Results of the first lactation after implementation of DP and dietary treatments on EB, milk yield, BCS, body weight (Van Knegsel et al., 2014), metabolites and metabolic hormones (Chen et al., 2015a), and fertility (Chen et al.,

2015b) were reported previously. For the second lactation after implementation of DP and dietary treatments, repeated measurement analyses consisting of weeks relative to calving with cows as the subject were performed using PROC MIXED in SAS 9.2 (SAS Institute, Inc., Cary, NC). A first-order autoregressive structure [AR(1)] was the best fit and was used to account for within-cow variation. Statistical analyses were performed separately for precalving and postcalving data.

Milk yield, DMI, EB, BW, BCS, plasma metabolites and hormones, and liver TAG were analyzed with DP length (0, 30, 60, or 0→67 d), diet (glucogenic or lipogenic), parity (3, 4, or > 4), time, and their 2 way interactions included as fixed effects (model 1). For precalving analyses, preliminary analyses showed that the interactions of diet with DP length, time, and parity were not significant ($P > 0.05$). For postcalving analyses, preliminary analyses showed that interactions of DP length with diet and parity, and of diet with parity were not significant ($P > 0.05$). These variables were therefore excluded from their respective models.

To investigate the differences in milk yield and EB between the first and second lactation in older cows (parity > 2) with different DP lengths, lactation (1 or 2), DP length (0, 30, or 60 d), diet (glucogenic or lipogenic), time, and the interactions between lactation and DP length were assessed as fixed effects (model 2).

Preliminary analysis showed that precalving BCS was related to milk composition, EB, and plasma metabolites and varied among DP length treatments. To analyze whether the precalving BCS had an explanatory effect on postcalving milk yield, EB, and metabolic status, cows were classified into 2 groups based on average BCS (< 3.75 or ≥ 3.75) during months -2 and -1 relative to calving. Milk yield, milk composition, DMI, EB, BW, plasma metabolites and hormones, and liver TAG postcalving were analyzed with BCS (< 3.75 or ≥ 3.75), DP length (0, 30, 60, or 0→67 d), diet (glucogenic or lipogenic), parity (3, 4, or > 4), time, and the interactions between BCS classification and other fixed effects included as fixed effects (model 3). Preliminary analysis showed that the interaction of BCS with diet was not significant ($P > 0.05$), and was therefore excluded from the model.

For comparison of DP lengths, P -values are presented after a Bonferroni adjustment. Values are presented as least squares means and standard error of the

means, unless otherwise stated. Differences are considered significant if $P < 0.05$, and trends are discussed if $P \leq 0.10$.

4.4 Results

4.4.1 Dry Period Length before the Second Calving

Actual days dry before the second calving were 0 ± 0 d, 40 ± 5 d, 64 ± 2 d, and 67 ± 8 d (means $\pm$ SEM) for cows with a 0, 30, 60, or 0→67 d DP, respectively.

4.4.2 Preclaving Milk yield, Energy Balance, Body Condition Score, Plasma Metabolites, Hormones, and Liver Triacylglycerol

During the last 8 weeks before the second calving, cows with a 0 d or 0→67 d DP had greater BCS compared with cows with a 60 d DP (Table 4.1). Cows with a 0 or 30 d DP produced 7.2 ± 0.8 or 4.1 ± 0.5 kg of milk per day, respectively, and 8.6 ± 0.9 or 5.3 ± 0.6 of FPCM per day, respectively (Figure 4.1 A). Cows with a 60 d DP had greater EB compared with cows with other DP lengths (Figure 4.1 B). Cows with a 0→67 d DP had lower DMI compared with cows with other DP lengths.

Cows with a 0 d DP had greater plasma concentrations of BHB and urea compared with cows with other DP lengths. Cows with a 0 d DP had greater plasma insulin concentration compared with cows with a 60 d DP. In addition, cows with a 0→67 d DP had greater plasma FFA concentration compared with cows with a 60 d DP.

4.4.3 Postclaving Milk yield, Energy Balance, Body Condition Score

During 9 weeks after the second calving, cows with a 0 or 30 d DP produced 5.0 or 4.3 kg less milk per day, respectively, compared with cows with a 60 d DP (Table 4.2). Cows with a 0 d DP had higher natural logarithm of SCC (LnSCC), and greater BCS compared with cows with a 60 d DP. Cows with a 60 d DP produced greater FPCM compared with cows with a 30 d DP. In addition, cows with a 0→67 d DP had greater BW, and lower DMI compared with cows with a 60 d DP, but had lower EB compared with cows with a 0 or 30 d DP. Cows with a 0→67 d DP had lower energy intake (**EI**) and greater BCS compared with cows with a 30 or 60 d DP. Cows fed a glucogenic diet had lower milk fat percentage, and tended to have greater

Table 4.1. Dry matter intake, energy balance, metabolites and hormones of dairy cows precalving during a dry period of 0, 30, 60, or 0→67 d¹ and fed either a glucogenic (G) or a lipogenic (L) diet (LSM ± SEM).

	Dry period length (d)				SEM ²	<i>P</i> -values ³		
	0	30	60	0→67		DPL	Diet	Time ⁴
Cows, n	20	41	50	19				
DMI (kg/d)	16.3 ^a	15.0 ^a	15.2 ^a	12.9 ^b	0.4	<0.01	0.24	<0.01
EB ⁵ (kJ/kg ^{0.75} ·d)	118 ^b	94 ^b	175 ^a	68 ^b	15	<0.01	0.63	<0.01
Body weight (kg)	808.6 ^{ab}	776.8 ^b	764.6 ^b	828.6 ^a	12.8	<0.01	0.98	<0.01
BCS ⁶	3.8 ^{ab}	3.5 ^b	3.2 ^c	4.2 ^a	0.1	<0.01	0.83	0.43
Cows, n	16	33	44	19				
FFA (mmol/L)	0.15 ^{ab}	0.17 ^{ab}	0.15 ^b	0.24 ^a	0.02	0.02	0.63	<0.01
BHB ⁷ (mmol/L)	0.55 ^a	0.41 ^b	0.42 ^b	0.40 ^b	0.04	<0.01	0.58	0.01
Glucose (mmol/L)	3.75	3.80	3.73	3.66	0.05	0.22	0.30	<0.01
Urea (mmol/L)	3.56 ^a	2.77 ^b	2.56 ^b	2.31 ^b	0.12	<0.01	0.40	0.04
IGF-1 (ng/mL)	162.39	151.46	147.71	164.27	7.13	0.25	0.74	<0.01
Insulin (μIU/mL)	27.97 ^a	21.17 ^{ab}	20.65 ^b	22.51 ^{ab}	1.68	0.02	0.58	<0.01
Cows, n	10	23	22	13				
Liver TAG ⁸ (mg/g of wet weight)	15.36	16.67	15.53	21.55	2.08	0.16	0.58	NM ⁹

^{a,b} Values within a dry period length in the same row with different superscripts differ ($P < 0.05$).

¹The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

²SEM = pooled standard error of the least squares means.

³DPL = dry period length. The *P*-value of parity and interaction of DPL × parity and diet × parity not shown in table.

⁴Weeks -8, -7, -6, -5, -4, -3, -2, and -1 relative to calving for dry matter intake (DMI), energy balance (EB), and body weight; months -2 and -1 relative to calving for body condition score (BCS); weeks -3, -2, and -1 relative to calving for plasma concentrations of FFA, BHB, glucose, urea, IGF-1, and insulin; week -2 relative to calving for liver triacylglycerol concentration.

⁵calculated with the VEM system (Van Es, 1975).

⁶Body condition score on a scale of 1 - 5.

⁷Statistical analysis was based on natural log-transformed data; back-transformed data are presented.

⁸TAG = triacylglycerol.

⁹NM = not included in the model.

milk yield ($P = 0.06$) compared with cows fed a lipogenic diet. Diet did not affect DMI, EB, BW, or BCS in early lactation.

4.4.5 Postcalving Plasma Metabolites, Hormones, and Liver Triacylglycerol

During 9 weeks after the second calving, cows with a 0 d DP had greater plasma insulin and IGF-1 concentrations but lower liver TAG concentration compared with cows with other DP lengths (Table 4.3). Cows with a 0→67 d DP had greater plasma concentrations of FFA, BHB, and lower plasma urea concentration compared with cows with other DP lengths. In addition, interaction of DP length $\times$ time was present for plasma FFA concentration showing that plasma FFA concentration increased from week 3 to 4 in cows with a 0→67 d DP, but not in cows with other DP lengths (Figure 4.2). Interaction of diet $\times$ time showed that liver TAG concentration decreased from week 2 to 4 in cows fed a glucogenic diet, but not in cows fed a lipogenic diet (Figure 4.3). Cows fed a glucogenic diet had greater plasma concentrations of glucose, IGF-1, and insulin, but lower plasma concentrations of FFA, BHB, and urea compared with cows fed a lipogenic diet. Postcalving, time effects were significant for plasma FFA, BHB, glucose, urea, IGF-1, insulin, and liver TAG concentrations.

4.4.6 Relationship between Body Condition Score and Milk yield, Energy Balance, and Metabolic Status

In early lactation, cows with precalving BCS ≥ 3.75 had greater FPCM, milk fat percentage, BW, and plasma concentrations of FFA and BHB, but lower EB, compared with cows with precalving BCS < 3.75 independent of DP length (Table 4.4). Interaction of DP length $\times$ BCS classification was present for EI. Cows with a 0→67 d DP had lower EI (984 ± 47 kJ/kg^{0.75}·d) compared with cows with a 60 d DP ($1,108 \pm 13$ kJ/kg^{0.75}·d) when cows had precalving BCS < 3.75 , whereas cows with a 30 d DP (956 ± 21 kJ/kg^{0.75}·d) had lower EI compared with cows with a 60 d DP ($1,032 \pm 24$ kJ/kg^{0.75}·d) when cows had precalving BCS ≥ 3.75 . Furthermore, cows with precalving BCS ≥ 3.75 tended to have lower DMI ($P = 0.07$) and forage intake ($P = 0.07$) compared with cows with precalving BCS < 3.75 .

When adding precalving BCS classification as a fixed effect in the model, DP length effects were no longer present for milk fat percentage ($P = 0.78$), body weight ($P = 0.47$), or plasma concentrations of FFA ($P = 0.08$), BHB ($P = 0.42$), and

(A)

(B)

Figure 4.1. Milk yield (kg/d) and energy balance (kJ/kg^{0.75}·d) for cows with a 0 d (●), 0→67 d (■), 30 d (○), or 60 d (□) dry period during week -8 to 9 relative to calving. Values represent means and error bars represent the error of the means per dry period length per week. The statistical effects of dry period length (DPL), time (T), and dry period length × time (DPL × T) postcalving are indicated.

urea ($P = 0.11$). Dry period length effects changed for EB and concentrate intake after correcting for precalving BCS. After correcting for precalving BCS, cows with a 0 d DP had greater EB in early lactation compared with cows with a 60 d DP. Cows with a 30 d DP had lower concentrate intake compared with cows with a 60 d DP. Moreover, the effects of DP length were unchanged after correction for precalving BCS for milk yield, FPCM, milk lactose and protein percentage, LnSCC, DMI, forage intake, EI, EB, plasma concentrations of glucose, IGF-1, and insulin, and liver TAG concentration.

4.4.7 Comparison of Milk Yield, Dry Matter Intake, and Energy Balance Between the First and Second lactation

Table 4.2. Milk yield, milk composition, DMI, energy balance, BCS of dairy cows in early lactation after a dry period of 0, 30, 60, or 0→67 d^l and fed either a glucogenic (G) or a lipogenic (L) diet (LSM ± SEM).

	Dry period length (d)				SEM ²		Ration		SEM	P-values ³					
	0	30	60	0→67			G	L		DPL	Diet	Time ⁴	Parity	DPL×T	D×T
Cows, n	20	41	50	19			66	64							
Milk yield (kg/d)	37.8 ^b	38.5 ^b	42.8 ^a	40.6 ^{ab}	1.0		40.8	39.1	0.7	<0.01	0.06	<0.01	0.04	0.23	0.02
FPCM ⁵ (kg/d)	41.0 ^{ab}	40.9 ^b	44.4 ^a	43.6 ^{ab}	1.0		42.8	42.1	0.7	<0.01	0.43	<0.01	0.16	0.65	0.07
Lactose (%)	4.50	4.51	4.51	4.49	0.03		4.51	4.49	0.02	0.94	0.21	<0.01	<0.01	0.87	0.35
Fat (%)	4.61 ^{ab}	4.54 ^{ab}	4.41 ^b	4.80 ^a	0.08		4.48	4.70	0.06	0.02	<0.01	<0.01	0.25	0.98	0.08
Protein (%)	3.58 ^a	3.53 ^a	3.39 ^{ab}	3.30 ^b	0.06		3.47	3.43	0.04	<0.01	0.38	<0.01	0.44	0.86	0.96
LnSCC ⁶ (×10 ³ cells/mL)	5.1 ^a	4.8 ^{ab}	4.6 ^b	5.0 ^{ab}	0.12		4.9	4.9	0.09	0.02	0.84	<0.01	<0.01	0.47	0.60
DMI (kg/d)	21.3 ^{ab}	20.8 ^{ab}	21.9 ^a	19.7 ^b	0.4		21.1	20.8	0.3	<0.01	0.35	<0.01	0.27	0.02	0.22
Concentrate (kg/d)	6.9	6.9	6.9	6.9	0.0		6.9	6.9	0.0	0.20	0.13	<0.01	0.20	0.13	0.83
Forage (kg/d)	14.4 ^{ab}	14.0 ^{ab}	14.9 ^a	12.8 ^b	0.4		14.2	13.8	0.3	<0.01	0.29	<0.01	0.32	0.06	0.17
EI ⁷ (kJ/kg ^{0.75} ·d)	1,031 ^{ab}	1,037 ^a	1,092 ^a	948 ^b	19		1,035	1,021	14	<0.01	0.17	<0.01	0.13	0.04	0.19
EB ⁸ (kJ/kg ^{0.75} ·d)	-206 ^a	-234 ^a	-270 ^{ab}	-338 ^b	24		-262	-263	16	<0.01	0.98	<0.01	0.53	0.71	0.06
Body weight (kg)	727.4 ^{ab}	699.6 ^{ab}	692.2 ^b	737.7 ^a	11.1		712.6	715.9	7.7	0.01	0.75	<0.01	0.48	<0.01	0.02
BCS ⁹	3.0 ^{ab}	2.7 ^b	2.3 ^c	3.4 ^a	0.1		2.8	2.8	0.1	<0.01	0.82	<0.01	0.06	0.34	0.93

^{a,b} Values within a dry period length in the same row with different superscripts differ ($P < 0.05$). (Continued on the next page)

In the first 9 weeks of the second lactation compared with the first lactation (Table 4.5), older cows (parity > 2) with a 0 d DP had lower EB ($P < 0.01$), similar milk yield ($P = 0.18$), and similar DMI ($P = 0.17$); older cows with a 30 d DP had lower EB ($P < 0.01$), similar milk yield ($P = 0.61$), and lower DMI ($P < 0.01$); older cows with a 60 d DP had similar negative EB ($P = 0.52$), similar milk yield ($P = 0.14$), and similar DMI ($P = 0.24$).

In the first 9 weeks of the first lactation, milk yield losses were 14.2 and 4.1 kg/d for young cows (parity = 2) with a 0 or 30 d DP, and were 9.9 or 5.4 kg/d for older cows with a 0 d or 30 d DP, respectively, compared with cows with a 60 d DP. In the first 9 weeks of the first lactation, difference of EB between cows with a 0 and 60 d DP was 311 kJ/kg^{0.75}·d for young cows, and was 195 kJ/kg^{0.75}·d for older cows. In the first 9 weeks of the second lactation, milk yield losses were 5.0 or 4.3 kg/d for older cows with a 0 or 30 d DP, respectively, compared with cows with a 60 d DP. In the first 9 weeks of the second lactation, difference of EB between cows with a 0 and 60 d DP was 64 kJ/kg^{0.75}·d. After correction for precalving BCS in the second lactation, milk yield losses of older cows with a 0 or 30 d DP were changed to 5.5 or 2.9 kg/d, respectively. In the second lactation after correction by precalving BCS, difference of EB between cows with a 0 and 60 d DP was 101 kJ/kg^{0.75}·d.

4.5 Discussion

At the onset of lactation of the second lactation, cows with a 0 d DP had greater BCS compared with cows with a 60 d DP. This is because cows with a 0 d

Table 4.2 continued

¹The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

²SEM = standard error of the least squares means.

³DPL = dry period length; D = diet; T = time.

⁴Weeks 1, 2, 3, 4, 5, 6, 7, 8, and 9 postcalving for all variables except body condition score (BCS, month 1 and 2).

⁵Fat- and protein-corrected milk.

⁶LnSCC = natural logarithm of SCC.

⁷Energy intake; calculated with the VEM system (Van Es, 1975).

⁸Energy balance; calculated with the VEM system (Van Es, 1975).

⁹Body condition score on a scale of 1 - 5.

Figure 4.2. Plasma FFA concentration (mmol/L) of cows with a 0 d (●), 0→67 d (■), 30 d (○), or 60 d (□) dry period during week -3 to 8 relative to calving. Values represent means and error bars represent the error of the means per DP length per week. The statistical effects of dry period length (DPL), time (T), and dry period length × time (DPL × T) postcalving are indicated.

DP produced less milk in the first lactation, but had similar energy intake compared with cows with a 60 d DP. This excess energy intake in the first lactation led to excessive weight gain in cows with a 0 d DP (BCS = 3.8) during the last 2 months before the second calving. The high peripartal BCS could be related to the less pronounced improvement of EB in cows with a 0 d DP in the second lactation compared with the first lactation. Roche et al. (2013) found that the extent of negative EB in early lactation increased with calving BCS. Indeed, after correction for precalving BCS in the current study, the EB contrast between cows with a 0 d DP (greater EB) and 60 d DP (lower EB) increased. Thus, a reduction in energy intake in the mid and late lactation periods may be necessary to prevent excessive weight gain in dairy cows when the DP is omitted. To our knowledge, the effects of a reduced energy intake (e.g., a reduced dietary energy content) on milk yield and EB after 0 d DP are unknown. We can hypothesized that reduced dietary energy density in cows with a 0 d DP during mid and late lactation may improve EB, cow health, and welfare due to the 0 d DP, and increases economic net herd returns by reducing feed costs.

The less pronounced improvement of EB in the second lactation could also be related to smaller milk yield losses after 0 d DP compared with the first lactation. During the first 9 weeks, cows with a 0 d DP had a 25% lower milk yield in the first

lactation and only a 12% lower milk yield in the second lactation compared with cows with a 60 d DP. This discrepancy could be related to the different distribution of parities in dairy cows between the first and second lactations. In the second lactation of the current study, all cows were older cows (parity > 2). Annen et al. (2004) found that omitting the DP reduced milk yield in the subsequent lactation in young cows (parity = 2), but not in older cows. Watters et al. (2008) suggested that fewer days dry are required to maximize milk yield in the subsequent lactation when cows increase in parity number. Moreover, in the first lactation of the current study, milk yield losses for cows with a 0 d DP were less in older cows compared with young cows (22 vs. 34%). This parity sensitivity to omission of the DP could be due to continued mammary development in young cows during lactation (Collier et al., 2012). In addition, in the current study, cows with a 0 d DP had greater FPCM yield from week -8 to 9 relative to calving compared with cows with a 60 d DP. This result is in line with the finding in the first lactation of the current study, indicating that omitting the DP shifts milk yield from the postcalving to the precalving period (Van Knegsel et al., 2014).

Figure 4.3. Liver triacylglycerol (TAG) (mg/g of wet weight) for cows fed a glucogenic or lipogenic diet. Values represent means and error bars represent the error of the means in weeks -2, 2, and 4 relative to calving. The statistical effects of diet (D) time (T), and diet $\times$ time (D $\times$ T) postcalving are indicated.

Although the improvement of EB was less pronounced, omitting the DP decreased liver TAG concentration in early lactation of the second lactation compared with 60 d DP. Together with our previous study (Chen et al., 2015a), this result suggests that omitting the DP could reduce the risk of fatty liver in early

lactation over 2 subsequent lactations. Additionally, cows with a 0 d DP had greater plasma IGF-1 and insulin concentrations compared with cows with other DP lengths in the second lactation, which is in accordance with previous studies in the first lactation after implementation of DP length treatment (Andersen et al., 2005; De Feu et al., 2009; Chen et al., 2015a). Greater plasma IGF-1 and insulin concentrations could be caused by (1) lower milk yield and improved EB in cows with a 0 d DP in early lactation, or (2) carry-over effects of lactation diet-induced increase in plasma IGF-1 and insulin concentrations before the second calving in cows with a 0 d DP.

In the current study, omitting the DP increased LnSCC in early lactation of the second lactation compared with a 60 d DP. This finding is in agreement with the results of the first lactation of the current study (Van Knegsel et al., 2014). The rise in LnSCC in cows with a 0 d DP can be explained partly by a decreased milk yield and, thus, a reduced diluting effect (Steenefeld et al., 2013). In the current study, when milk yield was included as a covariate in the model to analyze SCC, the effect of DP length on LnSCC was no longer present. Also, omission of preventive use of antibiotics in the weeks precalving in cows with a 0 d DP could contribute to the increased LnSCC.

Before the second calving, DP omission was achieved in 20 out of 39 cows assigned to the 0 d DP group in the current study, whereas DP omission was achieved in 2 out of 21 cows assigned to the 0 d DP group in the study of Rémond et al. (1997). The greater success in achieving DP omission over 2 subsequent lactations in the current study could be related to high genetic potential for milk yield and high feeding level in the cows (Rémond et al., 1997). In addition, these results indicate that not all cows could be milked continuously over 2 subsequent lactations because of low milk yield in late pregnancy. A Short calving interval, which is related to greater milk yield in late pregnancy, could be a feasible approach to improve the achievement of DP omission. Earlier, several studies reported that omitting the DP reduced the interval from calving to resumption of ovarian cyclicity (Gümen et al., 2005; De Feu et al., 2009; Chen et al., 2015b). It can be hypothesized that when an earlier resumption of ovarian cyclicity is matched with an adjusted reproductive management (e.g., shorter voluntary waiting period or earlier insemination), it results in a shorter calving interval, which could lead to more cows achieving a 0 d DP during multiple lactations.

Table 4.3. Plasma metabolites, hormones, and liver TAG of dairy cows in early lactation after a dry period of 0, 30, 60, or 0→67 d¹ (LSM ± SEM).

	Dry period length (d)				SEM ²		Diet		SEM	P-values ³			
	0	30	60	0→67			G	L		DPL	Diet	Time ⁴	Parity
Cows, n	15	35	44	19	58	55							
FFA (mmol/L)	0.37 ^b	0.36 ^b	0.37 ^b	0.49 ^a	0.03	0.43	0.36	0.43	0.02	<0.01	<0.01	<0.01	<0.01
BHB ⁵ (mmol/L)	0.82 ^{ab}	0.80 ^b	0.73 ^b	1.08 ^a	0.07	0.98	0.73	0.98	0.05	<0.01	<0.01	<0.01	0.44
Glucose (mmol/L)	3.33	3.27	3.27	3.19	0.06	3.17	3.36	3.17	0.04	0.54	<0.01	<0.01	0.24
Urea (mmol/L)	2.87 ^a	2.84 ^a	2.74 ^{ab}	2.39 ^b	0.09	2.81	2.61	2.81	0.06	0.01	0.02	<0.01	0.38
IGF-1 (ng/mL)	81.58 ^a	62.36 ^b	62.15 ^b	62.65 ^b	3.39	71.79	62.60	62.60	2.34	<0.01	<0.01	<0.01	<0.01
Insulin (μIU/mL)	14.91 ^a	11.90 ^b	10.24 ^b	9.83 ^b	0.69	12.67	10.78	10.78	0.47	<0.01	<0.01	<0.01	0.68
Cows, n	10	25	24	12	36	35							
Liver TAG ⁶ (mg/g of wet weight)	44.1 ^b	90.7 ^{ab}	96.7 ^a	117.2 ^a	11.9	88.9	85.5	85.5	8.3	<0.01	<0.01	0.02	0.01

^{a,b} Values within a dry period length in the same row with different superscripts differ ($P < 0.05$).¹The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.²SEM = standard error of the least squares means.³DPL = dry period length; D = Diet; T = Time.⁴Weeks 1, 2, 3, 4, 5, 6, 7, and 8 postcalving for plasma concentrations of FFA, BHB, glucose, urea, IGF-1, and insulin; week 2 and 4 postcalving for liver triacylglycerol concentration.⁵Statistical analysis was based on natural log-transformed data; back-transformed data are presented.⁶TAG = triacylglycerol.

Table 4.4. Milk yield, DMI, energy balance, metabolites and hormones in early lactation for dairy cows categorised according to BCS before calving¹ (LSM $\pm$ SEM).

	BCS		SEM ²		Dry period length ³ (d)					SEM ²				P-values ⁴			
	<3.75	≥ 3.75	80	50	0	20	41	30	60	50	19	0 $\rightarrow$ 67	BCS	DPL	Diet	Time ⁵	B $\times$ DPL
Cows, n																	
Milk yield (kg/d)	39.5	40.6	39.5	40.6	37.4 ^b	40.0 ^b	42.9 ^a	41.0 ^{ab}				1.1	0.41	<0.01	0.15	<0.01	0.72
FPCM ⁶ (kg/d)	41.6	44.1	41.6	44.1	41.1 ^b	41.5 ^b	45.3 ^a	43.5 ^{ab}				1.1	0.04	<0.01	0.37	<0.01	0.99
Lactose (%)	4.49	4.52	4.49	4.52	4.50	4.51	4.51	4.49				0.02	0.29	0.96	0.14	<0.01	0.51
Fat (%)	4.44	4.81	4.44	4.81	4.60	4.64	4.58	4.70				0.09	<0.01	0.78	0.01	<0.01	0.33
Protein (%)	3.45	3.43	3.45	3.43	3.62 ^a	3.49 ^{ab}	3.41 ^{ab}	3.24 ^b				0.07	0.83	<0.01	0.37	<0.01	0.09
LnSCC ⁷ ($\times 10^3$ cells/mL)	4.8	4.8	4.8	4.8	5.0 ^{ab}	4.9 ^a	4.5 ^b	4.9 ^{ab}				0.2	0.69	0.02	0.86	<0.01	0.07
DMI (kg/d)	21.3	20.4	21.3	20.4	21.5 ^{ab}	20.4 ^{ab}	21.6 ^a	19.7 ^b				0.4	0.07	<0.01	0.53	<0.01	0.09
EB ⁸ (kJ/kg ^{0.75} ·d)	1064	979	1064	979	1,039 ^{ab}	1,007 ^b	1,070 ^a	970 ^b				18	<0.01	<0.01	0.38	<0.01	0.02
EB ⁹ (kJ/kg ^{0.75} ·d)	-231	-327	-231	-327	-201 ^a	-271 ^{ab}	-302 ^b	-341 ^b				26	<0.01	<0.01	0.60	<0.01	0.09
Body weight (kg)	691.3	736.4	691.3	736.4	725.3	713.1	701.6	715.4				12.2	<0.01	0.47	0.95	<0.01	0.23
Cows, n																	
FFA (mmol/L)	0.36	0.45	0.36	0.45	0.37	0.40	0.38	0.48				0.02	<0.01	0.08	0.04	<0.01	0.33
BHB ¹⁰ (mmol/L)	0.70	0.98	0.70	0.98	0.78	0.77	0.88	0.85				0.12	<0.01	0.42	<0.01	<0.01	0.37
Glucose (mmol/L)	3.25	3.22	3.25	3.22	3.31	3.23	3.21	3.18				0.07	0.68	0.63	<0.01	<0.01	0.03
Urea (mmol/L)	2.80	2.65	2.80	2.65	2.89	2.80	2.74	2.46				0.10	0.19	0.11	0.03	<0.01	0.45
IGF-1 (ng/mL)	62.49	67.40	62.49	67.40	79.56 ^a	60.85 ^b	63.62 ^b	55.74 ^b				3.86	0.24	<0.01	<0.01	<0.01	0.06
Insulin (μ U/mL)	11.40	11.81	11.40	11.81	14.87 ^a	12.04 ^b	10.26 ^b	9.24 ^b				0.83	0.64	<0.01	<0.01	<0.01	0.91
Cows, n																	
Liver TAG ¹¹ (mg/g of wet weight)	80.2	105.4	80.2	105.4	42.7 ^b	95.4 ^a	110.4 ^a	124.6 ^a				12.3	0.19	<0.01	0.82	0.02	0.09

^{a,b} Values within a dry period length in the same row with different superscripts differ ($P < 0.05$). (Continued on the next page)

The cows that did not achieve a 0 d DP (0→67 d) had greater BCS (BCS = 4.2) during the last 2 months before the second calving compared with cows with other DP lengths, because of a lower milk yield and similar energy intake in the first lactation. Roche et al. (2009) suggested that an optimal BCS during calving should be between 3.0 and 3.25, and that $BCS \geq 3.5$ at calving is related to a decrease in DMI, more severe negative EB, and increased risk for metabolic disorders during the periparturient period. Indeed, cows with a 0→67 d DP had low energy intake and severe negative EB in the current study. A severe negative EB in cows with a 0→67 d DP in early lactation results in elevated body fat mobilization and an increase in plasma FFA concentration. Free fatty acids can enter the pathway of esterification and be stored as TAG, or be incompletely oxidized to ketone bodies in liver (Drackley, 1999), which led to increased plasma BHB and liver TAG concentrations in cows with a 0→67 d DP in early lactation of the second lactation. However, after correction for precalving BCS, plasma concentrations of FFA and BHB in cows with a 0→67 d DP was no longer different from cows with other DP lengths. This result implies that poor metabolic status caused by negative EB in cows with a 0→67 d DP was mainly due to high BCS rather than DP length treatments.

In the first 9 weeks of the second lactation, milk yield and FPCM did not differ between cows with a 0→67 d DP and cows with a 60 d DP in the current study. This high milk yield in cows with a 0→67 d DP could be due to a long DP

Table 4.4 continued

¹Mon -1 and -2 relative to calving.

²SEM = pooled standard error of the least squares means.

³The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

⁴DPL = dry period length; B = body condition score category. The *P*-value of parity and interaction of B $\times$ Time and B $\times$ Parity not shown in table.

⁵Week 1, 2, 3, 4, 5, 6, 7, 8, and 9 postcalving for DMI, EB, and BW; month 1 and 2 postcalving for BCS; week 1, 2, 3, 4, 5, 6, 7, and 8 postcalving for plasma concentrations of FFA, BHB, glucose, urea, IGF-1, and insulin; week 2 and 4 postcalving for liver triacylglycerol concentration.

⁶Fat- and protein-corrected milk.

⁷LnSCC = natural logarithm of SCC.

⁸Energy intake; calculated with the VEM system (Van Es, 1975).

⁹Energy balance; calculated with the VEM system (Van Es, 1975).

¹⁰Statistical analysis was based on natural log-transformed data; back-transformed data are presented.

¹¹TAG = triacylglycerol.

Table 4.5. Milk yield, DMI, and energy balance (EB) in early lactation¹ of young (parity = 2) and older (parity > 2) dairy cows in first and second lactation (LSM $\pm$ SEM).

	First lactation						Second lactation ³						Second lactation (Corrected by BCS)					
	Parity = 2			Parity > 2			Parity > 2			Parity > 2			Parity > 2			Parity > 2		
	0	30	60	SEM ²	0	30	60	SEM	0	30	60	0 $\rightarrow$ 67	SEM	0	30	60	0 $\rightarrow$ 67	SEM
Cows, n	20	20	20		36	35	36		18	39	50	18		18	39	50	18	
Milk yield (kg/d)	27.5	37.6	41.7	1.1	35.1	39.6	45.0	1.0	37.8	38.5	42.8	40.6	1.1	37.4	40.0	42.9	41.0	1.3
DMI (kg/d)	22.1	21.9	22.2	0.4	22.2	22.4	22.4	0.3	21.3	20.8	21.9	19.7	0.4	21.5	20.4	21.6	19.7	0.4
EB ⁴ (kJ/kg ^{0.75} -d)	106	-134	-205	23	-68	-154	-263	24	-206	-234	-270	-338	24	-201	-271	-302	-341	26

¹Week 1, 2, 3, 4, 5, 6, 7, 8, and 9 postcalving.²SEM = pooled standard error of the least squares means.³The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0 $\rightarrow$ 67 d DP group (actual days dry: 67 $\pm$ 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.⁴Energy balance; calculated with the VEM system (Van Es, 1975).

in these cows, which allows replacement of damaged or senescent mammary epithelial cells before the subsequent lactation (Capuco et al., 1997). In agreement with our study, Rémond et al. (1997) reported that the cows that had an average 7-d DP in the first lactation but had an average 54 d DP in the second lactation, had similar milk yield compared with cows with a conventional 60 d DP. These results imply that milk yield level could be recovered in cows with a 0 d DP in previous lactation by drying off these cows again.

In early lactation of the second lactation, shortening the DP (30 d) resulted in less milk yield compared with a conventional DP, which is in line with results in the first lactation of the current study (Van Knegsel et al., 2014) and in the study of Rastani et al. (2005). However, cows with a 30 d DP produced 297 kg of additional FPCM precalving because of an extended previous lactation. As a result, cows with a 30 d DP had a greater FPCM yield from week -8 to 9 relative to calving compared with cows with a 60 d DP.

Although shortening the DP reduced milk yield in early lactation, the improvement of EB in early lactation was less pronounced in the second lactation compared with the first lactation. The reduced improvement of EB was related to similar milk yield but lower DMI in cows with a 30 d DP in the second lactation compared with the first lactation. In addition, shortening the DP had no effects on LnSCC or metabolic status in the second lactation, which is in line with previous studies implementing a shortened DP for 1 lactation (Rastani et al., 2005; Watters et al., 2008; Van Knegsel et al., 2014). Furthermore, 31 out of 41 cows assigned to 30 d DP length treatment group had a DP length between 20 and 40 d before the second calving (3 cows had DP length ≤ 20 d and 7 cows had DP length ≥ 40 d), indicating that implementing the strategy of shortening the DP is possible for 2 subsequent lactations in most dairy cows.

In the second lactation, feeding a glucogenic diet reduced milk fat percentage, but tended to increase milk yield in early lactation compared with a lipogenic diet. Thus, the amount of energy partitioned to milk was similar, which led to similar EB in early lactation between cows fed a glucogenic diet and a lipogenic diets. Feeding a glucogenic diet compared with a lipogenic diet improved metabolic status independent of DP length, as indicated by decreased plasma FFA, BHB, and urea concentrations, and increased plasma IGF-1, insulin, and glucose concentrations. The high plasma glucose and insulin concentrations in cows fed a

glucogenic diet could be related to its high starch content. Starch can be either fermented in the rumen to produce high amounts of propionate or digested in the small intestine and absorbed as glucose (Van Knegsel et al., 2007a). Propionate is the major precursor for gluconeogenesis in dairy cows. In addition, a lipogenic diet is high in fiber and stimulates the ruminal production of lipogenic VFA (acetate and butyrate), which is related to increased plasma FFA and BHB concentrations (Moallem et al., 1997; Drackley et al., 1998; Drackley et al., 2003).

Dietary effects on plasma metabolites were clearer in the second lactation compared with the first lactation after implementation of DP length treatments. There may be 2 explanations. First, feeding a glucogenic diet has more pronounced effects in older cows than in young cows in early lactation (Van Knegsel et al., 2007b). Second, cows had a more severe negative EB in the second lactation compared with the first lactation. It can be hypothesized that a glucogenic diet could decrease fat mobilization and improve metabolic status in older cows and cows with severe negative EB.

Our results suggest that shortening the DP for 2 subsequent lactations is possible for most cows with limited milk yield losses in the subsequent lactation. Omitting the DP is an option for cows with high persistency of lactation and high milk yield, but omitting the DP increases the risk of excessive weight gain in the subsequent lactation. In future studies, it will be necessary to test whether decreasing dietary energy density in mid and late lactation can prevent excessive weight gain and whether an earlier resumption of ovarian cyclicity and shorter calving interval can improve milk yield in late pregnancy and increase the proportion of cows that achieve a 0 d DP for multiple lactations.

4.6 Conclusions

Omitting the DP (0 d) in the second lactation did not result in improvement of EB as much as in the first lactation after implementation of DP length treatment, which was related to high BCS at the onset of lactation and smaller milk yield losses in cows with 0 d DP in the second lactation. In spite of the less pronounced improvement of EB, omitting the DP improved metabolic status in the early lactation of second lactation. Before the second calving, 19 out of 39 cows in the 0 d DP group did not achieve a 0 d DP and were assigned to a new group (0→67 d). Cows with a 0→67 d DP had more severe negative EB and poorer metabolic status compared

with cows with a 0, 30, or 60 d DP. In the second lactation, omitting or shortening (30 d) the DP decreased milk yield compared with conventional DP (60 d), but additional milk produced precalving could compensate for milk yield losses in the first 9 weeks postcalving. Independent of DP length, a glucogenic diet, compared with a lipogenic diet, improved metabolic status without affecting EB in early lactation of the second lactation.

4.7 Acknowledgments

The authors thank the Dutch Dairy Board (PZ, the Netherlands), Product Board Animal Feed (PDV; the Netherlands), and CRV (the Netherlands) for financing the experiment. The first author thanks the Chinese Scholarship Council (CSC) for financial support. The authors thank the staff of the Dairy Campus (Lelystad, the Netherlands) and Veterinary Physiology Group of the University of Bern (Switzerland) for their technical support and laboratory analyses during the experiment. Additionally, the authors thank Danny de Koning for editing this manuscript.

References

- Andersen, J. B., T. G. Madsen, T. Larsen, K. L. Ingvarlsen, and M. O. Nielsen. 2005. The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. *J. Dairy Sci.* 88:3530-3541.
- Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. *J. Dairy Sci.* 87:3746-3761.
- Bachman, K. C. and M. L. Schairer. 2003. Invited review: Bovine studies on optimal lengths of dry periods. *J. Dairy Sci.* 86:3027-3037.
- Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in Holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477-487.
- Centraal Veevoederbureau (CVB). 2005. Gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. CVB, Lelystad, the Netherlands.
- Chen, J., J. J. Gross, H. A. van Dorland, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2015a. Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation. *J. Dairy Sci.* 98:1033-1045.
- Chen, J., N. Soede, H. van Dorland, G. Remmelink, R. Bruckmaier, B. Kemp, and A. van Knegsel. 2015b. Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths. *Theriogenology* 84:1387-1396.
- Collier, R. J., E. L. Annen-Dawson, and A. Pezeshki. 2012. Effects of continuous lactation and short dry periods on mammary function and animal health. *Animal* 6:403-414.
- De Feu, M., A. Evans, P. Lonergan, and S. Butler. 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status, and postpartum ovarian function in Holstein-Friesian dairy cows. *J. Dairy Sci.* 92:6011-6022.
- Drackley, J. K., D. W. LaCount, J. P. Elliott, T. H. Klusmeyer, T. R. Overton, J. H. Clark, and S. A. Blum. 1998. Supplemental fat and nicotinic acid for Holstein cows during an entire lactation. *J. Dairy Sci.* 81:201-214.

- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259-2273.
- Drackley, J. K., T. M. Cicela, and D. W. LaCount. 2003. Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. *J. Dairy Sci.* 86:1306-1314.
- Gümen, A., R. R. Rastani, R. R. Grummer, and M. C. Wiltbank. 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *J. Dairy Sci.* 88:2401-2411.
- Moallem, U., M. Kaim, Y. Folman, and D. Sklan. 1997. Effect of calcium soaps of fatty acids and administration of somatotropin in early lactation on productive and reproductive performance of high producing dairy cows. *J. Dairy Sci.* 80:2127-2136.
- Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004-1014.
- Remond, B., J. Rouel, N. Pinson, and S. Jabet. 1997. An attempt to omit the dry period over three consecutive lactations in dairy cows. *Ann. Zootech.* 46:399-408.
- Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *J. Dairy Sci.* 92:5769-5801.
- Steenefeld, W., Y. Schukken, A. Van Kneegsel, and H. Hogeveen. 2013. Effect of different dry period lengths on milk production and somatic cell count in subsequent lactations in commercial Dutch dairy herds. *J. Dairy Sci.* 96:2988-3001.
- Tamminga, S., W. M. Van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. *Livest. Prod. Sci.* 40:139-155.
- Van Es, A. J. 1975. Feed evaluation for dairy cows. *Livest. Prod. Sci.* 2:95-107.
- Van Kneegsel, A., H. Van den Brand, E. Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga, and B. Kemp. 2007a. Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. *J. Dairy Sci.* 90:1477-1485.

- Van Knegsel, A. T. M., H. Van den Brand, J. Dijkstra, W. M. Van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007b. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. *J. Dairy Sci.* 90:3397-3409.
- Van Knegsel, A. T. M., G. J. Remmelink, S. Jorjong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *J. Dairy Sci.* 97:1499-1512.
- Watters, R. D., J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Crump, P. W. Clark, and R. R. Grummer. 2008. Effects of dry period length on milk production and health of dairy cattle. *J. Dairy Sci.* 91:2595-2603.
- Watters, R. D., M. C. Wiltbank, J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Fricke, and R. R. Grummer. 2009. Effect of dry period length on reproduction during the subsequent lactation. *J. Dairy Sci.* 92:3081-3090.

CHAPTER 5

Relationships between uterine health and metabolism in dairy cows with different dry period lengths

**J. Chen,* N. M. Soede,* G. J. Remmelink, ‡ R. M. Bruckmaier, § B.
Kemp,* A. T. M. van Knegsel***

*Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands; ‡Livestock Research, Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, the Netherlands. §Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland.

Submitted (2016)

5.1 Abstract

The first objective of this study was to evaluate effects of dry period (**DP**) length and dietary energy source on ovarian activity, uterine health status, pregnancy rate, and days open in dairy cows in the second subsequent lactation after implementation of DP length and dietary treatments. The second objective was to determine relationships of uterine health status with ovarian activity, milk yield, energy balance (**EB**), and metabolic status in dairy cows. Holstein-Friesian dairy cows ($n = 167$) were assigned randomly to 1 of 3 DP lengths (0, 30, or 60 d) and 1 of 2 early lactation diets (glucogenic and lipogenic diet) for 2 subsequent lactations. Cows were fed a glucogenic or lipogenic diet from 10 d before the expected calving date until 100 d after calving. Results of the first lactation were reported previously. Milk samples were collected three times a week until 100 DIM postcalving for determination of ovarian activity. Vaginal discharge was scored in week 2 and 3 after calving to evaluate uterine health status and cows were classified as having a healthy uterine environment [**HU**, vaginal discharge score (**VDS**) = 0 or 1 in both week 2 and 3], a recovering uterine environment (**RU**, VDS = 2 or 3 in week 2 and VDS = 0 or 1 in week 3), or a non-recovering uterine environment (**NRU**, VDS = 2 or 3 in week 3). Cows were monitored for milk yield, dry matter intake (**DMI**), and blood was sampled weekly from calving to week 3 postcalving to determine EB and metabolic status. Dry period length was not related with uterine health status in early lactation, ovarian activity variables, pregnancy rate, or days open in dairy cows. Independent of DP length, cows fed a glucogenic diet had earlier onset of luteal activity, and lower overall pregnancy rate compared with cows fed a lipogenic diet. In the first 3 weeks after calving, cows with a NRU had lower milk yield and lower DMI than cows with a HU or RU. Cows with a RU had lower plasma glucose and insulin concentrations than cows with a NRU or HU. In conclusion, DP length did not influence fertility measures and uterine health status in the second subsequent lactation after implementation of DP length and dietary treatments. Independent of DP length, feeding a glucogenic diet shortened the interval from calving to first ovulation but decreased overall pregnancy rate compared with a lipogenic diet. In addition, the uterine health status was related to milk yield and metabolic status, independent of DP length.

Keywords: continuous milking, progesterone, uterine health, lipogenic nutrient, glucogenic nutrient

5.2. Introduction

In modern high-producing dairy cows, low fertility is a substantial concern (Lucy, 2001). Recently, several studies reported that shortening the conventional dry period (**DP**) of 60 d to 30 d or omitting the DP completely could improve fertility in dairy cows by improving the resumption of ovarian cyclicity (Chen et al., 2015b), decreasing the interval from calving to first ovulation (Watters et al., 2009), and decreasing days open (Gümen et al., 2005) in subsequent lactation. The beneficial effects of shortening or omitting the DP on fertility were mainly related to a reduced milk yield in the subsequent lactation. Lower milk yield resulted in improved energy balance (**EB**) and improved metabolic status (Gümen et al., 2005; Chen et al., 2015b). For application of this DP management strategy in practice, it is necessary to determine the long-term effects of a short or no DP on performance, health and fertility in dairy cows. Previously, we found that milk yield was increased and the improvement of EB and metabolic status in early lactation was less pronounced in the second subsequent lactation after implementation of the DP length treatments compared with the first subsequent lactation (Chen et al., 2016). Because of the reduced improvement of EB, it can be hypothesized that the beneficial effects of shortening or omitting the DP on fertility is also less pronounced in the second subsequent lactation after implementation of the DP length treatments compared with the first subsequent lactation.

In the first subsequent lactation after implementation of the DP length and dietary treatments, feeding a glucogenic diet improved EB after a short DP (Van Knegsel et al., 2014) and to decrease plasma BHB concentration (Chen et al., 2015a), but had no influence on fertility (Chen et al., 2015b) compared with a lipogenic diet. In the second subsequent lactation after implementation of the DP length treatments, feeding a glucogenic diet considerably improved metabolic status in early lactation of the second subsequent lactation compared with a lipogenic diet, as indicated by greater plasma glucose, insulin-like growth factor-1 (**IGF-1**), and insulin concentrations, and lower plasma free fatty acids (**FFA**) and β -hydroxybutyrate (**BHB**) concentrations. Increased plasma insulin and IGF-1 concentrations have been positively associated with the growth and maturation of follicles (Lucy, 2000; Gong et al., 2002). Thus, it may be expected that feeding a glucogenic diet improves fertility of cows in the second subsequent lactation compared with a lipogenic diet.

In this study, cows were followed for 2 subsequent lactations with same DP management strategy and dietary strategy in both lactations. Results of the first subsequent lactation were reported before on EB (Van Knegsel et al., 2014), metabolic hormones (Chen et al., 2015a), and fertility (Chen et al., 2015b). In the second subsequent lactation after implementation of DP length and dietary treatments, we additionally evaluated uterine health status, as reflected by vaginal discharge. Vaginal discharge is related to the bacterial load in the uterine lumen (Williams et al., 2005) and is widely used to diagnose clinical endometritis in dairy cows (LeBlanc et al., 2002; Prunner et al., 2014). Clinical endometritis compromises reproductive performance as it is related with lower pregnancy rates, more services per pregnancy, and more days open (Kasimanickam et al., 2004). Relationships of uterine health status with ovarian activity, energy balance, and metabolic status in early lactation are not entirely clear. The primary objective of this study was to evaluate effects of DP length and dietary energy source on ovarian activity, uterine health status, pregnancy rate, and days open in dairy cows in the second subsequent lactation after implementation of DP length and dietary treatments. The second objective was to determine relationships of uterine health status with ovarian activity, energy balance, milk yield, and metabolic status in dairy cows.

5.3. Material and Methods

5.3.1. Animals and Experimental Design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. The registration number of the experimental protocol is 2010026. A total of 167 Holstein-Friesian dairy cows (60 primiparous and 107 multiparous) were selected from the Dairy Campus Research dairy herd (WUR Livestock Research, Lelystad, the Netherlands). The experimental design, DP lengths, and dietary contrast have been reported previously (Van Knegsel et al., 2014). Briefly, dairy cows were blocked by parity, expected calving date, milk yield in previous lactation, and BCS. Within blocks, cows were assigned randomly to a 3 x 2 factorial design with 3 DP lengths (0, 30, or 60 d) and 2 early lactation diets (glucogenic or lipogenic). Cows with a 30 or 60 d DP were treated with an intramammary antibiotic at drying off (Supermastidol, Virbac Animal Health, Barneveld, the Netherlands). Cows with a 0 d DP were milked twice daily until

calving. Before calving, dry cows were fed a dry cow diet, while lactating cows were fed a lactation diet supporting 25 kg of milk. From 10 d before the expected calving date, cows were fed 1 kg/d of a glucogenic or lipogenic concentrate. From calving date until 100 DIM, the concentrates supply increased stepwise by 0.5 kg/d until concentrates supply reached 8.5 kg/d. After 100 DIM, all cows were fed the standard lactation concentrate. Details on diet composition were presented earlier (Van Knegsel et al., 2014). The main ingredient for the glucogenic concentrate was corn and the main ingredients for the lipogenic concentrate were sugar beet pulp, palm kernel, and rumen protected palm oil. Forage was offered ad libitum throughout the study, comprising of grass silage, corn silage, wheat straw, and rapeseed meal or soybean meal (51:34:2:13, DM basis). Diets were formulated to be isocaloric (net energy basis; Dutch net energy evaluation (**VEM**) system (Van Es, 1975) and equal in intestinal digestible protein and degraded protein balance (**DVE/OEB**) system (Tamminga et al., 1994). Cows were housed in a free stall with a slatted floor and cubicles, and were milked twice daily (at 0500 and 1630 h).

Cows were planned to have the same DP length and dietary treatments over 2 subsequent lactations. Results of the first subsequent lactation have been reported on EB and milk yield (Van Knegsel et al., 2014), metabolites and metabolic hormones (Chen et al., 2015a), and fertility (Chen et al., 2015b). After the first subsequent lactation, 20 cows with a 0 d DP, 15 cows with a 30 d DP, and 6 cows with a 60 d DP were excluded from experiment because of fertility or health problems. The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

5.3.2. Reproductive Management

The voluntary waiting period was 50 d. Estrus detection was based on Lely Qwes-HR Activity Tags (Lely, Maassluis, the Netherlands) mounted on the neck collar of dairy cows. Activity data were recorded in a 2 h interval and artificial insemination (**AI**) was performed every 12 h after detection of oestrus. Pregnancy

was confirmed by ultrasonography around 60 d after AI. First-service conception rate was determined by the number of cows diagnosed pregnant after first AI divided by the number of cows inseminated. The overall pregnancy rate was determined by the number of pregnant cows divided by the number of cows inseminated during whole lactation.

5.3.3. Milk Yield, Body Weight, and Energy Balance

Milk yield and feed intake were recorded daily (Insentec BV, Marknesse, the Netherlands) per cow and averaged per week during the first 3 weeks after calving. Body weight was recorded after each milking and averaged per week during the first 3 weeks after calving. Energy balance was calculated per week according to the VEM system (Van Es, 1975; CVB, 2005) as the difference between VEM supplied with feed and VEM required for maintenance and milk yield.

5.3.4. Milk Sampling and Progesterone Assay

Milk samples were collected three times a week on Monday, Wednesday, and Friday during morning milking until 100 DIM and stored at -20°C . Milk progesterone (**P4**) concentration was measured using a commercial enzyme-linked immunosorbent assay kit (Ridgeway Science Ltd, Gloucestershire, UK). The mean intra- and inter-assay coefficients of variation were 5.1% and 8.5%, respectively. The sensitivity of the assay was 0.1 ng/mL. Two or more succeeding milk samples with concentration of $\text{P4} \geq 2 \text{ ng/mL}$ indicated the onset of luteal activity. Ovarian cycle length was defined as the interval between onset of luteal activity in one ovarian cycle and onset of luteal activity in the next ovarian cycle. Cows with incomplete P4 data within 100 DIM were excluded for ovarian activity analysis (4 cows with a 0 d DP, 5 cows with a 30 d DP, 5 cows with a 60 d DP, and 1 cows with a 0→67d DP). All cows were examined for ovarian cysts monthly from week 4 after calving, and cows treated for ovarian cysts (Fertagyl, Intervet Inc., Millsboro, DE, USA) were included in the analysis until the time of treatment.

5.2.5. Definitions of Normal and Abnormal Resumption of Ovarian Cyclicity

The definitions of normal and abnormal resumption of ovarian cyclicity have been reported previously (Chen et al., 2015b). Briefly, the resumption of ovarian cyclicity for each cow within 100 DIM after calving was classified into one

of four categories based on the P4 profile according to (Pushpakumara et al., 2003): (1) Normal resumption of ovarian cyclicity: onset of luteal activity (**OLA**) occurring at 45 DIM or less, followed by regular ovarian cycles of 18 to 24 d in length. (2) Delayed first ovulation or anovulation: OLA occurring at more than 45 DIM post-calving. (3) Prolonged luteal phase: OLA occurring at 45 DIM or less, but is followed by one or more ovarian cycles with length of luteal phase for 20 d or more; (4) Cessation of cyclicity: absence of luteal activity for more than 14 d between two ovarian cycles. Cows that experienced more than one abnormal resumption type were classified according to the first type to occur post-calving. Cows treated with Fertagyl within 100 DIM that prohibited evaluation of resumption of ovarian cyclicity were excluded from analysis of resumption of ovarian cyclicity.

5.2.6. Vaginal Discharge Collection and Scoring

Vaginal discharge was scored in week 2 and 3 after calving from 98 cows for the assessment of the uterine environment according to (Williams et al., 2005). Vaginal discharge was scored based on a 4-point scoring system: score 0 = clear translucent mucus, score 1 = clear translucent mucus with discrete flecks of purulent exudate present, score 2 = discharge containing < 50% white or off-white mucopurulent material, and score 3 = discharge containing > 50% purulent material. The uterine health status was defined based on the vaginal discharge score: (1) healthy uterine environment (**HU**): vaginal discharge score = 0 or 1 in both week 2 and 3 (n = 19). (2) Recovering uterine environment (**RU**): vaginal discharge score = 2 or 3 in week 2 and vaginal discharge score = 0 or 1 in week 3 (n = 32). (3) Non-recovering uterine environment (**NRU**): vaginal discharge score = 0 or 1 in week 2 and vaginal discharge score = 2 or 3 in week 3 (n = 5), or vaginal discharge score = 2 or 3 in both week 2 and 3 (n = 42).

5.3.7. Blood Sampling and Analysis

Blood sampling and analysis have been reported previously (Chen et al., 2015a). Briefly, blood samples were taken weekly from calving to week 3 after calving. Concentrations of glucose and urea were measured using commercial kits no. 61269 and no. 61974 from Bio. Mérieux (Marcy l'Etoile, France). 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). Insulin-like growth factor-1 and insulin were measured using kit no. A15729 from Beckman Coulter (Fullerton, CA, USA) and kit no. PI-12K from EMD Millipore Corporation (Billerica, MA, USA), respectively.

5.3.8. Statistical Analysis

In the current study, the number of cows per treatment is shown in Table 5.1. Firstly, the LOGISTIC procedure of SAS (Version 9.2; SAS Institute, Inc., Cary, NC) was used to analyse resumption of ovarian cyclicity, uterine health status, first-service conception rate, overall pregnancy rate, and Fertagyl use (model 1). The MIXED procedure of SAS was used to analyse days to OLA, luteal phase length, cycle length, and maximal and mean P4 concentrations, calving to first AI, days open, and services per pregnancy (model 2). Fixed effects in model 1 and 2 were DP length (0, 30, 60, or 0→67 d), diet (glucogenic or lipogenic), parity (3 or >3), and their 2 way interactions.

Table 5.1. Distribution of cows per dry period length and diet.

	Dry period length ¹ (d)				Diet ²		Total
	0	30	60	0→67	G	L	
Cows, n							
Cows in experiment	20	41	50	19	66	64	130
- Cows with complete P4 profile within 100 DIM	16	36	45	18	58	57	115
- Cows with onset of luteal activity	15	30	36	18	53	46	99
- Cows with first AI	18	37	45	18	62	56	118
- Cows subjected to vaginal discharge sampling	13	33	38	14	48	50	98

¹The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

²G = glucogenic diet; L = lipogenic diet.

Secondly, cows were classified based on uterine health status (HU, RU, or NRU). The LOGISTIC procedure of SAS was used to analyse the relation between uterine health status and resumption of ovarian cyclicity (model 3). The MIXED procedure of SAS was used to analyse the relation of uterine health status with days

to OLA, luteal phase length, cycle length, and maximal and mean P4 concentrations (model 4). Fixed effects in model 3 and 4 were uterine health status, DP length, diet, parity, and interactions of uterine health status with other fixed effects.

Thirdly, the MIXED procedure of SAS with repeated measures analysis was used to analyse the relation of uterine health status with milk yield, DMI, EB, plasma metabolites and hormones (model 5). The cow was considered as the repeated subject. A first-order autoregressive structure [AR(1)] was the best fit and was used to account for within-cow variation. Fixed effects in model 5 were uterine health status, DP length, diet, parity, weeks after calving (week 1, 2, and 3), and interactions of uterine health status with other fixed effects.

Preliminary analyses showed that all interactions were nonsignificant ($P > 0.05$) in all models, and were therefore excluded. For comparison of DP lengths and uterine health status, P -values are presented after a Tukey's adjustment. Values are presented as least squares means and standard error of the means. Differences are considered significant if $P < 0.05$, and trends are discussed if $P < 0.10$.

5.4. Results

5.4.1. Effects of Dry Period Length and Diet on Resumption of Ovarian Cyclicity, Uterine Health Status, Characteristics of Ovarian Activities, and Reproductive Performance

In the second subsequent lactation after implementation of DP length and dietary treatments, resumption of ovarian cyclicity in dairy cows was not affected by DP length or diet (Table 5.2). Uterine health status, characteristics of ovarian cycles, and reproductive performance were not affected by DP length (Table 5.3). Cows fed a glucogenic diet had earlier OLA, and lower overall pregnancy rate compared with cows fed a lipogenic diet. Uterine health status was not affected by diet.

5.4.2. Effects of Uterine Health Status on Resumption of Ovarian Cyclicity and Characteristics of Ovarian Cycles

Cows with a HU (0.0%) tended to have a lower ($P = 0.06$) incidence

Table 5.2. Incidence of normal or abnormal resumption of ovarian cyclicity within 100 DIM for cows after 0, 30, 60, or 0→67¹ days dry period and fed a glucogenic (G) or lipogenic (L) diet within 100 DIM of the second subsequent lactation.

Variable	Dry period length (d)					Diet			P-value	
	0	30	60	0→67		G	L		DPL ²	Diet
Normal resumption of ovarian cyclicity (%)	42.9 (6/14)	32.1 (9/28)	37.8 (14/37)	54.5 (6/11)		40.0 (17/46)	40.9 (18/44)		0.59	0.57
Abnormal resumption of ovarian cyclicity:										
Type I: delayed first ovulation or anovulation (%)	7.1 (1/14)	28.6 (8/28)	29.7 (11/37)	9.1 (1/11)		17.4 (8/46)	29.6 (13/44)		0.29	0.21
Type II: prolonged luteal phase (%)	35.7 (5/14)	35.7 (10/28)	27.0 (10/37)	36.4 (4/11)		37.0 (17/46)	27.3 (12/44)		0.88	0.33
Type III: cessation of cyclicity (%)	14.3 (2/14)	3.6 (1/28)	5.4 (2/37)	0.0 (0/11)		8.7 (4/46)	2.3 (1/44)		0.53	0.15
Fertagyl use ³ (%)	12.5 (2/16)	22.2 (8/36)	17.8 (8/45)	38.9 (7/18)		20.7 (12/58)	22.8 (13/57)		0.16	0.59
									0.86	

^{a,b} Values within dry period length in the same row with different superscripts differ ($P < 0.05$).¹The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.²DPL = dry period length.³Cows treated with Fertagyl within 100 DIM that prohibited evaluation of resumption of ovarian cyclicity.

Table 5.3. Uterine health status¹, characteristics of ovarian cycle, and reproductive performance of dairy cows after 0, 30, 60, or 0→67² days dry period and fed a glucogenic (G) or lipogenic (L) diet (LSM ± SEM).

Variable	Dry period length (d)				SEM	Diet		SEM	P-value		
	0	30	60	0→67		G	L		DPL ³	Diet	Parity
Uterine health status											
Healthy (%)	30.8 (4/13)	15.2 (5/33)	21.1 (8/38)	14.3 (2/14)		25.0 (12/48)	14.0 (7/50)		0.67	0.17	0.77
Recovering (%)	15.4 (2/13)	33.3 (11/33)	39.5 (15/38)	28.6 (4/14)		33.3 (16/48)	32.0 (16/50)		0.55	0.80	0.47
Non-recovering (%)	53.8 (7/13)	51.5 (17/33)	39.5 (15/38)	57.1 (8/14)		41.7 (20/48)	54.0 (27/50)		0.63	0.19	0.68
First ovarian cycle											
Calving to onset of luteal activity (days)	27.2	28.8	27.8	28.4	2.9	25.3	31.0	2.1	0.98	0.04	0.08
Luteal phase length (days)	16.6	17.1	17.6	16.3	2.2	16.2	17.6	1.5	0.97	0.51	0.74
Ovarian cycle length (days)	27.2	25.4	24.1	26.3	2.5	24.7	26.9	1.7	0.82	0.35	0.69
P4 _i max ⁴ (ng/mL)	15.81	15.69	14.38	18.81	2.20	16.18	16.16	1.56	0.56	0.99	0.27
P4 _i mean ⁵ (ng/mL)	7.65	7.66	6.81	9.35	1.00	7.86	7.90	0.70	0.38	0.97	0.12
Fertagyl use ⁶ (%)	6.2 (1/16)	13.9 (5/36)	8.9 (4/45)	0.0 (0/18)		5.4 (3/58)	12.3 (7/57)		0.67	0.52	0.36

¹Healthy: vaginal discharge score = 0 or 1 in both week 2 and 3; Recovering: vaginal discharge score = 2 or 3 in week 2 and vaginal discharge score = 0 or 1 in week 3; Non-recovering: vaginal discharge score = 2 or 3 in week 3. (Continued on the next page)

Table 5.3 continued

Variable	Dry period length (d)				SEM	Diet		SEM	P-value	
	0	30	60	0→67		G	L		DPL ³	Diet
Reproductive performance										
Calving to first AI (days)	71.6	88.9	86.2	82.0	5.7	78.5	85.9	3.9	0.19	0.17
First-service conception rate ⁷ (%)	23.1 (3/18)	32.3 (10/37)	24.3 (9/45)	35.7 (5/18)		26.7 (12/62)	30.0 (15/56)		0.73	0.29
Overall pregnancy rate ⁸ (%)	72.2 (13/18)	83.8 (31/37)	82.2 (37/45)	77.8 (14/18)		72.6 (45/62)	89.3 (50/56)		0.69	0.03
days open (days)	144.5	147.3	142.2	146.1	14.3	145.9	144.1	9.7	0.99	0.89
Services per pregnancy	3.1	2.6	2.7	3.0	0.4	3.0	2.7	0.3	0.79	0.50
Fertagyl use ⁹ (%)	22.2 (4/18)	45.9 (17/37)	40.0 (18/45)	44.4 (8/18)		32.3 (20/62)	48.2 (27/56)		0.33	0.07

²The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

³DPL = dry period length.

⁴Maximal P4 concentration.

⁵Mean P4 concentration.

⁶Cows treated with Fertagyl before ovulation within 100 DIM.

⁷Number of cows diagnosed pregnant after first AI/ number of cows inseminated.

⁸Number of cows diagnosed pregnant during whole lactation / number of cows inseminated during whole lactation.

⁹Cows treated with Fertagyl during whole lactation.

of prolonged luteal phase compared with cows with a RU (44.0 %) or NRU (34.3 %, Table 5.4). In addition, cows with a HU had lower mean P4 concentration and tended to have lower maximal P4 concentration compared with cows with a RU or NRU ($P = 0.07$).

5.4.3. Relationships of Uterine Health Status with Milk Yield, Dry Matter Intake, Energy Balance, Metabolites and Hormones

Cows with a HU or RU had greater milk yield and greater DMI in the first 3 weeks after calving compared with cows with a NRU (Table 5.5). Cows with a HU or NRU had greater plasma glucose concentration compared with cows with a RU. Cows with a HU had greater plasma insulin concentration and tended to ($P = 0.05$) have greater plasma urea concentration compared with cows with a RU.

5.5. Discussion

This is the first study reporting effects of omitting or shortening the DP on fertility in the second lactation in dairy cows. In the current study, DP length did not influence the resumption of ovarian cyclicity and reproductive performance in the second subsequent lactation after implementation of DP length treatments. This result is inconsistent with the findings in the first subsequent lactation of the current experiment (Chen et al., 2015b), in which omitting the DP increased the incidence of normal resumption of ovarian cyclicity and tended to reduce interval from calving to OLA. Also, Gümen et al. (2005) and Watters et al. (2009) reported that omitting or shortening the DP led to an earlier ovulation after calving in the first subsequent lactation after implementation of DP length treatments. In addition, cows with an omitted or shortened DP had a greater first-service conception rate, less services per pregnancy, and shorter days open compared with cows with a conventional DP in the first subsequent lactation (Gümen et al., 2005; Watters et al., 2009). These beneficial effects of omitting or shortening the DP on resumption of ovarian cyclicity and reproductive performance in cows were related to improved EB and metabolic status in early lactation (Gümen et al., 2005; Watters et al., 2009; Chen et al., 2015b). In the second subsequent lactation, however, omitting the DP resulted in a less pronounced improvement of EB and metabolic status in early lactation compared with the first subsequent lactation (Chen et al., 2016), which could at least partly explain the disappearance of effects

Table 5.4. Incidence of normal or abnormal resumption of ovarian cyclicity and characteristics of the ovarian cycle within 100 DIM of dairy cows categorised according to uterine health status in week 2 and 3 after calving (LSM $\pm$ SEM).

Variable	Uterine health status ¹			SEM	P-value		
	HU	RU	NRU		Uterine health status	DPL ²	Diet Parity
Resumption of ovarian cyclicity							
Normal resumption of ovarian cyclicity (%)	57.1 (8/14)	36.0 (9/25)	28.6 (10/35)		0.12	0.86	0.28 0.21
Abnormal resumption of ovarian cyclicity:							
Type I: delayed first ovulation or anovulation (%)	21.4 (3/14)	16.0 (4/25)	37.1 (13/35)		0.10	0.22	0.31 0.68
Type II: prolonged luteal phase (%)	0.0 (0/14)	44.0 (11/25)	34.3 (12/35)		0.06	0.69	0.19 0.83
Type III: cessation of cyclicity (%)	21.4 (3/14)	4.0 (1/25)	0.0 (0/35)		0.12	0.31	0.10 0.07
Fertagyl use ³ (%)	17.6 (3/17)	21.9 (7/32)	22.2 (10/45)		0.96	0.46	0.32 0.65
First ovarian cycle							
Cows, n	14	30	40				
Cows with onset of luteal activity, n	13	29	36				
Days to onset of luteal activity (days)	27.7	25.8	32.7	3.2	0.16	0.79	0.07 0.20
First luteal phase length (days)	12.7	18.3	18.7	2.2	0.16	0.98	0.70 0.17
First ovarian cycle length (days)	22.4	28.9	28.5	2.6	0.20	0.63	0.63 0.25
P4, max ⁴ (ng/mL)	10.4	18.5	16.1	2.2	0.07	0.91	0.98 0.41
P4, mean ⁵ (ng/mL)	5.3 ^b	9.3 ^a	7.6 ^{ab}	1.0	0.03	0.71	0.96 0.35
Fertagyl use ⁶ (%)	17.6 (3/17)	6.3 (2/32)	11.1 (5/45)		0.98	0.74	0.61 0.40

^{a,b} Values within uterine health status with different superscripts differ ($P < 0.05$). (Continued on the next page)

of DP length on fertility in the second subsequent lactations after implementation of DP length treatments.

The less pronounced improvements of EB and metabolic status were caused by increased milk yield and decreased DMI in the second subsequent lactation after omitting the DP compared with the first subsequent lactation. The increased milk yield could be related to increased parity in cows with an omitted DP in the second subsequent lactation (Chen et al., 2016). The decreased DMI could be related to the high BCS at the onset of the second subsequent lactation caused by the lower milk yield but similar energy intake in cows with an omitted DP in the first subsequent lactation. Thus, it can be hypothesized that when dietary energy intake is reduced and fattening is limited during mid and late lactation of the first subsequent lactation, an improvement in fertility after omitting the DP could be expected also in the second subsequent lactation after implementation of DP length treatment.

Independent of DP length, a glucogenic diet decreased interval from calving to first ovulation, as indicated by a shorter interval from calving to OLA. Previously, we found that feeding a glucogenic diet considerably improved metabolic status during the first 8 weeks after calving in the second subsequent lactation, as indicated by increased plasma concentrations of IGF-1, insulin, and glucose (Chen et al., 2016). Gong et al. (2002) reported that high starch diet increased plasma insulin concentration and reduced the intervals from calving to first ovulation. Those authors proposed that the increased insulin improved maturation of dominant follicles and increased the chance of these dominant follicles ovulating (Gong et al., 2002). Also, Lucy (2000) suggested that IGF-1 act synergistically with gonadotropins to promote growth and differentiation of follicles. The greater plasma concentrations of insulin and IGF-1 in cows fed a

Table 5.4 continued

¹Healthy uterine environment (HU): vaginal discharge score (VDS) = 0 or 1 in both week 2 and 3; recovering uterine environment (RU): VDS = 2 or 3 in week 2 and vaginal discharge score = 0 or 1 in week 3; non-recovering uterine environment (NRU): VDS = 2 or 3 in week 3.

²DPL = dry period length.

³Cows treated with Fertagyl within 100 DIM that prohibited evaluation of resumption of ovarian cyclicity.

⁴Maximal P4 concentration during first ovarian cycle.

⁵Mean P4 concentration during first ovarian cycle.

⁶Cows treated with Fertagyl before ovulation within 100 DIM.

Table 5.5. Milk yield, DMI, energy balance, metabolites and hormones¹ of dairy cows categorised according to uterine health status (LSM $\pm$ SEM).

Variable	Uterine health status ²			SEM	<i>P</i> -value				
	HU	RU	NRU		Uterine health	DPL ³	Diet	Parity	Week
Cows, n	19	32	47						
Milk yield (kg/d)	36.8 ^a	36.8 ^a	32.4 ^b	0.8	<0.01	<0.01	0.67	0.34	<0.01
DMI (kg/d)	18.0 ^a	17.5 ^a	16.1 ^b	0.4	<0.01	0.07	0.82	0.48	<0.01
EB ⁴ (kJ/kg ^{0.75} .d)	-410	-448	-388	31	0.26	0.02	0.80	0.90	<0.01
Cows, n	17	26	43						
FFA (mmol/L)	0.56	0.66	0.57	0.05	0.15	0.11	0.98	0.02	<0.01
BHB ⁵ (mmol/L)	0.92	1.07	0.86	0.16	0.17	0.11	0.05	0.09	<0.01
Glucose (mmol/L)	3.34 ^a	2.87 ^b	3.16 ^a	0.09	<0.01	0.53	0.04	0.51	<0.01
Urea (mmol/L)	2.88	2.48	2.56	0.11	0.05	0.02	0.45	0.49	<0.01
IGF-1 (ng/mL)	57.53	46.48	50.94	4.39	0.24	0.25	0.19	0.43	<0.01
Insulin (μ IU/mL)	9.22 ^a	6.82 ^b	8.51 ^{ab}	0.66	0.03	0.18	0.11	0.09	0.02

^{a,b} Values within uterine health status with different superscripts differ ($P < 0.05$).

¹Milk yield, DMI, energy balance, plasma concentrations of FFA, BHBA, Glucose, IGF-1, or insulin were averaged for the first 3 week in the second subsequent lactation after implementation of dry period treatments.

²Healthy uterine environment (HU): vaginal discharge score = 0 or 1 in both week 2 and 3; recovering uterine environment (RU): vaginal discharge score = 2 or 3 in week 2 and vaginal discharge score = 0 or 1 in week 3; non-recovering uterine environment (NRU): vaginal discharge score = 2 or 3 in week 3.

³DPL = dry period length.

⁴Energy balance; calculated with the Dutch net energy evaluation (VEM) system (Van Es, 1975).

⁵Analyses were based on natural log-transformed data; back-transformed data are presented.

glucogenic diet as reported in Chen et al. (2016) may explain why cows fed a glucogenic diet had an earlier OLA compared with cows fed a lipogenic diet in the second subsequent lactation.

In spite of the earlier onset of luteal activity in cows fed a glucogenic diet, cows fed a glucogenic diet had lower overall pregnancy rate compared with cows fed a lipogenic diet (72.6 vs. 89.3%, $P = 0.03$). Diet had no effects on first-service conception rate, days open, and services per pregnancy in the current study. These

results are not in line with previous studies reporting that cows with an early first ovulation had more ovulation cycles before first AI (Butler and Smith, 1989), greater first-service conception rate (Galvão et al., 2010b), and fewer number of services per pregnancy (Lucy et al., 1992). Garnsworthy et al. (2009) suggested that high insulin concentration can be beneficial for the resumption of estrus cycles after calving, but might have detrimental effects on oocyte developmental competence. Fouladi-Nashta and Campbell (2006) reported that high concentration of insulin reduced cleavage rate in oocytes. In addition, it was suggested that fat supplementation increases plasma concentration of P4, reduces secretion of prostaglandin metabolites, and, as a consequence, increases pregnancy rate in dairy cows (reviewed by Staples et al. (1998)). In the current study, it is possible that feeding a glucogenic diet that stimulated greater plasma insulin concentration in early lactation resulted in an earlier ovulation, but had an adverse effect on pregnancy rate during the breeding period compared with a lipogenic diet that increased fatty acid supply.

In the current study, DP length or dietary energy source had no effects on uterine health status, which was reflected by VDS in week 2 and 3 post-calving. Risk factors for purulent vaginal discharge were twins, dystocia, and metritis (Dubuc et al., 2010). Neither dry period length nor dietary energy source influenced the incidence of twins, dystocia, and metritis in the current study (data not shown), which may explain the lack of effect of DP length on uterine health status. To analyse the relation of uterine health status with resumption of ovarian cyclicity, DMI, milk yield, EB, and metabolic status, cows were classified based on uterine health status. In the current study, there were no cows with a HU that had a prolonged luteal phase within 100 DIM, compared with 44.0% and 34.3 % of cows with a RU or NRU, respectively. Similarly, Shrestha et al. (2004) demonstrated that cows with uterine infection, as indicated by delayed uterine involution and abnormal vaginal discharge, had high incidence of prolonged luteal phase. Prolonged luteal phase is one of the most common ovarian disturbance and adversely affects fertility by reducing pregnancy rate and extending days open (Shrestha et al., 2004; Ranasinghe et al., 2011). In cows with uterine infections, high concentration of lipopolysaccharide in peripheral plasma stimulates the secretion and accumulation of prostaglandin E₂ (PGE) rather than prostaglandin F_{2α} (PGF_{2α}), because PGE plays a key role to control inflammation in dairy cows

(Sheldon et al., 2009). This switch of endometrial production from luteolytic $\text{PGF}_{2\alpha}$ to luteotropic PGE in dairy cows may cause delayed luteolysis and, therefore, prolongs the luteal phase (Sheldon et al., 2009).

Independent of DP length, cows with a NRU had lower milk yield compared with cows with a HU or RU in the first 3 weeks after calving. The decreased milk yield may be caused by the reduced DMI (McDougall et al., 2011), which could be related to persistent inflammation in cows with a NRU. The increased proinflammatory cytokines (e.g. interleukin- 1β , interleukin-6, and tumor necrosis factor α) during inflammation have been shown to reduce the DMI (Johnson and Finck et al., 2001). In addition, Urton et al. (2005) also found that cows with metritis spent less time feeding and had lower DMI. Due to the decreased DMI and milk yield, cows with a NRU had similar EB and metabolic status compared with cows with a HU. These results confirm the hypothesis that resumption of ovarian cyclicity is not only related to EB and metabolic status, but also related to other factors, such as uterine infection (Chen et al., 2015b). Additionally, cows with a HU and RU had similar level of milk yield and DMI, but cows with a HU had better metabolic status compared with cows with a RU, as indicated by greater plasma concentrations of glucose and insulin. The lower plasma glucose concentration in cows with a RU may be related to an impaired bacterial clearance capacity of polymorphonuclear neutrophils (**PMN**) because of a decrease in PMN glycogen concentration (Galvão et al., 2010a).

Sheldon et al. (2006) suggested that examination for clinical endometritis based on purulent vaginal discharge should be at 21 d or later after calving, and classifying cows as having clinical endometritis before 21 d may reflect the disease less accurately because cows may spontaneously solve the bacterial contamination. In the current study, a group of cows was recovered from purulent vaginal discharge within the first 3 weeks in lactation (cows with a HU or RU), which was related with higher milk yield and DMI compared with cows that did not recover within the first 3 weeks in lactation (cows with a NRU). In addition, cows with a HU tended to have lower incidence of prolonged luteal phase compared with cows with a RU or NUR. These findings imply that vaginal discharge within the first 3 weeks in lactation could not only be a possible indicator for fertility, and also for milk production performance in dairy cows.

5.6 Conclusions

Shortening or omitting the DP did not improve uterine health status in early lactation or fertility in dairy cows compared with a conventional DP in the second subsequent lactation after implementation of DP length and dietary treatments. Independent of DP length, feeding a glucogenic diet shortened the interval from calving to first ovulation, but decreased overall pregnancy rates compared with a lipogenic diet. During the first 3 weeks after calving, a non-recovering uterine environment, indicated by vaginal discharge score in week 2 and 3, was related to a decreased DMI and milk yield, and a healthy uterine environment was related to high milk yield, better metabolic status, and low incidence of prolonged luteal phase. It implies that determination of uterine health status based on vaginal discharge score within 3 weeks in lactation could be an indicator for both fertility and milk production performance of dairy cows in early lactation.

5.7 Acknowledgements

The authors thank the Dutch Dairy Board (PZ, the Netherlands), Product Board Animal Feed (PDV, the Netherlands), and CRV (the Netherlands) for financing the experiment. The authors would like to thank the Chinese Scholarship Council (CSC, China) for financial support. Additionally, the authors thank Renske Overeem, Kristina Simon, Ger de Vries Reilingh, the staff of the Dairy Campus (Lelystad, the Netherlands), and the Veterinary Physiology Group of the University of Bern (Switzerland) for their technical support and laboratory analyses during the experiment.

References

- Butler, W. and R. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767-783.
- Centraal Veevoederbureau (CVB). 2005. Gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. CVB, Lelystad, the Netherlands.
- Chen, J., J. Gross, H. van Dorland, G. Rummelink, R. Bruckmaier, B. Kemp, and A. van Kneegsel. 2015a. Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation. *J. Dairy Sci.* 98:1033-1045.
- Chen, J., N. Soede, H. van Dorland, G. Rummelink, R. Bruckmaier, B. Kemp, and A. van Kneegsel. 2015b. Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths. *Theriogenology* 84:1387-1396.
- Chen, J., G. Rummelink, J. Gross, R. Bruckmaier, B. Kemp, and A. van Kneegsel. 2016. Effects of dry period length and dietary energy source on milk yield, energy balance, and metabolic status of dairy cows over 2 consecutive years: Effects in the second year. *J. Dairy Sci.*
- Dubuc, J., T. Duffield, K. Leslie, J. Walton, and S. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. *J. Dairy Sci.* 93:5764-5771.
- Fouladi-Nashta, A. and K. Campbell. 2006. Dissociation of oocyte nuclear and cytoplasmic maturation by the addition of insulin in cultured bovine antral follicles. *Reproduction* 131:449-460.
- Gümen, A., R. Rastani, R. Grummer, and M. Wiltbank. 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *J. Dairy Sci.* 88:2401-2411.
- Galvão, K., M. Flaminio, S. Brittin, R. Sper, M. Fraga, L. Caixeta, A. Ricci, C. Guard, W. Butler, and R. Gilbert. 2010a. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. *J. Dairy Sci.* 93:2926-2937.
- Galvão, K., M. Frajblat, W. Butler, S. Brittin, C. Guard, and R. Gilbert. 2010b. Effect of early postpartum ovulation on fertility in dairy cows. *Reprod. Domest. Anim.* 45:e207-e211.

- Garnsworthy, P., A. Fouladi-Nashta, G. Mann, K. Sinclair, and R. Webb. 2009. Effect of dietary-induced changes in plasma insulin concentrations during the early post partum period on pregnancy rate in dairy cows. *Reproduction* 137:759-768.
- Gong, J., W. Lee, P. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Reproduction* 123:419-427.
- Kasimanickam, R., T. Duffield, R. Foster, C. Gartley, K. Leslie, J. Walton, and W. Johnson. 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology* 62:9-23.
- LeBlanc, S., T. Duffield, K. Leslie, K. Bateman, G. P. Keefe, J. Walton, and W. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.* 85:2223-2236.
- Lucy, M., C. Staples, W. Thatcher, P. Erickson, R. Cleale, J. Firkins, J. Clark, M. Murphy, and B. Brodie. 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Anim. Prod.* 54:323-331.
- Lucy, M. 2000. Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in cattle. *J. Dairy Sci.* 83:1635-1647.
- Lucy, M. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *J. Dairy Sci.* 84:1277-1293.
- McDougall, S., H. Hussein, D. Aberdein, K. Buckle, J. Roche, C. Burke, M. Mitchell, and S. Meier. 2011. Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. *Theriogenology* 76:229-240.
- Prunner, I., H. Pothmann, K. Wagener, M. Giuliadori, J. Huber, M. Ehling-Schulz, and M. Drillich. 2014. Dynamics of bacteriologic and cytologic changes in the uterus of postpartum dairy cows. *Theriogenology* 82:1316-1322.
- Pushpakumara, P., N. Gardner, C. Reynolds, D. Beever, and D. Wathes. 2003. Relationships between transition period diet, metabolic parameters and fertility in lactating dairy cows. *Theriogenology* 60:1165-1185.

- Ranasinghe, R., T. Nakao, K. Yamada, K. Koike, A. Hayashi, and C. Dematawewa. 2011. Characteristics of prolonged luteal phase identified by milk progesterone concentrations and its effects on reproductive performance in Holstein cows. *J. Dairy Sci.* 94:116-127.
- Sheldon, I., G. Lewis, S. LeBlanc, and R. Gilbert. 2006. Defining postpartum uterine disease in cattle. *Theriogenology* 65:1516-1530.
- Sheldon, I., S. Price, J. Cronin, R. Gilbert, and J. Gadsby. 2009. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. *Reprod. Domest. Anim.* 44:1-9.
- Shrestha, H., T. Nakao, T. Higaki, T. Suzuki, and M. Akita. 2004. Resumption of postpartum ovarian cyclicity in high-producing Holstein cows. *Theriogenology* 61:637-649.
- Staples, C., J. Burke, and W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. *J. Dairy Sci.* 81:856-871.
- Tamminga, S., W. Van Straalen, A. Subnel, R. Meijer, A. Steg, C. Wever, and M. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. *Livest. Prod. Sci.* 40:139-155.
- Urton, G., M. Von Keyserlingk, and D. Weary. 2005. Feeding behavior identifies dairy cows at risk for metritis. *J. Dairy Sci.* 88:2843-2849.
- Van Es, A. 1975. Feed evaluation for dairy cows. *Livest. Prod. Sci.* 2:95-107.
- Van Knegsel, A., G. Remmelink, S. Jorjong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *J. Dairy Sci.* 97:1499-1512.
- Watters, R., M. Wiltbank, J. Guenther, A. Brickner, R. Rastani, P. Fricke, and R. Grummer. 2009. Effect of dry period length on reproduction during the subsequent lactation. *J. Dairy Sci.* 92:3081-3090.
- Williams, E., D. Fischer, D. Pfeiffer, G. England, D. Noakes, H. Dobson, and I. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* 63:102-117.

CHAPTER 6

Effects of dry period length and dietary energy source on lactation curve characteristics over two subsequent lactations

**J. Chen,* A. Kok *† G. J. Remmelink, ‡ J. J. Gross, § R. M. Bruckmaier,
§ B. Kemp,* A. T. M. van Knegsel***

*Adaptation Physiology Group, Department of Animal Science, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands; †Animal Production Systems Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands; ‡Livestock Research, Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, the Netherlands; §Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland.

Journal of Dairy Science (2016) 99:9287-9299

6.1 Abstract

This study aimed to evaluate the effects of dry period (**DP**) length and dietary energy source on lactation curve characteristics over 2 subsequent lactations. The second objective was to evaluate relationships of energy balance (**EB**) or metabolic status in early lactation with lactation curve characteristics in dairy cows. Holstein-Friesian dairy cows ($n = 167$) were assigned randomly to 1 of 3 DP lengths (0, 30, or 60 d) and 1 of 2 early lactation diets (glucogenic or lipogenic) for 2 subsequent lactations. In the second subsequent lactation following DP length treatments, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group when they had a milk yield of < 4 kg/d at least 30 d before expected calving date. Fat- and protein-corrected milk (**FPCM**) was calculated weekly per cow in both lactations. Blood samples were taken weekly from calving to week 4 after calving. A Wilmink lactation curve for each lactation was modeled based on FPCM yield records from calving through 305 days in milk (**DIM**). During the first subsequent lactation following DP length treatments, cows with a 0 or 30 d DP had lower peak yield (**PY**), later time of peak yield (**PT**), and lower FPCM_{305} than cows with a 60 d DP. In the second lactation, cows with a 0 or 30 d DP had lower PY than cows with a 60 d DP. In both lactations, lactation persistency (**LP**) was not affected by DP length and none of lactation curve characteristics were affected by diet. In older cows (parity > 2), total FPCM yield and average daily FPCM yield throughout 2 subsequent lactations did not differ among DP lengths. In addition, average EB in the first 4 weeks after calving had a negative relationship with PY and FPCM_{305} , and a positive relationship with LP. Average plasma concentrations of free fatty acids and β -hydroxybutyrate had a positive relationship with PY and FPCM_{305} , and a negative relationship with LP. In conclusion, shortening or omitting the DP affected lactation curve characteristics in both 2 subsequent lactations after implementation of DP length treatments. Considering 2 subsequent lactations after implementation of DP length treatments, shortening or omitting the DP did not compromise FPCM yield compared with a conventional DP of 60 d in older cows. In addition, severe negative EB and poor metabolic status were related with greater PY, FPCM_{305} , and lower LP.

Key words: dry period length, milk yield, lactation persistency, long-term effect

6.2 Introduction

A dry period (**DP**) of 6 to 8 weeks is an established practice to maximize milk yield in the subsequent lactation in most dairy farms. This conventional DP management has been applied since the beginning of the past century (Arnold and Becker, 1936), and is likely inappropriate for modern dairy cows because of increased milk production potential (Zobel et al., 2015). In recent years, interest in shortening length of the DP for modern dairy cows was increased. Although shortening the DP to about 30 d or omitting the DP improves energy balance (**EB**) (Rastani et al., 2005), metabolic status (Chen et al., 2015), and fertility (Watters et al., 2009) in the first subsequent lactation, the reduction in milk yield hinders the application of shortening or omitting the DP in practice. A meta-analysis including 22 studies showed that across studies the average milk loss was 4.5% after a shortened DP (28-35 d) and 19.1% after omitting the DP compared with a conventional DP (49-63 d) (van Knegsel et al., 2013). However, most of these studies only investigated effects of the short or omitted DP on milk yield in the first subsequent lactation immediately following the DP length treatments. It is important to determine the long-term effect of short or omitted DP on milk yield, because sustainable dairy farming implies long-living dairy cows with multiple lactations. In our previous study (Chen et al., 2016), we found that differences in milk yield between cows with an omitted DP and cows with a conventional DP were less pronounced in the first 9 weeks of the second subsequent lactation compared with the first subsequent lactation after implementation of DP length treatments (5.0 vs. 9.6 kg/d for the second vs. first lactation, respectively). Therefore, it is possible that omitting the DP for 2 subsequent lactations results in less 305-d milk yield losses in the second subsequent lactation compared with the first subsequent lactation following DP length treatments.

A lactation curve is a graphical representation of daily milk yield during a complete lactation and is widely used to determine the effects of management (Vance et al., 2012; Steeneveld et al., 2013) and metabolic disorders (Wittrock et al., 2011; Hostens et al., 2012) on milk yield. The shape of lactation curve is characterized by an increase from initial milk yield directly after calving to peak yield (**PY**), and a decrease after PY to the end of lactation (lactation persistency, **LP**). To our knowledge, only 2 studies are available concerning the effects of shortening or omitting the DP on lactation curve characteristics in the first

subsequent lactation, and the results are conflicting. Mantovani et al. (2010) reported that omitting the DP resulted in an earlier time of peak yield (**PT**), lower PY, and lower LP in a small study (n = 17). On the contrary, Atashi et al. (2013) found that a shortened DP (0 to 35 d) was associated with later PT, lower PY, and higher LP in a field study, compared with a conventional DP (50 to 60 d). In addition, both studies did not investigate effects of DP length on lactation curve characteristics in multiple lactations.

Recently, Hostens et al. (2012) showed in a field study that cows with one or more metabolic disorders in early lactation had a lower PY, a delayed PT, and a higher LP compared with healthy cows. It can be hypothesized that not only metabolic disease itself, but also metabolic status, indicated by plasma metabolites and metabolic hormones, is related with lactation curve characteristics. Indeed, an increased blood free fatty acids (**FFA**) and β -hydroxybutyrate (**BHB**) concentrations in early lactation were related to decreased 305-d milk yield (Duffield et al., 2009; Ospina et al., 2010). Data are scarce concerning the relationships of energy balance (**EB**) or metabolic status with PY, PT, and LP. Additionally, we found that a glucogenic diet improved EB and metabolic status in early lactation compared with a lipogenic diet (van Knegsel et al., 2007). However, the effects of dietary energy sources on PT, PY, LP, and 305-d milk yield in dairy cows with different DP lengths have not been studied yet.

The primary objective of this study was to evaluate the effects of DP length and dietary energy sources on lactation curve characteristics over 2 subsequent lactations. A further objective was to evaluate relationships between metabolic status in early lactation and lactation curve characteristics in dairy cows with different DP lengths and early lactation diets.

6.3 Materials and Methods

6.3.1 Animals and Experimental Design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. A total of 167 Holstein-Friesian dairy cows (60 primiparous and 107 multiparous) were selected from the Dairy Campus Research dairy herd (WUR Livestock Research, Lelystad, the Netherlands) for this experiment. The selected cows were served by a Holstein sire and met to the

following selection criteria: (1) having an expected calving interval less than 490 d, (2) having a daily milk yield greater than 16 kg/d at 90 d before the expected calving date, (3) having no mastitis or high SCC ($>250,000$ cells/mL) at last 2 test days before drying off. The experimental design, DP lengths, and dietary contrast have been reported previously (van Knegsel et al., 2014). Briefly, dairy cows were blocked by parity, expected calving date, milk yield in previous lactation, and body condition score (**BCS**). Within blocks, cows were assigned randomly to 1 of 3 DP lengths (0, 30, or 60 d) and 1 of 2 early lactation diets (glucogenic or lipogenic). The calving interval during previous lactation before the initiation of the experiment were 390 ± 5 , 398 ± 6 , and 396 ± 7 d (means $\pm$ SEM) for cows with a 0, 30, or 60 d DP, respectively. The milk yield during previous lactation before the initiation of the experiment were 32.8 ± 0.6 , 32.8 ± 0.6 , and 32.8 ± 0.5 kg/d (means $\pm$ SEM) for cows with a 0, 30, or 60 d DP, respectively. Before calving, dry cows were fed a dry cow diet, lactating cows were fed a lactation diet supporting 25 kg of milk. All cows were fed 1 kg/d of glucogenic or lipogenic concentrate from 10 d before the expected calving date. From calving to 100 DIM, the concentrate supply was increased stepwise by 0.5 kg/d until it reached 8.5 kg/d at 17 DIM. After 100 DIM, all cows were fed the standard lactation concentrate. The main ingredient for the glucogenic concentrate was corn and the main ingredients for the lipogenic concentrate were sugar beet pulp, palm kernel, and rumen protected palm oil. Forage was offered ad libitum throughout the study and was composed of grass silage, corn silage, wheat straw, and rapeseed meal or soybean meal (51:34:2:13, DM basis). Cows were housed in a free stall with a slatted floor and cubicles. During lactation, cows were milked twice daily (0500 and 1630 h). Cows with a 30 or 60 d DP were treated with an intramammary antibiotic at drying off (Supermastidol, Virbac Animal Health, Barneveld, the Netherlands).

We planned for cows to have the same DP length and diet over 2 subsequent lactations. During the first subsequent lactation following DP length treatments, 17 cows with a 0 d DP, 14 cows with a 30 d DP, and 6 cows with a 60 d DP were excluded from experiment because of fertility or health problems. The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d). Cows were allocated to this new group and dried off without use of intramammary

antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.

6.3.2 Fat- and Protein-corrected Milk Yield, Composition, and Lactation Curves

Milk yield was recorded daily per cow throughout 2 subsequent lactations. Milk samples for fat, protein, lactose, and SCC analysis (ISO 9622, Qlip, Zutphen, the Netherlands) were collected 4 times per week and averaged per week. Fat- and protein-corrected milk (**FPCM**) yield was calculated as $[(0.337 + 0.116 \times \text{fat \%} + 0.06 \times \text{protein \%}) \times \text{milk yield (kg/d)}]$ (CVB, 2012). A lactation curve for each lactation was modeled using the exponential model described by Wilmink (1987):

$$Y_t = a + b \times t + c \times \exp(-k \times t),$$

where Y_t is FPCM yield of a cow at t DIM, a is a regression coefficient related to maximum daily level of production, b is a regression coefficient related to the rate of FPCM yield decrease after PY, c is a regression coefficient related to the rate of FPCM yield increase toward PY, and k is a factor related to the time of PT. In the present analysis, factor k was estimated on the whole data set with a nonlinear regression model for the first and second lactations separately and fixed for further analyses ($k = 0.06$ for the first lactation and $k = 0.07$ for the second lactation). Parameters were estimated based on FPCM yield records from calving through 305 DIM for each lactation. Cows with FPCM yield records less than 260 DIM were excluded to ensure accurate estimates ($n = 8, 4$, and 1 for cows with a 0, 30, or 60 d DP in the first lactation and $n = 3, 7, 6$, and 2 for cows with a 0, 30, 60, or 0→67 d DP in the second lactation). Cows with positive estimated value of c , which implies that PY occurred before calving, were excluded from the analysis ($n = 1, 5$, and 7 for cows with a 0, 30, or 60 d DP in the first lactation and $n = 1, 4, 2$, and 0 for cows with a 0, 30, 60, or 0→67 d DP in the second lactation).

Time of PY was estimated using the first derivative of the Wilmink function, and the PY was the FPCM yield at PT. The cumulative 305-d FPCM yield (**FPCM₃₀₅**) was calculated by summing the estimated daily FPCM yield from calving until 305 DIM. Estimated FPCM yield was set to zero when it was negative. The FPCM yield in the last 60 d pre-calving (**FPCM₆₀**) was actual FPCM yield produced in the last 60 d pre-calving. The 365-d FPCM yield (**FPCM₃₆₅**) was calculated by summing **FPCM₃₀₅** and **FPCM₆₀**. Lactation persistency was calculated as daily milk reduction

(kg/d) by dividing the difference in estimated FPCM yield at 260 DIM and 100 DIM by 160 d.

The cumulative FPCM yield from calving in the first lactation through 60 d before the calving in the second lactation (**FPCM_{1st}**) was calculated by summing estimated daily FPCM yield from calving in the first lactation through 60 d before the calving in the second lactation (for cows with calving interval < 365 d) or by summing FPCM₃₀₅ in the first lactation and actual FPCM yield from 305 d in the first lactation through 60 d before the calving in the second lactation (for cows with calving interval > 365 d). The total FPCM yield in 2 subsequent lactations was calculated by summing FPCM yield from 3 periods: (1) the FPCM₋₆₀ in the first lactation; (2) the FPCM_{1st} in the first lactation; (3) the 365-d FPCM yield in the second lactation. The average daily FPCM yield in 2 subsequent lactations was calculated by dividing the total FPCM yield in 2 subsequent lactations by the number of days from 60 d before the calving in the first lactation to 305 d after the calving in the second lactation.

6.3.3 Energy Balance

Energy balance calculation was reported previously (van Knegsel et al., 2014). Briefly, EB was calculated per week according to the VEM system (Van Es, 1975; CVB, 2005) as the difference between VEM supplied with feed and VEM required for maintenance and milk yield.

6.3.4 Blood Sampling and Analysis

Blood sampling and analysis was reported previously (Chen et al., 2015). Briefly, blood samples were taken weekly from 95 cows in the first lactation and from 113 cows in the second lactation from calving to week 4 post-calving. Plasma samples were analyzed for indicators of metabolic status. Concentration of glucose was measured using commercial kits no. 61269 from Bio. Mérieux (Marcy l'Etoile, France). Concentration 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. Insulin-like growth factor-1 (**IGF-1**) and insulin were measured using kit no. A15729 from Beckman Coulter (Fullerton, CA, USA) and kit no. PI-12K from EMD Millipore Corporation (Billerica, MA, USA), respectively.

6.3.5 Statistical Analysis

The PROC MIXED procedure of SAS (Version 9.2; SAS Institute, Inc., Cary, NC, USA) was performed to analyze the effect of DP length and dietary energy source on lactation curve characteristics for the first and second subsequent lactations separately. Fat- and protein-corrected milk yield, PT, PY, and LP were analyzed with DP length (0, 30, or 60 d for the first lactation; 0, 30, 60, or 0→67 d for the second lactation), diet (glucogenic or lipogenic), parity (2 or > 2 for the first lactation; 3 or > 3 for the second lactation), and their 2-way interactions included as fixed effects. Non-significant interactions were excluded from the model via backward stepwise elimination if $P > 0.05$. For the analyses of the first lactation, preliminary analyses showed that the interaction of DP length with diet was not significant ($P > 0.05$). For the analyses of the second subsequent lactation, preliminary analyses showed that interactions of diet with DP length and diet with parity were not significant ($P > 0.05$). For analyses of average daily FPCM yield in 2 subsequent lactations, preliminary analyses showed that interactions of diet with DP length and diet with parity were not significant ($P > 0.05$). For comparison of DP lengths, P -values were presented after a Tukey's adjustment. Values were presented as least squares means with their standard errors.

To determine the relationships of metabolic status with lactation curve characteristics (PY, PT, LP, and FPCM₃₀₅), data from the first and second lactations were combined (243 lactations in total). Data were analyzed as repeated measurements with cow as the subject. A compound symmetry covariance structure was the best fit and was used to account for within-cow variation. Dry period length (0, 30, 60, or 0→67 d), parity (2 or >2), and interactions of DP length or parity with metabolic variables were included in model as fixed effects. Average EB, plasma concentrations of FFA, BHB, glucose, IGF-1, or insulin in the first 4 weeks after calving were included as covariate in the model one by one to obtain regression coefficients (β). To determine the relationships between PY, PT, LP, and FPCM₃₀₅, DP length, parity, and interactions of DP length or parity with lactation curve characteristics were included in model as fixed effects. Peak yield, PT, and LP were included as covariate in the model one by one to obtain regression coefficients (β). A preliminary backward stepwise regression was performed for each analysis and nonsignificant interactions ($P > 0.05$) were excluded from the model.

6.4 Results

6.4.1 Actual Dry Period Length

Actual days dry before the calving in the first lactation were 2 ± 1 d, 30 ± 1 d, 64 ± 2 d, and 61 ± 1 d (means $\pm$ SEM) for cows with a 0, 30, or 60 d DP, respectively. Actual days dry before the calving in the second lactation were 0 ± 0 d, 42 ± 7 d, 65 ± 2 d, and 67 ± 8 d (means $\pm$ SEM) for cows with a 0, 30, 60, or 0→67 d DP, respectively.

6.4.2 Fat- and Protein-Corrected Milk yield, Peak Yield, Peak Time, and Lactation Persistency in the First Lactation

In the first lactation, cows with a 0 d DP had lower FPCM₃₀₅ and PY, but greater FPCM₆₀ than cows with a 60 d DP, with cows with a 30 d DP being intermediate (Table 6.1). Cows with a 0 d DP had lower FPCM₃₆₅ than cows with a 30 or 60 d DP. Cows with a 30 or 60 d DP had similar FPCM₃₆₅. Cows with a 0 d DP had later PT than cows with a 60 d DP. Lactation persistency was not affected by DP length, and none of lactation curve characteristics were affected by diet.

An interaction of DP length $\times$ parity was present for FPCM₃₀₅, FPCM₆₀, and PY. In young cows (parity = 2), cows with a 0 d DP produced 3,067 kg less FPCM₃₀₅, and had a 10.9 kg lower PY than cows with a 60 d DP ($P < 0.01$). In older cows (parity > 2), cows with a 0 d DP produced 1,937 kg less FPCM₃₀₅, and had a 6.3 kg lower PY than cows with a 60 d DP ($P < 0.01$). In addition, omitting the DP resulted in more FPCM₆₀ (1,226 vs. 884 kg, $P < 0.01$) in young cows than older cows. Furthermore, FPCM₃₀₅ was lower for cows with a 30 d DP than cows with a 60 d DP in older cows (9,261 vs. 10,601 kg, $P = 0.04$), but not in young cows ($P = 0.55$).

6.4.3 Fat- and Protein-Corrected Milk yield, Peak Yield, Peak Time, and Lactation Persistency in the Second Lactation

In the second lactation, cows with a 0 or 30 d DP had greater FPCM₆₀ than cows with a 60 or 0→67 d DP (Table 6.2). Cows with a 0 or 30 d DP had lower PY and cows with a 0 d DP tended to have lower FPCM₃₀₅ ($P = 0.09$) than cows with a 60 d DP. Lactation persistency and FPCM₃₆₅ were not affected by DP length. None of the lactation curve characteristics were affected by diet or parity.

Table 6.1. Fat- and protein-corrected milk (FPCM) yield, peak yield (PY), time of peak yield (PT), and lactation persistency (LP) in the first subsequent lactation following dry period length treatments in dairy cows fed either a glucogenic or a lipogenic diet (LSM $\pm$ SEM).

Variable ¹	Dry period length (d)			SEM ²		Parity		SEM		P-value			
	0	30	60			2	>2			DPL ³	Diet	Parity	DPL $\times$ Parity ⁴
Cows, n	47	46	48			58	83						
FPCM ₃₀₅ (kg)	8,277 ^c	9,952 ^b	10,779 ^a	184		9,709	9,629	150		<0.01	0.36	0.71	0.02
FPCM ₆₀ (kg)	1042 ^a	596 ^b	17 ^c	36		612	492	30		<0.01	0.18	<0.01	0.01
FPCM ₃₆₅ (kg)	9,318 ^b	10,548 ^a	10,796 ^a	186		10,321	10,120	152		<0.01	0.52	0.35	0.08
PT (DIM)	39.3 ^a	36.2 ^{ab}	32.5 ^b	1.4		39.2	32.8	1.1		<0.01	0.77	<0.01	0.34
PY (kg/d)	38.2 ^c	44.1 ^b	46.8 ^a	0.7		41.4	44.7	0.6		<0.01	0.34	<0.01	0.01
LP	-0.102	-0.104	-0.102	0.004		-0.088	-0.118	0.003		0.91	0.76	<0.01	0.72

^{a,b,c} Values within dry period length in the same row with different superscripts differ ($P < 0.05$).

¹FPCM₃₀₅ = 305-d FPCM yield; FPCM₆₀ = FPCM yield during 60 d precalving; FPCM₃₆₅ = FPCM₃₀₅ + FPCM₆₀.

²SEM = pooled standard error of the least squares means.

³DPL = dry period length.

⁴Young cows (parity = 2): 0 d, n = 20; 30 d, n = 19; 60 d, n = 19; Older cows (parity > 2): 0 d, n = 27; 30 d, n = 27; 60 d, n = 29.

Table 6.2. Fat- and protein-corrected milk (FPCM) yield, peak yield (PY), time of peak yield (PT), and lactation persistency (LP) in the second subsequent lactation following dry period length treatments in dairy cows fed either a glucogenic or a lipogenic diet (LSM $\pm$ SEM).

Variable ¹	Dry period length ² (d)					Parity		SEM	P-value	
	0	30	60	0→67	SEM ³	3	>3		DPL ⁴	Diet Parity
Cows, n	17	32	42	16		45	62			
FPCM ₃₀₅ (kg)	9,735	9,942	10,392	10,362	205	10,101	10,115	151	0.09	0.68 0.95
FPCM ₋₆₀ (kg)	599 ^a	326 ^b	18 ^c	28 ^c	31	230	256	23	<0.01	0.54 0.39
FPCM ₃₆₅ (kg)	10,334	10,269	10,410	10,389	201	10,330	10,371	148	0.95	0.74 0.84
PT (DIM)	37.1	34.7	31.9	33.9	1.6	34.0	34.8	1.2	0.15	0.38 0.64
PY (kg/d)	43.9 ^b	44.7 ^b	47.5 ^a	47.2 ^{ab}	0.9	45.8	45.9	0.7	0.02	0.60 0.96
LP	-0.107	-0.108	-0.118	-0.118	0.005	-0.113	-0.113	0.004	0.22	0.55 0.99

^{a,b,c} Values within dry period length in the same row with different superscripts differ ($P < 0.05$).
¹FPCM₃₀₅ = 305-d FPCM yield, FPCM₋₆₀ = FPCM yield during 60 d precalving; FPCM₃₆₅ = FPCM₃₀₅ + FPCM₋₆₀.
²The second subsequent lactation started with 130 cows (39 cows with a 0 d DP, 41 cows with a 30 d DP, and 50 cows with a 60 d DP). However, 19 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 $\pm$ 8 d). Cows were allocated to this new group and dried off without use of intramammary antibiotics when they had a milk yield of < 4 kg/d at least 30 d before expected calving date.
³SEM = pooled standard error of the least squares means.
⁴DPL = dry period length.

6.4.4 Average Daily Fat- and Protein-Corrected Milk yield throughout 2 Subsequent Lactations

Throughout 2 subsequent lactations following DP length treatments, total FPCM yield and average daily FPCM yield were not different among cows with a 0, 30, or 60 d DP (Table 6.3). Cows with a 0→67 d DP had lower total FPCM yield and average daily FPCM yield than cows with a 30 or 60 d DP. Concerning the first lactation, cows with a 0 or 67 d DP had lower FPCM_{1st} than cows with a 30 or 60 d DP. An interaction of DP length × parity was present for total FPCM yield and average daily FPCM yield throughout 2 subsequent lactations following DP length treatments. In young cows, cows with a 0 or 0→67 d DP had lower total FPCM yield compared with cows with a 30 or 60 d DP (Figure 6.1 A), and cows with a 0→67 d DP had lower average daily FPCM yield than cows with a 30 or 60 d DP (Figure 6.1 B). In older cows, there was no effect of DP length on total FPCM yield ($P = 0.49$) and average daily FPCM yield ($P = 0.50$).

Figure 6.1. (A) Total fat-and protein-corrected milk (FCPM) yield from 60 d before first calving through 305 d after second calving in young (parity = 2) and older cows (parity > 2) after a dry period (DP) of 0, 30, 60, or 0→67 d. (B) Average daily FCPM yield from 60 d before first calving through 305 d after second calving in young and older cows after a DP of 0, 30, 60, or 0→67 d. Values represent LSM ($\pm$ SEM) per treatment per parity class; Bars with different letters (a, b) within parity differ ($P < 0.05$).

Table 6.3. Total and average daily fat-and protein-corrected milk (FPCM) yield from 60 d before calving in the first subsequent lactation through 305 d after calving in the second subsequent lactation following dry period length treatment in dairy cows fed either a glucogenic or a lipogenic diet (LSM ± SEM).

	Dry period length ¹ (d)					SEM ²			P-value			DPL ×	
	0	30	60	0→67	16				DPL ³	Diet	Parity	DPL ×	Parity
Cows, n	16	28	37	16	16								
FPCM ₆₀ in the first subsequent lactation (kg)	1,184 ^a	604 ^c	15 ^d	920 ^b	49				<0.01	0.16	<0.01	0.01	
FPCM _{1st} ⁴ (kg)	8,986 ^b	10,756 ^a	11,387 ^a	8,251 ^b	387				<0.01	0.18	0.86	0.04	
FPCM ₃₆₅ in the second subsequent lactation (kg)	10,294	10,302	10,386	10,330	226				0.99	0.53	0.46	0.26	
Total FPCM yield (kg)	20,464 ^{ab}	21,662 ^a	21,787 ^a	19,501 ^b	498				<0.01	0.23	0.86	0.04	
Calving interval (d)	376.2	415.1	410.3	408.3	15.4				0.34	0.18	0.50	0.65	
Total days ⁵ (d)	741.2	780.1	775.3	773.3	15.6				0.34	0.18	0.50	0.65	
Average daily FPCM yield (kg/d)	27.6 ^{ab}	27.9 ^a	28.2 ^a	25.2 ^b	0.5				<0.01	0.89	0.33	0.04	

^{a,b,c,d} Values within dry period length in the same row with different superscripts differ ($P < 0.05$).
¹Cows with a 0, 30, or 60 d dry period were the cows with same dry period in 2 subsequent lactations; Cows with a 0→67 d were the cows had 0 d dry period before the calving in the first subsequent lactation, but were dried off before the calving in the second subsequent lactation.
²SEM = pooled standard error of the least squares means.
³DPL = dry period length.
⁴FPCM_{1st}: FPCM yield from calving in the first subsequent lactation through 60 d before the calving in the second subsequent lactation following DP length treatments.
⁵Total days: Days from 60 d before the calving in the first subsequent lactation through 305 d after the calving in second subsequent lactation following DP length treatments.

6.4.5 Relationships between Peak Yield, Peak Time, Lactation Persistency, and 305-d Fat- and Protein-Corrected Milk yield

When 2 subsequent lactations after implementation of DP length treatments were combined, PT had a positive relationship with LP ($\beta = 0.0006$; $P < 0.01$). Peak yield had a negative relationship with LP ($\beta = -0.003$; $P < 0.01$) and a positive relationship with FPCM₃₀₅ ($\beta = 217$; $P < 0.01$). Interactions of PY $\times$ DP length were present for LP (Figure 6.2 A, $P < 0.01$) and FPCM₃₀₅ (Figure 6.2 B, $P < 0.01$). No correlation was found between LP and FPCM₃₀₅ ($P = 0.66$).

Figure 6.2. Estimated associations of peak yield with lactation persistency (A), and peak yield with FPCM₃₀₅ (B) in cows with a 0, 30, 60, or 0→67 d dry period (DP). The values of estimated peak yield were truncated according to actual minimum and maximum values in each DP length group.

6.4.6 Relationships of Energy Balance or Metabolic Status with Fat- and Protein-Corrected Milk Lactation Curve Characteristics

Average EB and plasma insulin concentration in the first 4 weeks after calving had a positive relationship with PT, whereas average plasma FFA concentration in the first 4 weeks after calving had a negative relation with PT (Table 6.4). Average EB, plasma concentrations of glucose, insulin, and insulin-like growth factor-1 (**IGF-1**) in the first 4 weeks after calving had a negative relationship with PY and FPCM₃₀₅. Average plasma FFA and BHB concentrations in the first 4 weeks after calving had a positive relationship with PY and FPCM₃₀₅. In addition, average EB in the first 4 weeks after calving had a positive relationship with LP, whereas average plasma FFA and BHB concentrations in the first 4 weeks after calving had a negative relationship with LP. Interactions of IGF-1 $\times$ parity (Figure 6.3 A) and

Table 6.4. Regression coefficients (β) of energy balance (EB), plasma metabolites, or hormones¹ related to peak yield (PY), time of peak yield (PT), lactation persistency (LP), and 305-d fat-and protein-corrected milk (FPCM) yield²

	PT ⁴ (DIM)	PY ⁵ (kg/d)	LP	FPCM ₃₀₅ ⁶ (kg)
Energy balance ³ (kJ/kg ^{0.75} ·d)	0.02**	-0.02**	0.00006**	-7.7**
FFA (mmol/L)	-7.10*	9.52**	-0.025**	2418.6**
BHB (mmol/L)	0.42	2.03**	-0.011**	842.6*
Glucose (mmol/L)	2.23	-3.88**	0.010	-1754.9**
IGF-1 (ng/mL)	-0.16	-0.05*	0.00004	-22.2*
Insulin (μIU/mL)	0.28**	-0.32**	0.00051	-75.0**

** $P < 0.01$; * $P < 0.05$.

¹Energy balance, plasma concentrations of FFA, BHB, Glucose, IGF-1, or insulin were average for the first 4 weeks in the first subsequent lactation or second subsequent lactation following DP length treatments.

²Total lactations for EB and SCC: 0 d, n = 63; 30 d, n=76; 60 d, n = 86; 0→67 d, n = 16. Total lactations for plasma metabolites and hormones: 0 d, n = 37; 30 d, n=52; 60 d, n = 59; 0→67 d, n = 16.

³Energy balance was calculated with the VEM system (Van Es, 1975).

⁴Interactions of IGF-1 × dry period length (DPL, $P = 0.02$) and IGF-1 × parity ($P = 0.02$) were present.

⁵Interactions of FFA × DPL ($P = 0.04$), and IGF-1 × DPL ($P = 0.04$) were present.

⁶Interactions of EB × DPL ($P < 0.01$), FFA × DPL ($P < 0.01$), BHB × DPL ($P = 0.02$), glucose × DPL ($P < 0.01$), IGF-1 × DPL ($P < 0.01$), FFA × parity ($P < 0.01$), glucose × parity ($P < 0.01$) were present.

IGF-1 × DP length (Figure 6.4 A) were present for PT. A negative relationship between plasma IGF-1 concentration and PT was present in cows with a 0→67 d DP ($P = 0.02$) or young cows ($P = 0.01$), but not in cows with other DP lengths or older cows.

Interactions of FFA × DP length (Figure 6.4 B), and IGF-1 × DP length (Figure 6.4 C) were present for PY. A positive relationship between plasma FFA concentration and PY was present in cows with a 0 or 30 d DP ($P < 0.01$), but not in cows with a 60 or 0→67 d DP. A negative relationship between plasma IGF-1 concentration and PY was only present in cows with a 0 d DP ($P < 0.01$).

Interactions of EB × DP length (Figure 6.4 D), FFA × DP length(Figure 6.4 E), BHB × DP length (Figure 6.4 F), glucose × DP length (Figure 6.4 G), glucose × parity (Figure 6.3 B), FFA × parity (Figure 6.3 C), and IGF-1 × DP length

(Figure 6.4 H) were present for FPCM_{305} . Negative relationships between EB and FPCM_{305} ($P < 0.01$), between plasma glucose concentration and FPCM_{305} ($P < 0.01$), and positive relationship between plasma FFA concentration and FPCM_{305} ($P < 0.05$) were present in cows with a 0, 30, or 0→67 d DP, but not in cows with a 60 d DP. A positive relationship between plasma BHB concentration and FPCM_{305} was present in cows with a 0 or 0→67 d DP ($P = 0.04$), but not in cows with other DP lengths. A negative relationship between plasma IGF-1 concentration and FPCM_{305} was present in cows with a 0 d DP ($P < 0.01$), but not in cows with other DP lengths.

Figure 6.3. (A) Estimated associations of plasma IGF-1 concentrations with peak time in young (parity = 2) or older (parity > 2) cows. (B) Estimated associations of plasma glucose concentrations with 305-d fat-and protein-corrected milk (FPCM_{305}) in young or older cows. (C) Estimated associations of plasma FFA concentrations with FPCM_{305} in young or older cows. Plasma concentrations of IGF-1, FFA, and glucose were average for first 4 weeks after calving. The values of estimated plasma concentrations of IGF-1, glucose, and FFA were truncated according to actual minimum and maximum values in each parity group.

6.5 Discussion

In the current study, shortening or omitting the DP resulted in later PT, lower PY, lower FPCM₃₀₅, and had no effect on LP in 2 subsequent lactations after implementation of DP length treatments. Peak yield was positively correlated with FPCM₃₀₅, but no correlation was found between LP and FPCM₃₀₅. This implies that FPCM₃₀₅ yield losses after a 0 or 30 d DP compared with a 60 d DP were not related to LP in dairy cows. Atashi et al. (2013) found an increased LP after a shortened (0 to 35 d) DP compared with a conventional (51 to 60 d) DP in primiparous cows in a field study. In that field study, cows were not planned to have a shorter DP and cows with high persistency were probably the cows with a longer lactation period (shorter DP) due to a greater milk yield in late lactation. This could explain the increased LP in cows with a short DP in the study of Atashi et al. (2013). By contrast, Mantovani et al. (2010) reported that omitting the DP decreased LP. In that study, cows with a conventional or omitted DP had a PY of 36.8 and 31.3 kg, respectively, and a LP of –0.062 and –0.078, respectively. The differences in the effects of DP length on LP between our study and this previous finding might be related to the relatively low PY and high LP in dairy cows in the study of Mantovani et al. (2010), but further studies are needed to confirm that finding.

The decreased PY and later PT in cows with a 0 or 30 d DP could be related to the high proportion of senescent mammary epithelial cells in these cows in early lactation. Capuco et al. (2001) suggested that the increase in milk yield until peak is due to continued differentiation of mammary secretory cells and increased secretory activity per cell in early lactation. Omitting the DP increases carryover of senescent mammary epithelial cells into the subsequent lactation (Capuco et al., 1997; Annen et al., 2007; Annen et al., 2008), which decreases secretory ability of mammary gland cells (Collier et al., 2012). Bernier-Dodier et al. (2011) also suggested that the decreased milk yield after shortening the DP to 35 d is related to the incomplete mammary gland renewal during DP. In addition, the PT had a positive relation, and PY had a negative relationship with LP in the current study. Although cows with a 0 or 30 d DP had decreased PY and later PT, these cows did not have higher LP than cows with a 60 d DP in both 2 subsequent lactations. It is possible that the high proportion of senescent mammary epithelial cells in cows

Figure 6.4. Estimated associations of plasma IGF-1 concentration with time of peak time (A), plasma FFA concentration with peak yield (B), plasma IGF-1 concentration with peak yield (C), energy balance with 305-d fat-and protein-corrected milk (FPCM₃₀₅) (D), plasma FFA concentration with FPCM₃₀₅ (E), plasma BHB concentration with FPCM₃₀₅ (F), plasma glucose concentration with FPCM₃₀₅ (G), and plasma IGF-1 concentration with FPCM₃₀₅ (H) in cows with a 0, 30, 60, or 0→67 d dry period (DP). (Continued on the next page)

Figure 6.4. (continued) Energy balance, plasma concentrations of FFA, IGF-1, glucose, and BHB were average for first 4 weeks after calving. The values of estimated energy balance, plasma concentrations of FFA, IGF-1, glucose, and BHB were truncated according to actual minimum and maximum values in each DP length group.

with a 0 or 30 d DP limited LP when PY decreased.

In the first lactation, omitting the DP in young cows caused a more pronounced decrease in PY and FPCM₃₀₅ compared with older cows, which is in agreement with Mantovani et al. (2010). Collier et al. (2012) suggested that the greater reduction in milk yield in young cows after omitting the DP could be related to greater requirements for both mammary development and mammary epithelial cell turnover between the first and second parity of a cow, compared with later parities. This might imply that omitting the DP is less suitable for young cows because of substantial milk yield losses. In addition, Santschi et al. (2011) found that shortening the DP decreased milk yield in the first lactation in young cows, but not in older cows in a field study. By contrast, in the first lactation of the current study, shortening the DP decreased FPCM₃₀₅ compared with conventional DP in older cows, but not in young cows. Taking into account the additional FPCM yield precalving, however, shortening the DP resulted in similar FPCM₃₆₅ compared with a conventional DP in both young and older cows.

In the second lactation, the difference in PY, PT, and, FPCM₃₀₅ between cows with a 0 and 60 d DP were smaller than the first lactation, although the PY was still lower and FPCM₃₀₅ tended to be lower in cows with a 0 d DP than cows with a 60 d DP. Moreover, FPCM₃₆₅ of cows with a 0 d DP in the second lactation did not differ from that of cows with a 60 d DP (10,334 vs. 10,410 kg for 0 vs. 60

d DP). The smaller differences between cows with a 0 and 60 d DP on lactation curve characteristics were related to increased PY, FPCM₃₀₅, FPCM₃₆₅, and earlier PT in cows with a 0 d DP in the second lactation, which can be explained by the increased parity in dairy cows in the second lactation. It was suggested that older cows had less milk yield losses after omitting the DP compared with young cows (Annen et al., 2004; Watters et al., 2008; Chen et al., 2016). A recent field study, nevertheless, demonstrated that omitting the DP resulted in 560 kg greater FPCM₃₀₅ in the second lactation compared with the first lactation in older cows (Kok, et al., 2016a). In line with this, omitting the DP in older cows resulted in 1071 kg greater FPCM₃₀₅ in the second lactation compared with the first lactation in the current study. These results imply that increased parity is not the only cause for the increased milk yield in the second lactation after implementation of omitting the DP. It is possible that increased turnover of mammary epithelial cell during lactation in the first lactation also plays a role in this increased FPCM yield in cows with a 0 d DP in the second lactation. The turnover of mammary epithelial cells exists throughout lactation (Capuco et al., 2001). It can be hypothesized that the senescent mammary epithelial cells in cows with a 0 d DP was replaced with new cells in mid or late lactation of the first lactation, which increased the secretory capability of mammary gland in the second lactation.

To our knowledge, only one controlled study is available that reported the effects of omitting the DP on milk yield over 2 subsequent lactations (Remond et al., 1997). They found similar milk yield during 36 weeks of the second lactation between experimental (no DP) and control group (conventional DP of 8 weeks). In that study, however, only 2 out of 21 cows assigned to experimental group had a DP of 0 d prior to calving in the second lactation, which means most of these cows dried themselves off and in fact had a DP of variable length. Herewith, it can be suggested that those cows had more opportunity for replacement of senescent mammary epithelial cells during the pre-calving period. This implies that these cows in the study of Remond et al. (1997) were comparable with cows with a 0→67 d DP in the current study, which had similar FPCM₃₀₅, FPCM₃₆₅, and PY than cows with a 60 d DP in the second lactation.

Milk yield in last 60 d pre-calving in cows with a 0 or 30 d DP was lower in the second lactation compared with the first lactation. Kok et al., (2016a) reported that omission of the DP result in 172 kg lower FPCM₆₀ in the second lactation compared with the first lactation following DP length treatments in a field study. The

decreased PY and unchanged LP in cows with a 0 or 30 d DP in the first lactation led to decreased FPCM yield in late lactation, which can partly explain decreased FPCM₋₆₀ in cows with a 0 or 30 d DP in the second lactation. In addition, decreased FPCM₋₆₀ in the second lactation could also be related to increased parity of these cows. Primiparous cows generally have higher LP compared with multiparous cows (Miller et al., 2006). In late lactation of the first lactation, all cows were multiparous and the lower LP of those multiparous cows resulted in lower FPCM yield before the next lactation.

Kok et al. (2016) proposed the effective lactation yield instead of traditional 305-d milk yield to compare milk yield among cows with different DP lengths. The effective lactation yield was defined as the daily yield from 60 d before calving to 60 d before next calving (Kok et al., 2016). This new approach corrects for differences in calving interval and possible additional milk yield precalving. In the current study, complete data was available for the first lactation, but data from the second lactation stopped at 305 DIM. To correct for differences in calving interval in the first lactation, and assess the effect of milk yield over 2 lactations, we compared total FPCM yield and average daily FPCM yield throughout 2 subsequent lactations among DP lengths in the current study.

In older cows, total FPCM yield and average daily FPCM throughout 2 subsequent lactations did not differ among DP lengths. These results suggest that shortening the DP to 30 d or omitting the DP completely does not compromise FPCM yield compared with a conventional DP of 60 d in older cows in a long-term perspective. In young cows, cows that were able to milk continuously for 2 subsequent lactations (0 d DP) had lower total FPCM yield throughout 2 subsequent lactations than cows with a 60 d DP. In young cows, however, the average daily FPCM yield did not differ between cows with a 0 d DP and cows with a 60 d DP. This is because the cows with a 0 d DP had relatively less total days throughout 2 subsequent lactations than cows with a 60 d DP, due to a short calving interval (376.2 vs. 415.1 vs. 410.3 vs. 408.3 d for 0, 30, 60, and 0→67 d DP). To our knowledge, the present study is the first reporting the average daily FPCM yield throughout 2 subsequent lactations after different DP lengths. Considering the beneficial effects on EB (Rastani et al., 2005), metabolic status (Chen et al., 2015), and fertility (Watters et al., 2009), shortening or omitting the

DP can be a practical management strategy with no milk yield losses in older cows in a long-term perspective.

In the current study, elevated plasma FFA concentration, decreased EB and decreased plasma glucose concentration were related to increased FPCM₃₀₅ in cows with a 0 or 30 d DP, but not in cows with a 60 d DP. In cows with a 0 or 30 d DP, high-producing cows partition more energy to milk and, therefore, cause more severe negative EB and more pronounced body fat mobilization, which is related to increased plasma concentrations of FFA and BHB (Chen et al., 2015). Cows with a 60 d DP, however, had greater milk yield, more severe negative EB, greater plasma FFA and BHB concentrations than cows with a 0 or 30 d DP, especially in the first lactation in the current study (van Knegsel et al., 2014; Chen et al., 2015), which increased the risk of metabolic disorders. In earlier studies, cows that encountered one or more metabolic disorders (ketosis, milk fever, and displaced abomasum) had lower PY, lower 305-d milk yield, and higher LP (Appuhamy et al., 2007; Hostens et al., 2012). In the current study, cows with a 60 d DP had a more severe negative EB and greater plasma concentrations of FFA and BHB than cows with other DP lengths, which was related to an increased risk of metabolic disorders. Therefore, relationships of EB and metabolic status with FPCM₃₀₅ in cows with a 60 d DP may be influenced by metabolic disorders, which could partly explain the lack of relationships in these cows in our study. Furthermore, elevated plasma FFA and BHB concentrations and decreased EB were related to decreased LP. This relationship is not surprising because the increased plasma FFA and BHB concentrations and more negative EB were associated with increased PY, and consequently associated with a decreased LP.

Dietary energy source in early lactation did not influence lactation curve characteristics, although a glucogenic diet improved metabolic status compared with a lipogenic diet in the second lactation of the current study (Chen et al., 2016). Boerman et al., (2015) reported high-starch (glucogenic) diet increased milk yield, but reduced milk fat yield compared with high-fiber and high-fat (lipogenic) diet in mid-lactation cows (64 to 142 DIM). In line with this, we also found glucogenic diet tended to increased milk yield in the first 9 weeks (Chen et al., 2016), but FPCM yield did not differ between diets because the glucogenic diet decreased milk fat yield in the second lactation. In addition, the lipogenic or glucogenic diet was only supplied in first 100 DIM in the current study. The fat- and protein-correction of

milk yield and the limited period of dietary treatment may be related to the lack of difference on FPCM lactation curve characteristics between diets in the current study.

6.6 Conclusions

Shortening the DP to 30 d or omitting the DP affected lactation curve characteristics in 2 subsequent lactations after implementation of DP length treatments in dairy cows, indicated by later PT, lower PY, and lower FPCM₃₀₅ compared with a conventional DP of 60 d. These effects were more pronounced in the first subsequent lactation compared with the second subsequent lactation, and more pronounced in young cows compared with older cows. Dry period length had no effects on LP in both lactations. Considering 2 subsequent lactations after implementation of DP length treatments, shortening or omitting the DP does not compromise FPCM yield compared with a conventional DP in older cows. In young cows, omitting the DP decreased the total FPCM yield throughout 2 subsequent lactations. Metabolic status in early lactation was related with the shape of lactation curve, although relationships were dependent on DP length. Severe EB and poor metabolic status were related to elevated PY, FPCM₃₀₅, and low LP, especially in cows after a shortened or omitted DP.

6.7 Acknowledgments

The authors thank the Dutch Dairy Board (PZ, the Netherlands), Product Board Animal Feed (PDV; the Netherlands), and CRV (the Netherlands) for financing the experiment. The authors thanks the Chinese Scholarship Council (CSC) for financial support. The authors thank the staff of the Dairy Campus (Lelystad, the Netherlands) and Veterinary Physiology Group of the University of Bern (Switzerland) for their technical support and laboratory analyses during the experiment.

References

- Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. *J. Dairy Sci.* 87:3746-3761.
- Annen, E. L., A. C. Fitzgerald, P. C. Gentry, M. A. McGuire, A. V. Capuco, L. H. Baumgard, and R. J. Collier. 2007. Effect of continuous milking and bovine somatotropin supplementation on mammary epithelial cell turnover. *J. Dairy Sci.* 90:165-183.
- Annen, E. L., C. M. Stiening, B. A. Crooker, A. C. Fitzgerald, and R. J. Collier. 2008. Effect of continuous milking and prostaglandin E2 on milk production and mammary epithelial cell turnover, ultrastructure, and gene expression1. *J. Anim. Sci.* 86.
- Appuhamy, J. A. D. R. N., B. G. Cassell, C. D. Dechow, and J. B. Cole. 2007. Phenotypic relationships of common health disorders in dairy cows to lactation persistency estimated from daily milk weights. *J. Dairy Sci.* 90:4424-4434.
- Arnold, P. T. D. and R. B. Becker. 1936. Influence of preceding dry period and of mineral supplement on lactation. *J. Dairy Sci.* 19:257-266.
- Atashi, H., M. J. Zamiri, and M. Dadpasand. 2013. Association between dry period length and lactation performance, lactation curve, calf birth weight, and dystocia in Holstein dairy cows in Iran. *J. Dairy Sci.* 96:3632-3638.
- Bernier-Dodier, P., C. L. Girard, B. G. Talbot, and P. Lacasse. 2011. Effect of dry period management on mammary gland function and its endocrine regulation in dairy cows. *J. Dairy Sci.* 94:4922-4936.
- Boerman, J. P., S. B. Potts, M. J. VandeHaar, and A. L. Lock. 2015. Effects of partly replacing dietary starch with fiber and fat on milk production and energy partitioning. *J. Dairy Sci.* 98:7264-7276.
- Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477-487.
- Capuco, A. V., D. L. Wood, R. Baldwin, K. McLeod, and M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine

- lactation: relation to milk production and effect of bST. *J. Dairy Sci.* 84:2177-2187.
- Centraal Veevoederbureau (CVB). 2005. Veevoedertabel. Gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. CVB, Lelystad, the Netherlands.
- Centraal Veevoederbureau (CVB). 2012. CVB Tabellenboek Veevoeding. Voedernormen landbouwhuisdieren en voederwaarde veevoerders. Productschap Diervoeder, Den Haag, the Netherlands. 122 p.
- Chen, J., J. J. Gross, H. A. van Dorland, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2015. Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation. *J. Dairy Sci.* 98:1033-1045.
- Chen, J., G. J. Remmelink, J. J. Gross, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2016. Effects of dry period length and dietary energy source on milk yield, energy balance, and metabolic status of dairy cows over 2 consecutive years: Effects in the second year. *J. Dairy Sci.* 99:4826-4838.
- Collier, R. J., E. L. Annen-Dawson, and A. Pezeshki. 2012. Effects of continuous lactation and short dry periods on mammary function and animal health. *Animal* 6:403-414.
- Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. *J. Dairy Sci.* 92:571-580.
- Hostens, M., J. Ehrlich, B. Van Ranst, and G. Opsomer. 2012. On-farm evaluation of the effect of metabolic diseases on the shape of the lactation curve in dairy cows through the MilkBot lactation model. *J. Dairy Sci.* 95:2988-3007.
- Kok, A., C. E. van Middelaar, B. Engel, A. T. M. van Knegsel, H. Hogeveen, B. Kemp, and I. J. M. de Boer. 2016. Effective lactation yield: A measure to compare milk yield between cows with different dry period lengths. *J. Dairy Sci.*
- Mantovani, R., L. Marinelli, L. Bailoni, G. Gabai, and G. Bittante. 2010. Omission of dry period and effects on the subsequent lactation curve and on milk quality around calving in Italian Holstein cows. *Ital. J. Anim. Sci.* 9:101-108.

- Miller, N., L. Delbecchi, D. Petitclerc, G. F. Wagner, B. G. Talbot, and P. Lacasse. 2006. Effect of stage of lactation and parity on mammary gland cell renewal. *J. Dairy Sci.* 89:4669-4677.
- Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Associations of elevated nonesterified fatty acids and β -hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *J. Dairy Sci.* 93:1596-1603.
- Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004-1014.
- Remond, B., J. Rouel, N. Pinson, and S. Jabet. 1997. An attempt to omit the dry period over three consecutive lactations in dairy cows. *Ann. Zootech.* 46:399-408.
- Steeneveld, W., Y. H. Schukken, A. T. M. van Knegsel, and H. Hogeveen. 2013. Effect of different dry period lengths on milk production and somatic cell count in subsequent lactations in commercial Dutch dairy herds. *J. Dairy Sci.* 96:2988-3001.
- Van Es, A. J. H. 1975. Feed evaluation for dairy cows. *Livest. Prod. Sci.* 2:95-107.
- Van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. *J. Dairy Sci.* 90:3397-3409.
- Van Knegsel, A. T. M., S. G. A. van der Drift, J. Čermáková, and B. Kemp. 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: A systematic review. *Vet. J.* 198:707-713.
- Van Knegsel, A. T. M., G. J. Remmelink, S. Jorj Jong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *J. Dairy Sci.* 97:1499-1512.
- Vance, E. R., C. P. Ferris, C. T. Elliott, S. A. McGettrick, and D. J. Kilpatrick. 2012. Food intake, milk production, and tissue changes of Holstein-Friesian and

- Jersey $\times$ Holstein-Friesian dairy cows within a medium-input grazing system and a high-input total confinement system. *J. Dairy Sci.* 95:1527-1544.
- Watters, R. D., J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Crump, P. W. Clark, and R. R. Grummer. 2008. Effects of dry period length on milk production and health of dairy cattle. *J. Dairy Sci.* 91:2595-2603.
- Watters, R. D., M. C. Wiltbank, J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Fricke, and R. R. Grummer. 2009. Effect of dry period length on reproduction during the subsequent lactation. *J. Dairy Sci.* 92:3081-3090.
- Wilmink, J. B. M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. *Livest. Prod. Sci.* 16:335-348.
- Wittrock, J. M., K. L. Proudfoot, D. M. Weary, and M. A. G. von Keyserlingk. 2011. Short communication: Metritis affects milk production and cull rate of Holstein multiparous and primiparous dairy cows differently. *J. Dairy Sci.* 94:2408-2412.
- Zobel, G., D. M. Weary, K. E. Leslie, and M. A. G. von Keyserlingk. 2015. Invited review: Cessation of lactation: Effects on animal welfare. *J. Dairy Sci.* 98:8263-8277.

CHAPTER 7

General discussion

7.1. Introduction

The objective of this thesis was to evaluate the effects of dry period (**DP**) length (0, 30, or 60 d) and dietary energy source (glucogenic or lipogenic) on milk yield, energy balance (**EB**), metabolism, and fertility over 2 subsequent lactations. In the current study, shortening (30 d) or omitting (0 d) the DP decreased milk yield but improve EB compared with a conventional DP, and glucogenic diet also improved EB independent of DP length in the first subsequent lactation after implementation of DP length and dietary treatments (van Knegsel et al., 2014). The first (Chapter 2) and second (Chapter 3) studies of the this thesis showed that omitting the DP but not shortening the DP improved metabolic status in early lactation and resulted in an earlier resumption of ovarian cyclicity compared with a conventional DP (60 d) in the first subsequent lactation. Independent of DP length, feeding a glucogenic diet also improved metabolic status in early lactation, as indicated by lower plasma β -hydroxybutyrate (**BHB**) concentration, compared with a lipogenic diet in early lactation. The third (Chapter 4) and fourth (Chapter 5) studies focused on the long-term effects of DP length and dietary energy source in dairy cows. These studies showed that omitting the DP or feeding a glucogenic diet improved metabolic status in the second subsequent lactation after implementation of DP length and dietary treatments, although effects of DP length on metabolic status were less pronounced in the second lactation compared with the first lactation. No effects of DP length or dietary energy source were found on fertility in the second lactation. Finally, the lactation curve characteristics were determined in both lactations (Chapter 6) and results showed that shortening or omitting the DP decreased peak milk yield and 305-d fat- and protein-corrected milk yield (**FPCM**), but had no effects on lactation persistency (**LP**) in both lactations. Considering 2 subsequent lactations together, shortening or omitting the DP decreased FPCM yield compared with a conventional DP in young cows (parity = 2), but not in older cows (parity > 2).

The first part of this chapter will discuss the possible biological mechanisms behind the effects of shortening or omitting DP on milk yield, metabolism, and fertility. Secondly, long-term effects of shortening or omitting the DP on milk yield, EB, and metabolic status, including effects of body condition score (**BCS**) and parity, will be discussed. Thirdly, other factors (e.g. diet, genotype, or uterine health status) that affected EB and metabolic status of cows with different DP lengths will be

discussed. Fourthly, achievement of shortening or omitting the DP over multiple lactations will be discussed. Fifthly, application of shortening or omitting the DP and the economic impact of this DP management strategy in practice will be discussed. Lastly, concluding remarks are presented.

7.2. How Does Shortening or Omitting the Dry Period Affect Milk Yield, Dry Matter Intake, Energy Balance, Metabolic Status, and Fertility?

It seems that shortening or omitting the DP decreases milk yield, improves dry matter intake (DMI), and herewith improves EB in the subsequent lactation, which is related to improved metabolic status and fertility in dairy cows. The possible mechanisms underlying these effects of shortening or omitting the DP on milk yield, DMI, and EB, and the relationships of EB with metabolic and fertility are presented briefly.

7.2.1 Milk yield, Dry Matter Intake, and Energy Balance

It has been widely reported that shortening or omitting the DP decreases milk yield in the subsequent lactation, which decreases the energy requirement for milk yield in early lactation and, therefore, improves EB and metabolic status. In chapter 6, we reported that shortening or omitting the DP caused a 305-d FPCM yield losses of 7.6 and 23.2%, respectively, compared with a conventional DP in the first lactation after implementation of DP length treatments. It was suggested that the milk yield losses in cows with a shortened or omitted DP could be related to the relatively high proportion of senescent mammary epithelial cells in these cows (Capuco et al., 1997; Annen et al., 2007; Annen et al., 2008). Annen et al. (2007) evaluated the mammary ultrastructure in continuously milked glands (0 d DP) and showed that continuously milked glands, compared with mammary glands that had a 60 d dry period, contained large populations of resting and engorged alveoli rather than a majority of secretory alveoli at 20 d postcalving. These authors hypothesized that the increased prevalence of resting alveoli in continuously milked glands is caused by the carryover of old mammary epithelial cells from the previous lactation. In spite of decreased milk yield postcalving, additional milk produced precalving in cows with a shortened or omitted DP could, at least, partly compensate for the milk yield losses postcalving. In the first lactation of current experiment, cows with a shortened or omitted DP produced 1,025 and 579 kg more

FPCM precalving compared with cows with a conventional DP (van Knegsel et al., 2014). It confirmed the hypothesis that shortening or omitting the DP shifts at least part of milk yield from the postcalving to the precalving period and, therefore, decreases the energy required for milk yield in early lactation (van Knegsel et al., 2014). In the current study, because energy intake in early lactation was similar, the decreased energy requirement for milk yield in early lactation in cows with a shortened or omitted DP improved EB in early lactation.

Besides decreased milk yield, it was hypothesized that shortening or omitting the DP might improve EB by increasing DMI in early lactation because of less diet changes before calving (Grummer and Rastani, 2004). However, the effects of DP length on DMI are not consistent among studies. On the one hand, Rastani et al. (2005) reported that the omitting the DP improved EB due to increased DMI in early lactation in cows with ad libitum feeding. Goselink et al. (2012) reported that shortening or omitting the DP increased DMI in early lactation compared with a conventional DP in dairy cows. On the other hand, no effects of DP length were found on DMI in early lactation in the current study with controlled amount of concentrates feeding (van Knegsel et al., 2014) and in studies with ad libitum TMR feeding (Andersen et al., 2005; De Feu et al., 2009). The cause for the discrepancies among studies is not clear and more studies are needed to further investigate the relationship between DP length and DMI in early lactation. In addition, Goselink et al. (2012) found that shortening or omitting the DP increased rumen papillae surface area in first 2 weeks postcalving, which may imply a more stable ruminal environment and increased absorption capacity of volatile fatty acids (VFA). It appears that, besides decreased milk yield, shortening or omitting the DP might improve EB by increasing DMI, or increasing ruminal capacity for VFA absorption, or both in early lactation.

7.2.2 Metabolic Status

The reduction in milk yield in early lactation after omitting the DP decreases the glucose requirement of the mammary gland for milk production. Hereby, omitting the DP repartitions glucose from milk to body reserves, which is related to an increased plasma glucose concentration (Andersen et al., 2005; Rastani et al., 2005; Chapter 2). This increased plasma glucose concentration results in an increased plasma insulin concentration. Insulin plays a key role in the adaptation

process around parturition in dairy cows. In early lactation, a low plasma insulin concentration ensures that less glucose is utilized in muscle and adipose tissue and glucose is spared for milk production in mammary gland, which is not insulin-responsive (Bauman et al., 1983). Moreover, a low level of insulin coupled with a high level of growth hormone in early lactation facilitates lipolysis in adipose tissue to compensate for energy deficit in early lactation (Butler et al., 2003). Greater plasma insulin concentration in cows with an omitted DP, compared with cows with a conventional DP, suppresses lipolysis and therefore decreases plasma free fatty acids (FFA) concentration.

Circulating FFA from lipolysis can be completely oxidized to make energy available to the body in the form of ATP. In early lactation, however, the hepatic capacity of complete oxidation is not sufficient because glucogenic precursors are limiting. Therefore, circulating FFA from lipolysis are partially oxidized to form ketone bodies or esterified to be stored in the liver as triacylglycerol (TAG) (Grummer, 1993). Parts of the formed TAG are exported from the liver as very-low-density-lipoproteins (VLDL). Free fatty acids and VLDL can be used for milk fat synthesis (Bell, 1995). This explains the higher proportion of long-chain fatty acids in milk fat in cows with a conventional DP, compared with cows with an omitted DP in the current study (van Knegsel et al., 2014). Plasma BHB concentration did not differ among DP lengths, whereas cows with an omitted DP had lower plasma FFA and liver TAG concentrations (Chapter 2). Possibly, in the current study, the high plasma concentration of FFA as found in cows with a conventional DP was related to the increased degree of esterification and liver TAG concentration, but not related to the extent of β -oxidation and plasma BHB concentration in liver. Drackley (1999) also suggested that liver is prone to increase FFA esterification and TAG deposition around calving.

7.2.3 Fertility

In the current study, omitting the DP tended to reduce the interval from calving to onset of luteal activity (OLA) and increased the incidence of normal resumption of ovarian cyclicity, which was defined as OLA occurring at 45 DIM or less and followed by regular ovarian cycles of 18 to 24 days in length (Chapter 3). Gümen et al. (2005) reported that the days to first ovulation was negatively correlated with mean EB in the first 3 weeks ($r = -0.42$; $P < 0.01$) and EB nadir (r

= -0.45; $P < 0.01$). The severe negative EB in early lactation inhibits the follicular development by suppressing plasma insulin-like growth factor-1 (**IGF-1**) concentration and pituitary luteinizing hormone pulsatility, which decreases the numbers of large follicles (Lucy et al., 1991). This suppressed follicle growth and development may lead to inactive ovaries, ovarian cysts, and non-functional corpora lutea in dairy cows, which could be the reason for delayed first ovulation (Shrestha et al., 2004). However, cows with an earlier (< 21 DIM) OLA had similar EB compared with cows with later OLA (from 21 to 30 DIM, or > 30 DIM) in the current study (Chapter 3). Consistent with this result, other studies reported that interval to first ovulation was not related to EB (Reist et al., 2003; Spicer et al., 1990). It was suggested that other factors also contribute to the delayed first ovulation, such as uterine infection (Opsomer et al., 2000), mastitis (Huszenicza et al., 2005), and lameness (Petersson et al., 2006).

Although omitting the DP improved the resumption of ovarian cyclicity, no DP length effects were found on pregnancy rate, days open, or services per pregnancy in the first lactation after implementation of DP length treatments (Table 7.1). This may be explained by the following reasons: firstly, the first artificial insemination was performed around 75 DIM in all groups, in which most cows were in positive EB and had normal ovarian cyclicity. Secondly, the effects of DP length on interval to OLA might be too small (5.8 d between 0 and 60 d DP) to cause effects on reproductive performance in a long term. Thirdly, partly lacking of pedometer activity in some cows might have caused inaccurate oestrus detection. Similarly, De Feu et al. (2009) found that omitting the DP resulted in an earlier first ovulation without effects on reproductive performance. Some authors, however, reported a decrease in number of days open after shortening or omitting the DP in both a controlled experiment (Gümen et al., 2005) and a field trial (Kok et al., 2016a). Also, number of days open was numerically less in cows with an omitted DP compared with cows with a conventional DP in the current study. Fertility is a complex trait influenced by many factors (reproductive management, EB, climate, etc.), so further research with a large number of animals, more accurate heat detection, and earlier insemination is needed to assess the effects of DP length on fertility.

Table 7.1. Reproductive performance of dairy cows after 0, 30, or 60 days dry period and fed a glucogenic (G) or lipogenic (L) diet in the first lactation (LSM $\pm$ SEM).

Variable	Dry period length (d)			SEM ¹	P-value		
	0	30	60		DPL ²	Diet	Parity
Cows, n	56	55	56				
Calving to first AI ^{3,4}	73.1	75.4	78.8	3.0	0.40	0.38	0.12
First-AI pregnancy rate (%)	33.3 (18/54)	27.5 (14/51)	37.5 (21/56)		0.51	0.60	0.39
Overall pregnancy rate (%)	83.7 (41/49)	86.3 (44/51)	96.4 (53/55)		0.11	0.45	0.15
Days open ⁴	107.0	114.5	116.3	7.7	0.67	0.57	0.58
Services per pregnancy ⁴	1.8	2.1	1.9	0.2	0.44	0.90	0.04
Calving interval ⁴	392.3	398.0	403.6	8.7	0.65	0.39	0.34
Fertagyl use ⁵ (%)	19.6 (11/56)	21.8 (12/55)	19.6 (11/56)		0.94	0.73	0.63

¹SEM = pooled standard error of the least squares means.²DPL = dry period length.³AI = artificial insemination.⁴Statistical analysis was based on natural log-transformed data; back-transformed data are presented.⁵Cows treated with Fertagyl during the whole lactation.

7.3. Long-Term Effects of Shortening or Omitting the Dry Period

In Chapter 4, we reported that (1) shortening the DP caused less improvement in EB in the second lactation compared with the first lactation after implementation of DP length treatments, and (2) omitting the DP caused less milk yield losses, and less improvement in EB and metabolic status in the second lactation. The less improvement of EB in cows with a shortened DP was related to similar milk yield (38.6 vs. 38.5 kg/d for the first and second lactation, respectively), but lower DMI (22.2 vs. 20.8 kg/d for the first and second lactation, respectively) in the second lactation compared with the first lactation. In cows with an omitted DP, a selection effect might play a role in these differences between the first and second lactation. In the current study, 20 out of 39 cows assigned to the 0 d DP group achieved a 0 d DP over 2 subsequent lactations. We found that these cows that were able to be milked continuously for 2 subsequent lactations (0→0 d) had greater 365-d FPCM yield and greater persistency in the first lactation (Table 7.2) compared with cows that could not be milked continuously for 2 subsequent lactations (0→67 d). This implies that cows with a 0→0 d DP had a greater potential for milk yield compared with cows which dried themselves off

before the second calving. In the second lactation of the current study, cows with a 0→67 d DP were attributed to another group, which could partly explain the lower milk yield losses and less improvement in EB in the second lactation compared with the first lactation in cows with an omitted DP. In addition, differences between the first and second lactation after omitting the DP may also related to other 2 factors: BCS at calving and parity of the cows, which will be further discussed below.

7.3.1 Body Condition Score

In chapter 4, we showed that high precalving BCS, which was related to low DMI, in cows with an omitted DP was one of the reasons for less pronounced improvement of EB in the second lactation compared with the first lactation after implementation of DP omission. In the first lactation of the current study, cows with an omitted DP produced less milk and had similar energy intake, compared with cows with a conventional DP. Lower milk yield and similar energy intake led to excessive weight gain in these cows during the mid and late lactation. After correction for precalving BCS, the improvement of EB was more pronounced in early lactation of the second lactation in cows with an omitted DP compared with cows with a conventional DP. These results confirm that high precalving BCS is a risk factor for severe negative EB and metabolic disorders in the second lactation after implementation of DP length treatments. Fattening in mid or late lactation may eliminate the beneficial effects of omitting the DP on EB and metabolic status or even aggravate the negative EB in the subsequent lactation. We propose that it is necessary to reduce energy intake in mid and late lactation to prevent excessive weight gain when the DP is omitted.

Also, in the first lactation, precalving BCS interacted with the effect of DP length on milk yield, EB, and metabolic status (Figure 7.1). Omitting the DP in the first lactation had more pronounced improvement of EB and metabolic status in lean cows (precalving BCS < 3.25; 57 vs. -260 kJ/kg^{0.75}·d for 0 vs. 60 d DP, respectively) compared with fat cows (precalving BCS ≥ 3.25; -161 vs. -286 kJ/kg^{0.75}·d for 0 vs. 60 d DP, respectively). The less pronounced improvement in EB in fat cows was due to a high milk yield after omitting the DP compared with lean cows. In the first 8 weeks of the first lactation, FPCM yield losses were 13.2 and 4.3 kg/d for lean cows with a 0 or 30 d dry period, and were 4.5 or 1.3 kg/d for fat cows with a 0 or 30 d dry period, respectively, compared with cows with a 60 d dry period.

Table 7.2. Fat- and protein-corrected milk (FPCM) yield, peak yield (PY), time of peak yield (PT), and lactation persistency (LP) in the first lactation for dairy cows categorised according to achievement of dry period omission for 2 subsequent lactations (LSM $\pm$ SEM).

Variable ¹	Dry period achievement ²		SEM ³	P-values		
	0→0 d	0→67 d		Achievement	Diet	Parity
Cows, n	20	19				
FPCM ₃₀₅ (kg)	8855	7985	376	0.11	0.88	0.23
FPCM ₋₆₀ (kg)	1090	857	85	0.06	0.20	0.01
FPCM ₃₆₅ (kg)	9945	8842	366	0.04	0.88	0.56
PT (DIM)	38.2	36.2	2.0	0.49	0.69	0.01
PY (kg/d)	39.9	38.7	1.5	0.60	0.77	0.01
LP	-0.099	-0.114	0.005	0.04	0.66	<0.01

¹FPCM₃₀₅ = 305-d FPCM yield; FPCM₋₆₀ = FPCM yield during 60 d precalving; FPCM₃₆₅ = FPCM₃₀₅ + FPCM₋₆₀.

²0→0 d: cows had 0 d dry period for 2 subsequent lactations; 0→67 d: cows had the 0 d dry period in the first lactation, but had a milk yield of < 4 kg/d at least 30 d before the expecting calving date in the second lactation and were dried off (actual days dry: actual days dry: 67 $\pm$ 8 d).

³SEM = pooled standard error of the least squares means.

In modern high-producing dairy cows, BCS at calving was associated positively with milk yield in early lactation. Domezq et al. (1997) found that an increase of 1.0 point in BCS from drying off to calving was associated with 545.5 kg more milk during the first 120 d in lactation. Roche et al. (2007) suggested that the optimal BCS at calving for milk yield is approximately 3.5. It was suggested that greater milk yield from increased BCS at calving could be the results of increased mammary cell proliferation (Roche et al., 2007). In the current study, no differences in milk yield were found between fat and lean cows after a conventional DP. This may be because the BCS threshold of 3.25 is close to the optimal BCS for milk yield in cows after a conventional DP. However, BCS > 3.25 cows had 7.4 kg/d greater milk yield compare BCS < 3.25 cows after omitting the DP, which implies that optimal BCS at calving for milk yield might be higher in cows with an omitted DP compared with cows with a conventional DP. Accordingly, improvement of EB and metabolic status after omitting the DP was more pronounced in lean cows compared with fat cows in the first lactation.

Figure 7.1. (A) Milk yield (kg); (B) Fat- and protein-corrected (FPCM) milk yield (kg); (C) DMI (kg/d); (D) Energy balance (kJ/kg^{0.75}·d); (E) Plasma free fatty acids (FFA) (mmol/L); (F) Plasma β -hydroxybutyrate (BHB) (mmol/L); (G) Glucose (mmol/L); (H) Insulin (μ IU/mL) during first 8 weeks after calving for cows with precalving BCS < 3.25 or BCS $\geq$ 3.25 after 0, 30, or 60 d dry period in the first lactation after implementation of dry period length treatments. Values represent least squares means ($\pm$ SEM) per dry period length per BCS class; Bars with different letters (a, b) within BCS class differ ($P < 0.05$).

Additionally, interactions between BCS and DP length were present for FPCM yield during 60 d precalving (**FPCM₋₆₀**), peak yield, and LP in the first lactation (Table 7.3). Fat cows with an omitted DP had greater milk yield (42.6 vs. 36.8 kg, $P < 0.01$), lower FPCM₋₆₀ (752 vs. 1,164 kg, $P < 0.01$), and tended to have lower LP (-0.121 vs. -0.099 , $P = 0.07$) compared with lean cows with an omitted DP. These differences between fat and lean cows were not present in cows with a shortened or conventional DP. In chapter 6, we illustrated that peak yield was negatively related to LP. Thus, the lower LP could be related to the greater peak yield in fat cows compared with lean cows after omitting the dry period. It seems that the high milk yield in late lactation, which might be caused by the relatively high LP in previous lactation in lean cows, is the reason for greater FPCM₋₆₀ in lean cows compared with fat cows after omitting the DP. Due to this greater FPCM₋₆₀, the total FPCM yield from 60 d precalving until 305 d postcalving (FPCM₃₆₅) in lean cows did not differ from the fat cows after omitting the DP (9,123 vs. 9,672 kg for lean vs. fat cows with 0 d DP, $P = 0.71$). In the second lactation, precalving BCS had similar effects on lactation characteristics. However, interaction between precalving BCS and DP length on lactation characteristics could not be determined due to limited number of cows in the second lactation.

In conclusion, the less pronounced improvement of EB in the second lactation after omitting the DP could be related to high BCS at the onset of lactation. In addition, omitting the DP in lean cows (precalving BCS < 3.25) results in more pronounced improvement in EB and metabolic status in early lactation of the first lactation, which is related to the lower milk yield in lean cows compared with fat cows (precalving BCS ≥ 3.25) after omitting the DP.

7.3.2 Parity

Parity is another major factor influencing the effects of omitting DP on milk yield and EB in dairy cows. We showed that, compared with a conventional DP of 60 d, 305-d FPCM yield losses in young cows (parity = 2) was greater than older cows (parity > 2) after omitting the DP (28% vs. 18%) in the first lactation (Chapter 6). This is in line with previous studies reporting that omitting the DP caused less milk yield losses in older cows compared with young cows (Annen et al., 2004; Mantovani et al., 2010). Collier et al. (2012) suggested that parity sensitivity to omission of the dry period could be because the mammary is

Table 7.3. Fat- and protein-corrected milk (FPCM) yield, peak yield (PY), time of peak yield (PT), and lactation persistency (LP) for dairy cows categorised according to BCS before calving in the first lactation¹ (LSM $\pm$ SEM).

Variable ²	BCS		SEM ³	Dry period length (d)			SEM	P-value ⁴							
	<3.25	≥3.25		0	30	60		BCS	DPL	Diet	Parity	BCS×DPL			
Cows, n	93	48		47	46	48									
FPCM ₃₀₅ (kg)	9552	9837	159	8439	9893	10750	196	0.22	<0.01	0.36	0.44	0.11			
FPCM ₆₀ (kg)	591	434	28.6	958	579	1	35	<0.01	<0.01	0.07	<0.01	<0.01			
FPCM ₃₆₅ (kg)	10143	10271	163	9397	10472	10751	200	0.58	<0.01	0.57	0.43	0.43			
PT (DIM)	36.0	33.3	1.2	37.2	35.3	31.4	1.4	0.12	0.01	0.92	<0.01	0.28			
PY (kg/d)	42.9	44.7	0.6	39.7	44.6	47.1	0.7	0.04	<0.01	0.33	<0.01	<0.01			
LP	-0.105	-0.112	0.003	-0.110	-0.111	-0.106	0.004	0.15	0.62	0.83	<0.01	0.03			

¹Month -1 and -2 relative to calving.

²FPCM₃₀₅ = 305-d FPCM yield; FPCM₆₀ = FPCM yield during 60 d precalving; FPCM₃₆₅ = FPCM₃₀₅ + FPCM₆₀.

³SEM = pooled standard error of the least squares means.

⁴DPL = dry period length; B = Body condition score category.

still developing in young cows during lactation. This continued mammary development may be hampered by omitting the DP. Due to the greater milk yield losses, young cows with an omitted DP had more pronounced improvement in EB in early lactation compared with older cows with an omitted DP (van Knegsel et al., 2014). In addition, the less milk yield losses in cows with an omitted DP, which led to less pronounced improvement of EB in the second lactation compared with the first lactation, could be the result of the increased parity number.

Shortening the DP resulted in similar 305-d FPCM yield losses in older cows (9%) compared with young cows (6%) in the first lactation of the current study, which is in line with Rastani et al. (2005) who reported that shortening the DP decreased milk yield in both young and older cows. It might be speculated that 30 d DP is enough to sustain the mammary development in young cows. The similar milk yield losses between older cows and young cows after shortening the DP suggests that shortening the DP was less parity sensitive compared with omitting the DP. However, several studies reported that shortening the DP decreases milk yield only in young cows, but not in older cows (Annen et al., 2004; Pezeshki et al., 2007). The reasons for discrepancies among studies are not clear. Rastani et al. (2005) suggested that it could be related to differences in genetic potential for milk yield between cows among studies.

7.4. Other Factors Which Affected Energy Balance and Metabolic Status of Cows with Different Dry Period Lengths

Besides BCS and parity, in the current study, we found that several other factors also affected EB and metabolic status in the early lactation, independent or dependent of DP length in dairy cows.

7.4.1 Glucogenic vs. Lipogenic Nutrients

In the first lactation of current study, feeding a glucogenic diet in the early lactation improved EB by a reduction in milk fat content compared with a lipogenic diet, independent of DP length (van Knegsel et al., 2014). Also in earlier studies, feeding a glucogenic diet improved EB and metabolic status in early lactation compared with a lipogenic diet (Van Knegsel et al. 2007b, 2007d). In the first lactation of the current study, feeding a glucogenic diet improved EB, decreased plasma BHB concentration, but had no effects on plasma concentration of FFA,

glucose, IGF-1, and insulin compared with a lipogenic diet. In the second lactation of the current study, by contrast, feeding a glucogenic diet in early lactation had no effects on EB, but resulted in greater plasma glucose, insulin, IGF-1, and lower plasma FFA, and BHB concentrations compared with a lipogenic diet, independent of DP length (Chapter 4). This improved metabolic status in cows fed glucogenic diet was extensively discussed in Chapter 4. Briefly, in the second lactation, the increased milk yield and decreased milk fat percentage in cows fed a glucogenic diet resulted in the similar milk energy output and thus similar EB compared with cows fed a lipogenic diet. This improved metabolic status independent of EB could imply the more efficient generation of energy to the body in cows fed a glucogenic diet. In early lactation, increased availability of lipogenic nutrients (acetyl-coenzyme-A) due to mobilization of body fat and decreased availability of glucogenic nutrients (oxaloacetate) due to high lactose production causes an imbalanced ratio of oxaloacetate to acetyl-coenzyme-A. This imbalance leads to limited production of citrate to form ATP in the Krebs cycle and respiratory chain reaction (Van Knegsel et al., 2007a). Feeding a glucogenic diet, compared with a lipogenic diet, helps to restore the balance between lipogenic and glucogenic nutrients, and improves the efficiency of generation of energy for body as ATP. Therefore, cows fed a glucogenic diet could mobilize less body reserves to meet the energy deficiency compared with a lipogenic diet in early lactation

This more pronounced improvement of metabolic status in cows fed a glucogenic diet in the second lactation could be related to more severe negative EB due to the increased milk yield and reduced energy intake in cows in the second lactation compared with the first lactation. In addition, Van Knegsel et al. (2007c) suggested that feeding a glucogenic diet has more pronounced effects in older cows compared with young cows in early lactation. We hypothesized that feeding a glucogenic diet could decrease body fat mobilization and improve metabolic status in cows with a severe negative EB or older cows.

7.4.2 *DGAT1* Gene

The enzyme acyl-coenzyme A: diacylglycerol acyltransferase 1 (DGAT1) plays a key role in the final step in triglyceride synthesis (Grisart et al., 2002). Diacylglycerol acyltransferase 1 was shown to have a major effects on bovine milk yield and milk characteristics (Grisart et al., 2002). Bovenhuis et al. (2015) reported

that cows of *DGATI* AK genotype had higher values for total energy output in milk and might have a more negative EB compared with cows of *DGATI* AA and KK genotypes in primiparous cows. In the current experiment, we found that an interaction between *DGATI* genotype and DP length was present for both milk yield and EB (van Knegsel et al., 2013). Cows of the *DGATI* AK genotype had a less improved EB compared with cows of the *DGATI* AA and KK genotypes after omitting the DP because of less milk yield losses in early lactation. In future studies, it would be interesting to investigate the underlying mechanism of the interaction between *DGATI* genotype and DP length.

7.4.3 Uterine Health Status

In chapter 5, we reported that DP length had no effects on uterine health, as indicated by vaginal discharge score (VDS) in weeks 2 and 3 after calving in the second lactation. In addition, we found that uterine health status was related to milk yield and metabolic status in first 3 weeks after calving independent of DP length. A non-recovering uterine environment (NRU, VDS = 2 or 3 in week 3), was related to a lower DMI and milk yield. A healthy uterine environment (HU, VDS = 0 or 1 in both weeks 2 and 3) was related to a better metabolic status, as indicated by increased plasma glucose and insulin concentrations.

In previous studies, risk factors for purulent vaginal discharge were twins, retained placenta, and metritis (Dubuc et al., 2010; LeBlanc et al., 2002). In the current study, shortening or omitting the DP had no effect on incidence of twins ($n = 3, 1, 3$, and 0 for cows a $0, 30, 60$, or $0 \rightarrow 67$ DP, respectively), retained placenta ($n = 2, 7, 6$, and 0 for cows a $0, 30, 60$, or $0 \rightarrow 67$ DP, respectively), or metritis ($n = 3, 12, 11$, and 5 for cows a $0, 30, 60$, or $0 \rightarrow 67$ DP, respectively), which may explain the lack of effect of DP length on vaginal discharge score.

Furthermore, we found that the lower milk of cows with a NRU remained throughout lactation. Cows with a NRU had lower peak yield compared with cows with a HU, and had lower FPCM₃₀₅, FPCM₃₆₅, peak yield, but higher LP compared with cows with a recovering uterine environment (RU, VDS = 2 or 3 in week 2 and VDS = 0 or 1 in week 3) (Table 7.4; Figure 7.2). To our knowledge, this is the first study reporting the long-term effect of uterine health status in early lactation on lactation curve characteristics. In the current study, the decreased milk yield in early lactation in cows with a NRU did not result in an improved EB because of

the decreased DMI for cows with a poor uterine health status. Moreover, this decreased milk yield could not be compensated by a higher LP afterward. Due to limited number of cows in the current study, interaction between uterine health status and DP length on lactation curve characteristics could not be determined. We hypothesized that NRU may exacerbate the milk yield losses after shortening or omitting the DP without benefits on EB in early lactation in dairy cows. It would be interesting to determine whether purulent vaginal discharge or endometritis in early lactation interacts with DP length with respect to milk yield, EB, metabolic status, and fertility in a large scale study.

7.5. Achievement of Omitting or Shortening the Dry Period

The remarkable increase in milk yield per cow in modern dairy cows due to genetic selection and improved nutritional and farm management in recent years can be considered as the precondition for implementation of shortening or omitting the DP. Zobel et al. (2015) found that the milk yield at drying off of the modern cow is similar to the peak yield of a cow in 1975 (around 25 kg). Before the calving

Table 7.4. Fat- and protein-corrected milk (FPCM) yield, peak yield (PY), time of peak yield (PT), and lactation persistency (LP) for dairy cows categorised according to uterine health status (LSM $\pm$ SEM).

Variable ¹	Uterine health status ²			SEM ³	P-value		
	HU	RU	NRU		Uterine health	DPL ⁴	Diet
Cows, n	14	29	37				
FPCM ₃₀₅ (kg)	10456 ^{ab}	10522 ^a	9882 ^b	204	0.02	0.60	0.19
FPCM ₋₆₀ (kg)	184	200	195	27	0.93	<0.01	0.57
FPCM ₃₆₅ (kg)	10685 ^{ab}	10763 ^a	10118 ^b	201	0.02	0.69	0.22
PT (DIM)	33.8	32.8	35.1	1.6	0.49	0.55	0.47
PY (kg/d)	47.9 ^a	48.4 ^a	44.2 ^b	0.9	<0.01	0.33	0.07
LP	-0.121 ^{ab}	-0.123 ^a	-0.106 ^b	0.005	0.01	0.38	0.07

^{a,b} Values within uterine health status with different superscripts differ ($P < 0.05$).

¹FPCM₃₀₅ = 305-d FPCM yield; FPCM₋₆₀ = FPCM yield during 60 d precalving; FPCM₃₆₅ = FPCM₃₀₅ + FPCM₋₆₀.

²Healthy uterine environment (HU): vaginal discharge score (VDS) = 0 or 1 in both week 2 and 3; recovering uterine environment (RU): VDS = 2 or 3 in week 2 and vaginal discharge score = 0 or 1 in week 3; non-recovering uterine environment (NRU): VDS = 2 or 3 in week 3.

³SEM = pooled standard error of the least squares means.

⁴DPL = dry period length.

Figure 7.2. Estimated lactation curve of fat- and protein-corrected milk (FPCM) for dairy cows categorised according to uterine health status in the second lactation after implementation of dry period length treatment. Healthy uterine (HU): vaginal discharge score (VDS) = 0 or 1 in both weeks 2 and 3; Recovering uterine (RU): VDS = 2 or 3 in week 2 and VDS = 0 or 1 in week 3; Not-recovering uterine (NRU): VDS = 2 or 3 in week 3.

in the first lactation of the current study, the average daily FPCM yield at week 9 precalving in cows with a shortened or omitted DP were around 20 kg (19.5 ± 7.9 kg/d, means $\pm$ SD). This high milk yield in late lactation ensures that cows were able to produce milk until week 4 precalving or until calving.

7.5.1 Omitting or Shortening the Dry Period for the First Time

In most published studies, the short- or no-DP management strategy was only implemented once, and the consequences were only investigated in the lactation immediately following the DP treatment (i.e. the first lactation). Generally, shortening the DP to 30 or 35 d in dairy cows in the first lactation could be achieved for most cows across studies (Andersen et al., 2005; Pezeshki et al., 2007; Rastani et al., 2005), which is consistent with the current study, where the dry period is shortened to 30 d. The actual days dry of cows attributed to 0 d DP group in the first lactation was around 5 d across studies (Andersen et al., 2005; De Feu et al., 2009; Rastani et al., 2005). In the current study, the achievement of DP omission (2 ± 1 d) in the first lactation was slightly better compared with previous studies. Difference in management of the cows with 0 d DP before calving may play a role in this better achievement of DP omission. In previous studies,

milking was discontinued if milk yield precalving in cows assigned to 0 d DP group decreased below 2 kg/d (De Feu et al., 2009; Rastani et al., 2005) or 5 kg/d (Andersen et al., 2005). In the current study, cows assigned to 0 d DP group in the first lactation were milked even if milk yield was lower than 2 kg/d, which improved the achievement of DP omission. However, it should be mentioned that milking cows with little milk yield precalving might increase cost and labor in herds.

7.5.2 Omitting or Shortening the Dry Period for the Second Time

In the current study, 20 out of 39 cows assigned to 0 d DP group in the current study achieved DP omission for 2 subsequent lactations and 31 out of 41 cows assigned to 30 d DP group in the current study had a DP length between 20 to 40 d prior to the calving in the second lactation. To our knowledge, only one published study, under controlled experimental conditions, has investigated the effects of omitting the DP in dairy cows over 2 subsequent lactations (Remond et al., 1997). In that study, only 2 out of 21 cows assigned to 0 d DP group achieved DP omission for 2 consecutive lactations. These results indicate that implementing the DP omission for two subsequent lactations is more difficult compared with omitting the DP only once. To apply 0 d DP in practice, it is necessary to identify the characteristics of cows that are able to be milked continuously for more lactations. Therefore, cows assigned to 0 d DP group were grouped based on their achievement of DP omission in the second lactation (0→0 or 0→67 days dry) to analyse the differences between these two groups on milk yield, EB, metabolic status, and fertility in the first lactation (Table 7.2).

We found that there were two main factors that affected the success of DP omission in the second lactation. Firstly, cows with an omitted DP for 2 subsequent lactations (0→0 d) seemed to have greater milk yield in late lactation of the first lactation compared with cows with an omitted DP only for the first lactation, but dried themselves off before the second lactation (0→67 d). It can be explained by the similar PY but higher LP, which allowed a longer lactation in cows with a 0→0 d DP compared with cows with a 0→67 d DP (Table 7.2). Secondly, cows with a 0→0 d DP had 27 d less number of days open and therefore a shorter calving interval compared with cows with a 0→67 d DP ($P = 0.11$, Table 7.5). Shortened calving interval is important for the achievement of DP omission for the second time. The shortened calving interval allows cows enter into next lactation cycle earlier, which

means a greater milk yield in late lactation. The causes for the shortened calving interval in cows with a 0→0 d DP in the current study is not completely clear. More fertagyl use in cows with a 0→67 d DP (5.0 vs 26.3%, $P = 0.12$) compared with cows with a 0→0 d DP might reflect more reproductive problems in these cows.

Energy balance and metabolic status in early lactation of the first lactation did not differ between cows with a 0→0 d DP and cows with a 0→67 d DP (Table 7.6). This indicates that the achievement of DP omission could not be predicted by EB or metabolic status in early lactation. However, the numerically greater plasma glucose, IGF-1, and insulin concentration could be related to improved fertility and less number of days open in cows with a 0→0 d DP compared with cows with a 0→67 d DP. Further research with a larger number of cows is needed to clarify this relationship. In summary, high milk yield potential, especially high LP, and short calving interval were two main factors which affect the success of DP omission over multiple lactations (Figure 7.3).

7.6. The Application of Shortening or Omitting the Dry Period

One of the limiting factors in application of a shortened or omitted DP is the decreased milk yield in the subsequent lactation compared with a conventional DP. However, the reported milk yield losses after shortening or omitting the DP are mostly studies which focused on the first lactation after shortening or omitting the DP and did not consider the additional milk yield precalving.

7.6.1 Milk Yield Losses in Cows with a Shortened or Omitted Dry Period?

In chapter 6, we found that cows that were able to be milked continuously for 2 subsequent lactations (0→0 d) and cows with a shortened DP had similar average daily FPCM yield throughout 2 subsequent lactations (from 60 d before the calving in the first lactation to 305 d after the calving in the second lactation) compared with cows with a conventional DP. This is because cows with an shortened or omitted DP produced extra milk precalving and cows with a 0→0 d DP had relatively less total days needed to go through 2 subsequent lactations compared with cows with a 60 d DP, due to a shorter calving interval (376 vs. 415 vs. 410 vs. 408 d for 0→0, 30, 60, and 0→67 d DP, respectively, $P = 0.34$; Chapter 6). The shortened calving interval not only resulted in greater daily milk yield

Table 7.5. Reproductive performance and characteristics of the ovarian cycle in the first lactation for dairy cows categorised according to achievement of dry period omission for 2 subsequent lactations (LSM $\pm$ SEM).

Variable	Dry period achievement ¹		SEM ²	P-values ³		
	0→0	0→67		Achievement	Diet	Parity
Cows, n	20	18				
Calving to onset of luteal activity (days)	24.5	20.5	2.2	0.21	0.10	0.31
Luteal phase length (days)	15.3	18.9	2.2	0.24	0.87	0.07
Ovarian cycle length (days)	23.0	25.3	2.5	0.50	0.89	0.15
P4, max ³ (ng/mL)	12.18	10.45	1.35	0.37	0.24	0.02
P4, mean ⁴ (ng/mL)	7.37	6.06	0.77	0.23	0.17	0.06
Normal resumption of ovarian cyclicity (%)	60.0 (12/20)	53.3 (8/15)		0.74	0.64	0.83
Abnormal resumption of ovarian cyclicity:						
Type I: delayed first ovulation or anovulation (%)	10.0 (2/20)	0.0 (0/15)		0.28	0.20	0.83
Type II: prolonged luteal phase (%)	30.0 (6/20)	46.7 (7/15)		0.35	0.78	0.71
Type III: cessation of cyclicity (%)	0.0 (0/20)	0.0 (0/15)		na ⁵	na	na
Fertagyl use ⁶ (%)	0.0 (0/20)	21.1 (4/19)		0.09	0.85	0.50
Calving to first AI	72.4	78.4	4.3	0.33	0.13	0.13
First-AI conception rate (%)	45.0 (9/20)	42.1 (8/19)		0.85	0.94	0.58
Days open	102.6	129.2	11.6	0.11	0.84	0.87
Services per conception	1.8	2.3	0.3	0.28	0.62	0.24
Calving interval	383.4	410.2	11.9	0.12	0.87	0.88
Fertagyl use ⁷ (%)	5.0 (1/20)	26.3 (5/19)		0.12	0.75	0.83

¹0→0: cows had 0 d dry period for 2 subsequent lactations; 0→67: cows had the 0 d dry period in the first lactation, but had a milk yield of < 4 kg/d at least 30 d before the expecting calving date in the second lactation and were dried off.

²SEM = pooled standard error of the least squares means.

³Maximal P4 concentration.

⁴Mean P4 concentration.

⁵na: not available;

⁶Cows treated with Fertagyl within 100 DIM that prohibited evaluation of resumption of ovarian cyclicity.

⁷Cows treated with Fertagyl during the whole lactation.

Table 7.6. Milk yield, dry matter intake (DMI), body condition score (BCS), energy balance (EB), metabolites and hormones in early lactation¹ of the first lactation for dairy cows categorised according to achievement of dry period omission for 2 subsequent lactations (LSM $\pm$ SEM).

Variable	Dry period achievement ²		SEM ³	P-values				
	0→0	0→67		Achievement	Diet	Parity	Time	Achievement $\times$ time
Cows, n	20	19						
Milk yield (kg/d)	34.9	32.6	1.5	0.28	0.21	<0.01	<0.01	0.38
FPCM ⁴ (kg/d)	39.1	36.1	1.7	0.22	0.39	<0.01	<0.01	0.04
DMI (kg/d)	21.8	21.7	0.5	0.79	0.38	0.15	<0.01	0.22
Body weight (kg)	678.4	694.1	14.3	0.44	0.54	0.03	<0.01	0.75
BCS	2.8	3.1	0.1	0.12	0.95	0.12	0.63	0.97
EB ⁵ (kJ/kg ^{0.75} ·d)	-83	-52	40	0.58	0.56	<0.01	<0.01	0.63
Cows, n	12	7						
FFA ⁶ (mmol/L)	0.26	0.27	0.06	0.93	0.44	0.03	<0.01	0.07
BHB ⁷ (mmol/L)	0.61	0.64	0.05	0.61	0.78	0.05	0.21	0.39
Glucose (mmol/L)	3.83	3.58	0.14	0.32	0.74	0.12	0.04	0.03
Urea (mmol/L)	2.95	2.70	0.19	0.39	0.12	0.48	<0.01	0.67
IGF-1 (ng/mL)	114.08	98.89	13.64	0.48	0.64	<0.01	0.15	0.29
Insulin (μ IU/mL)	23.74	16.98	2.77	0.13	0.92	0.10	0.13	0.65

¹week 1, 2, 3, 4, 5, 6, 7, and 8 post-calving.

²0→0: cows had 0 d dry period for 2 subsequent lactations; 0→67: cows had the 0 d dry period in the first lactation, but had a milk yield of < 4 kg/d at least 30 d before the expecting calving date in the second lactation and were dried off; Excluded: cows had 0 d dry period in the first lactation, but were excluded before the calving in the second lactation.

³SEM = pooled standard error of the least squares means.

⁴Fat- and protein-corrected milk.

⁵Energy balance; calculated with the VEM system (Van Es, 1975).

⁶Free fatty acids.

⁷ β -hydroxybutyrate; statistical analysis was based on natural log-transformed data; back-transformed data are presented.

Figure 7.3. Estimated lactation curve of fat- and protein-corrected milk (FPCM) in the first lactation after implementation of dry period length treatment for dairy cows categorised according to achievement of dry period omission for 2 years. 0→0 d: cows had 0 d dry period for 2 subsequent lactations; 0→67 d: cows had the 0 d dry period in the first lactation, but had a milk yield of < 4 kg/d at least 30 d before the expecting calving date in the second lactation and were dried off.

before calving, but also reduced the total milk yield losses in cows with a shortened or omitted DP.

Omitting the DP improved the resumption of ovarian cyclicity, which was related with an improved EB and metabolic status in early lactation. Consequently, we propose that the optimizing reproductive management like shorter voluntary waiting period and earlier insemination postcalving or more accurate heat detection in cows with a shortened or omitted DP may shorten the calving interval further and therefore decrease the milk yield losses due to a shorter or omitted DP further.

For a more accurate evaluation of the effect of DP length on milk yield, Kok et al. (2016b) proposed a new approach to compare milk yield between cows with different DP lengths: effective lactation yield. The effective lactation yield was defined as the daily yield from 60 d before calving to 60 d before next calving, which corrects milk yield for differences in calving interval and additional milk yield precalving. In a field study, application of the effective lactation yield, compared

with traditional 305-d milk yield, decreased the milk yield losses for cows with an omitted or shortened DP from 7.0 and 2.3 kg to 3.1 and 0.9 kg of FPCM per cow per day, respectively (Kok et al., 2016b).

In addition, milk yield losses after omitting the DP are also affected by parity. In the current study, the average daily FPCM yield throughout 2 subsequent lactations did not differ among DP length in older cows, even when cows that could not be milked continuously for 2 subsequent lactations were not attributed to a new group (27.3 vs. 27.2 vs. 27.8 kg/d for 0, 30, or 60 d DP, respectively, $P = 0.79$). This means omitting the DP does not decrease milk yield in a long-term perspective in older cows, regardless of the achievement of DP omission before the calving of the second lactation.

7.6.2 The Application of Shortening or Omitting the Dry Period on Commercial Farms

In practice, some farmers have shifted their DP management strategy from a conventional DP to a shortened or omitted DP. In a field study involving 11 commercial dairy Dutch herds, omitting the DP resulted in a reduction in 305-d milk yield between 12 and 32% compared with the milk yield in the lactation before the implementation of 0 d DP strategy (Steenefeld et al., 2013), which is comparable with results from the current experiment. It was noteworthy that some farmers are satisfied with the no-DP strategy because of healthier cows, decreased work load due to less dietary and group transitions, and improved fertility, whereas two farmers stopped using it because of the lower milk yield (Steenefeld et al., 2013). In addition, omission of the DP could decrease the use of preventive antibiotics at drying off, which could reduce the veterinary costs and possibly decrease the risk of development of microbial resistance to antibiotics. Steenefeld et al. (2013) found a higher SCC (about 20,000 cells/ml) in cows with an omitted DP compared with cows with a conventional DP. The authors, however, indicated that the high SCC in cows with an omitted DP could be related to the dilution effects of milk yield on SCC. When milk yield was included as a covariate in the model to analyse SCC, the effect of DP length on SCC was no longer present (Steenefeld et al., 2013). Consistent with this, the SCC was not significantly greater in cows with an omitted DP compared with cows with a conventional DP after correcting for milk yield in both the first and second lactations of the current

experiment (Van Kneegsel et al., 2014; Chapter 4). Moreover, no DP effects on incidence of mastitis in early lactation were found in both lactations of the current study (First lactation: 16.1%, 12.7%, and 16.1% for cows with a 0, 30, or 60 d DP, respectively; Second lactation: 15.0%, 28.6%, 22.4%, and 26.3% for cows with a 0, 30, 60, or 0→67 d DP, respectively).

Santschi et al. (2011) investigated the economic impact of a short DP (35-d) compared with a conventional DP (60 d) with 850 cows from 13 commercial dairy herds in Canada. They concluded that an average Canadian herd would be economically beneficial from shortening the DP, because cows with a shortened DP had lower replacement rate and greater milk yield when taking into account the additional milk yield precalving compared with cows with a conventional DP. However, knowledge about the economic impact of omitting the DP is scarce, especially when additional milk yield precalving and shortened calving interval are taken into account. Field study with a large number of animals is needed to evaluate the economic impact of omitting the DP based on effective lactation yield. Additionally, other factors (such as cow health, fertility, work load, and antibiotics use) also should be taken into account for a complete economic evaluation of the short- or no-DP management strategy.

7.6. Conclusion

In conclusion, shortening or omitting the DP improved EB in early lactation compared with a conventional DP in dairy cows due to a decreased milk yield in the subsequent lactation. Additional milk produced precalving in cows with a shortened or omitted DP could partly compensate for milk yield losses, and milk yield losses might be further decreased if a shorter calving interval is realised when the DP is shortened or omitted. Shortening the DP did not influence metabolic status and fertility compared with a conventional DP in dairy cows in both the first and second lactations. Omitting the DP improved metabolic status and resulted in an increased proportion of cows with a normal resumption of ovarian activity in early lactation, but had no effects on days open or pregnancy rate compared with a conventional DP. A similar energy intake but lower milk yield in cows with an omitted DP, compared with cows with a conventional DP, led to excessive weight gain in the first lactation after implementation of DP length treatments. In the second lactation after implementation of DP length and dietary treatments, effects of omitting the DP on

milk yield reduction and improvements of EB and metabolic status were less pronounced compared with the first lactation, which was related to high BCS at the onset of the second lactation and reduced milk yield losses in cows with an omitted DP in the second lactation. Around half cows that assigned to the 0 d DP group were not able to be milked continuously for 2 subsequent lactations. These cows had a more severe negative EB and poor metabolic status compared with cows in other groups in the second lactation, which was due to both an increased milk yield and a decreased DMI related with a high BCS at calving in these cows. Feeding a glucogenic diet improved EB and metabolic status compared with lipogenic diet in the first and second lactations independent of DP length. Further research could assist in determining whether reduced dietary energy intake in cows with an omitted DP during mid and late lactation could prevent the excessive weight gain, and whether optimized reproductive management could shorten the calving interval in cows with a shortened or omitted DP and decreases the milk yield losses. Finally, effects of DP length on milk yield, EB, metabolic status, and fertility depend on many factors (e.g. parity, BCS, calving interval, genotype, lactation persistency). Therefore, further studies with a multidisciplinary approach are needed to determine whether it is possible to predict the optimal DP length for each individual cow.

References

- Andersen, J. B., T. G. Madsen, T. Larsen, K. L. Ingvarsten, and M. O. Nielsen. 2005. The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. *J. Dairy Sci.* 88:3530-3541.
- Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. *J. Dairy Sci.* 87:3746-3761.
- Annen, E. L., A. C. Fitzgerald, P. C. Gentry, M. A. McGuire, A. V. Capuco, L. H. Baumgard, and R. J. Collier. 2007. Effect of continuous milking and bovine somatotropin supplementation on mammary epithelial cell turnover. *J. Dairy Sci.* 90:165-183.
- Annen, E. L., C. M. Stiening, B. A. Crooker, A. C. Fitzgerald, and R. J. Collier. 2008. Effect of continuous milking and prostaglandin E2 on milk production and mammary epithelial cell turnover, ultrastructure, and gene expression. *J. Anim. Sci.* 86:1132-1144.
- Bauman, D. E., J. M. Elliot, and T. B. Mepham. 1983. Control of nutrient partitioning in lactating ruminants. *Biochemistry of lactation*, Elsevier Science Publishers BV, Amsterdam, the Netherlands, pp. 437-468.
- Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73:2804-2819.
- Bovenhuis, H., M. H. P. Visker, H. J. F. van Valenberg, A. J. Buitenhuis, and J. A. M. van Arendonk. 2015. Effects of the DGAT1 polymorphism on test-day milk production traits throughout lactation. *J. Dairy Sci.* 98:6572-6582.
- Butler, S. T., A. L. Marr, S. H. Pelton, R. P. Radcliff, M. C. Lucy, and W. R. Butler. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. *J. Endocrinol.* 176:205-217.
- Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477-487.

- Collier, R. J., E. L. Annen-Dawson, and A. Pezeshki. 2012. Effects of continuous lactation and short dry periods on mammary function and animal health. *Animal* 6:403-414.
- De Feu, M., A. Evans, P. Lonergan, and S. T. Butler. 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status, and postpartum ovarian function in Holstein-Friesian dairy cows. *J. Dairy Sci.* 92:6011-6022.
- Domecq, J. J., A. L. Skidmore, J. W. Lloyd, and J. B. Kaneene. 1997. Relationship Between Body Condition Scores and Milk Yield in a Large Dairy Herd of High Yielding Holstein Cows¹. *J. Dairy Sci.* 80:101-112.
- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259-2273.
- Dubuc, J., T. Duffield, K. Leslie, J. Walton, and S. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. *J. Dairy Sci.* 93:5764-5771.
- Gümen, A., R. R. Rastani, R. R. Grummer, and M. C. Wiltbank. 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *J. Dairy Sci.* 88:2401-2411.
- Goselink, R. M. A., J. T. Schonewille, G. Dunkirk, and A. T. M. Knegsel. 2012. Effect of dry period length on rumen adaptation in dairy cows. *J. Anim. Sci.* 90:667-667.
- Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, and P. Simon. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res.* 12:222-231.
- Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci.* 76:3882-3896.
- Grummer, R. R. and R. R. Rastani. 2004. Why reevaluate dry period length? *J. Dairy Sci.* 87, Supplement:E77-E85.
- Huszenicza, G. Y., S. Z. Jánosi, M. Kulcsar, P. Korodi, J. Reiczigel, L. Katai, A. R. Peters, and F. De Rensis. 2005. Effects of clinical mastitis on ovarian function in post-partum dairy cows. *Reprod Domest Anim* 40:199-204.

- Kok, A., A. T. M. van Kneegsel, C. E. van Middelaar, B. Engel, H. Hogeveen, B. Kemp, and I. J. M. de Boer. 2016a. Effect of dry period length on milk yield over multiple lactations. *J. Dairy Sci.*
- Kok, A., C. E. van Middelaar, B. Engel, A. T. M. van Kneegsel, H. Hogeveen, B. Kemp, and I. J. M. de Boer. 2016b. Effective lactation yield: A measure to compare milk yield between cows with different dry period lengths. *J. Dairy Sci.*
- LeBlanc, S., T. Duffield, K. Leslie, K. Bateman, G. P. Keefe, J. Walton, and W. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.* 85:2223-2236.
- Lucy, M. C., C. R. Staples, F. M. Michel, and W. W. Thatcher. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. *J. Dairy Sci.* 74:473-482.
- Mantovani, R., L. Marinelli, L. Bailoni, G. Gabai, and G. Bittante. 2010. Omission of dry period and effects on the subsequent lactation curve and on milk quality around calving in Italian Holstein cows. *Ital. J. Anim. Sci.* 9:101-108.
- Opsomer, G., Y. T. Gröhn, J. Hertl, M. Coryn, H. Deluyker, and A. De Kruif. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology* 53:841-857.
- Petersson, K. J., E. Strandberg, H. Gustafsson, and B. Berglund. 2006. Environmental effects on progesterone profile measures of dairy cow fertility. *Anim. Reprod. Sci.* 91:201-214.
- Pezeshki, A., J. Mehrzad, G. R. Ghorbani, H. R. Rahmani, R. J. Collier, and C. Burvenich. 2007. Effects of short dry periods on performance and metabolic status in Holstein dairy cows. *J. Dairy Sci.* 90:5531-5541.
- Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004-1014.
- Reist, M., D. K. Erdin, D. von Euw, K. M. Tschümperlin, H. Leuenberger, H. M. Hammon, C. Morel, C. Philipona, Y. Zbinden, N. Künzi, and J. W. Blum. 2003. Postpartum reproductive function: association with energy, metabolic

- and endocrine status in high yielding dairy cows. *Theriogenology* 59:1707-1723.
- Remond, B., J. Rouel, N. Pinson, and S. Jabet. 1997. An attempt to omit the dry period over three consecutive lactations in dairy cows. *Ann. Zootech.* 46:399-408.
- Roche, J. R., J. M. Lee, K. A. Macdonald, and D. P. Berry. 2007. Relationships among body condition score, body weight, and milk production variables in pasture-based dairy cows. *J. Dairy Sci.* 90:3802-3815.
- Santschi, D. E., D. M. Lefebvre, R. I. Cue, C. L. Girard, and D. Pellerin. 2011. Economic effect of short (35-d) compared with conventional (60-d) dry period management in commercial Canadian Holstein herds. *J. Dairy Sci.* 94:4734-4743.
- Shrestha, H. K., T. Nakao, T. Higaki, T. Suzuki, and M. Akita. 2004. Resumption of postpartum ovarian cyclicity in high-producing Holstein cows. *Theriogenology* 61:637-649.
- Spicer, L. J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-i in dairy cows: relationships among energy balance, body condition, ovarian activity, and estrous behavior. *J. Dairy Sci.* 73:929-937.
- Steeneveld, W., Y. H. Schukken, A. T. M. Van Knegsel, and H. Hogeveen. 2013. Effect of different dry period lengths on milk production and somatic cell count in subsequent lactations in commercial Dutch dairy herds. *J. Dairy Sci.* 96:2988-3001.
- Van Knegsel, A. T. M., H. Van den Brand, J. Dijkstra, and B. Kemp. 2007a. Effects of dietary energy source on energy balance, metabolites and reproduction variables in dairy cows in early lactation. *Theriogenology* 68:S274-S280.
- Van Knegsel, A. T. M., H. Van den Brand, J. Dijkstra, W. M. Van Straalen, M. J. W. Heetkamp, S. Tamminga, and B. Kemp. 2007b. Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. *J. Dairy Sci.* 90:1467-1476.
- Van Knegsel, A. T. M., H. Van den Brand, J. Dijkstra, W. M. Van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007c. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. *J. Dairy Sci.* 90:3397-3409.

- Van Knegsel, A. T. M., H. Van den Brand, E. A. M. Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga, and B. Kemp. 2007d. Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. *J. Dairy Sci.* 90:1477-1485.
- Van Knegsel, A. T. M., M. H. P. W. Visker, G. J. Remmelink, J. A. M. van Arendonk, and B. Kemp. 2013. DGAT1 Genotype affects the response of dairy cows to shortened or no dry period. Pages 36-36 in *Proc. Proceedings of the 15th International Conference on Production Diseases in Farm Animals*, 24-28 June 2013, Uppsala, Sweden.
- Van Knegsel, A. T. M., G. J. Remmelink, S. Jorjong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *J. Dairy Sci.* 97:1499-1512.
- Zobel, G., D. M. Weary, K. E. Leslie, and M. A. G. von Keyserlingk. 2015. Invited review: Cessation of lactation: Effects on animal welfare. *J. Dairy Sci.* 98:8263-8277.

•—————•

Summary

—————•

The early lactation period of dairy cows is characterized by a negative energy balance (**EB**) resulting from a fast increase in milk production postcalving while feed intake capacity is limited in this period. The severe negative EB results in extensive mobilization of body reserves in early lactation in dairy cows. A high mobilization of body reserves is related to an increased incidence and severity of metabolic disorders, like ketosis and liver fattening. Extensive research efforts have been made to improve the EB in early lactation in dairy cows. Many studies focused on increasing energy intake in cows via decreasing the forage to concentrate ratio, or the addition of dietary fats. Moreover, decreasing milking frequency has been proposed as a management strategy to improve EB by reducing milk yield in early lactation. However, increasing dietary energy density is potentially associated with rumen acidosis and decreased dry matter intake, and decreased milking frequency (e.g. once a day) causes substantial milk yield losses (ranging from 7 to 50%). Recent studies suggest that shortening or omitting the dry period (**DP**) shifts milk yield partially from the critical period postcalving to the period before calving. The reduction in milk yield in early lactation results in an improvement of EB, metabolic status, and fertility of dairy cows in early lactation. However, data on the effects of DP length on hepatic genes expression involved in carbohydrate or fatty acid metabolism in early lactation, and resumption and regularity of ovarian cycles after calving in dairy cows are scarce. Also, little information is available on long-term effects of shortening or omitting the DP (e.g. over 2 subsequent lactations). The main hypothesis of this thesis was that shortening the DP to 30 d or omitting the DP improves EB and consequently improves metabolic status and fertility of dairy cows in early lactation compared with a conventional DP of 60 d. In addition, it can be hypothesized that these beneficial effects of shortening or omitting the DP on EB, metabolic status, and fertility remain in the second subsequent lactation after shortening or omitting the DP. Earlier, it was reported that feeding a glucogenic diet in early lactation improved EB and metabolic status by reducing milk energy output compared with a lipogenic diet. Shortening or omitting the DP might reduce the requirement for glucogenic nutrients in early lactation because of the improved EB and metabolic status. Thus, it can be hypothesized that feeding a glucogenic diet may have less impact on EB and metabolic status in early lactation in dairy cows if cows have a better EB due to the shortened or omitted DP.

To test these hypotheses, an experiment was executed where Holstein-Friesian dairy cows ($n = 167$) were assigned randomly to 3 DP lengths (0, 30, or 60 d) and 2 early lactation diets (glucogenic or lipogenic diet). Cows were planned to have the same DP length and dietary treatments over 2 subsequent lactations. Before calving of the second subsequent lactation, 19 out of 39 cows in the 0 d DP group were attributed to 0→67 d DP group (actual days dry: 67 ± 8 d) because these cows had a milk yield of < 4 kg/d at least 30 d before expected calving date and were allowed to go dry.

In the first subsequent lactation after implementation of DP length and dietary treatments, cows with a 0 d DP had lower liver triacylglycerol (**TAG**) and plasma free fatty acids (**FFA**) concentrations, and greater plasma glucose, insulin-like growth factor-1 (**IGF-1**), and insulin concentrations compared with cows with a 60 d DP in early lactation (Chapter 2). Cows with a 0 d DP had lower hepatic mRNA expression of pyruvate carboxylase, which indicated lower rate of gluconeogenesis in the liver compared with cows with a 60 d DP. Shortening the DP to 30 d did not affect metabolic status or hepatic genes expression in early lactation compared with a 60 d DP. Feeding a glucogenic diet decreased plasma β -hydroxybutyrate (**BHB**) concentration compared with a lipogenic diet, independent of DP length. This study showed that omitting the DP improved metabolic status compared with a conventional DP in dairy cows, which could be associated with an improved EB because of a reduced milk energy output due to a lower milk yield in cows with a 0 d DP compared with cows with a 60 d DP. Independent of dry period length, glucogenic diet also improved the metabolic status, compared with the lipogenic diet.

The improved EB and metabolic status in early lactation is related to improved fertility in dairy cows. Therefore, the objective of chapter 3 was to evaluate effects of shortening or omitting the DP and dietary energy source on ovarian cyclicity in the first lactation after implementation of DP length and dietary treatments. The resumption of ovarian cyclicity was determined based on milk progesterone profile within 100 days in milk (**DIM**) in dairy cows. The normal resumption of ovarian cyclicity was defined as the onset of luteal activity occurring within 45 DIM and followed by regular ovarian cycles of 18 to 24 d in length. In this study, omitting the DP increased the percentage of cows with normal resumption of ovarian cyclicity (53.2%; 25 out of 47 cows) compared with a

conventional DP (26.0%; 13 out of 50 cows) in dairy cows. Shortening the DP did not affect the resumption of ovarian cyclicity compared with a conventional DP. In addition, feeding a glucogenic diet did not affect the resumption of ovarian cyclicity compared with a lipogenic diet. These results indicated that omitting the DP could possibly improve fertility in dairy cows compared with a conventional DP.

A similar energy intake but lower milk yield in cows with a 0 d DP in the first lactation led to greater weight gain in these cows compared with cows with a 60 d DP. Accordingly, cows with a 0 or 0→67 d DP had greater body condition score (BCS) than cows with a 60 d DP at the onset of the second lactation after implementation of DP length and dietary treatments (Chapter 4). Cows with a 0 or 30 d DP produced 5.0 and 4.3 kg less milk per day, respectively, in the first 9 weeks of the second lactation, which was less pronounced than the difference in the first lactation (31.1 vs. 38.6 vs. 43.4 kg/d for 0, 30, or 60 d DP, respectively). Cows with a 0 or 30 d DP had similar EB compared with cows with a 60 d DP in early lactation of the second lactation. Cows with a 0 d DP, but not cows with a 30 d DP, had greater plasma insulin and IGF-1, and lower liver TAG concentration compared with cows with a 60 DP. Cows with a 0→67d DP had a more severe negative EB, and greater plasma FFA and BHB concentrations compared with cows with other DP lengths. This could be caused by a lower dry matter intake in cows with a 0→67d DP in early lactation, which was related with high BCS at calving in these cows. Feeding a glucogenic diet did not affect EB, but increased plasma glucose, IGF-1, and insulin concentrations, and decreased plasma FFA, BHB, and urea concentrations compared with a lipogenic diet, independent of DP length. These results indicated that omitting the DP or feeding a glucogenic diet improved metabolic status in the second lactation after implementation of DP length and dietary treatments, although effects of omitting the DP are less pronounced than the first lactation.

In the second lactation, effects of DP length on luteal activity, pregnancy rate, days open, and uterine health status as indicated by vaginal discharge score in week 2 and 3 postcalving, were assessed (Chapter 5). Dry period length was not related with uterine health status, onset of luteal activity, resumption of ovarian cyclicity, pregnancy rate, or days open in dairy cows. Independent of DP length, feeding a glucogenic diet shortened the interval from calving to first ovulation compared with a lipogenic diet. In addition, we found that a healthy uterine environment was related to a high milk yield and a better metabolic status, as

indicated by greater plasma glucose and insulin concentrations, independent of DP length. This study showed that DP length did not influence fertility or uterine health status in the second lactation after implementation of DP length and dietary treatments. The shortened interval from calving to first ovulation in cows fed a glucogenic diet, compared with cows fed a lipogenic diet, could be related to the improved metabolic status in these cows.

Shape of the lactation curve for cows with different DP lengths was analysed with the Wilmink lactation curve function in both the first and second lactations after implementation of DP length and dietary treatments (Chapter 6). In the first lactation, cows with a 0 or 30 d DP had lower peak yield (**PY**), later time of peak yield (**PT**), and lower 305-d fat- and protein-corrected milk (**FPCM₃₀₅**) yield than cows with a 60 d DP. In the second lactation, cows with a 0 or 30 d DP had lower PY than cows with a 60 d DP. In the second lactation, the difference in PY, PT, and, FPCM₃₀₅ between cows with a 0 and 60 d DP were smaller compared with the first lactation. In both lactations, lactation persistency was not affected by DP length and none of the lactation curve characteristics were affected by dietary energy source. These results imply that the milk losses after shortening or omitting the DP were not related to the differences in lactation persistency in dairy cows. The milk yield losses reduced in cows with a 0 d DP in the second lactation (9,735 vs. 10,392 kg for 0 or 60 d DP, respectively) compared with the first lactation (8,277 vs. 10,779 kg for 0 or 60 d DP, respectively) in terms of the 305-d FPCM yield. Moreover, additional milk produced precalving could partly compensate for these milk yield losses in cows with 0 or 30 d DP.

Biological mechanisms behind the effects of shortening or omitting DP on milk yield, metabolism, and fertility were discussed in chapter 7. Additionally, the influences of BCS and parity on the effects of shortening or omitting the DP were described. Results indicated that the BCS at calving was related to EB and metabolic status in both lactations and omitting the DP. In addition, effects of omitting the DP on milk yield reduction and improvement of EB were stronger in young cows (parity = 2), compared with older cows (parity > 2). Both differences in BCS and parity could partly explain the differences in EB and metabolic status between the first and second lactation in cows with a 0 d DP. In the current experiment, around half of the cows (19 out of 39 cows) could not achieve DP omission for 2 subsequent lactations. The two main factors that may hinder the

success of DP omission over multiple lactations are low lactation persistency and long calving interval. Furthermore, some farmers apply a short or no DP because it results in healthier cows, decreased work load due to less dietary and group transitions, and improved fertility.

In conclusion, shortening or omitting the DP improved EB in early lactation compared with a conventional DP in dairy cows due to a decreased milk yield. Additional milk produced precalving in cows with a shortened or omitted DP could partly compensate for the milk yield losses in these cows. Omitting the DP improved metabolic status in early lactation and resumption of ovarian cyclicity compared with a conventional DP, which could be associated with the improved EB and metabolic status. This implies that omitting the DP could possibly reduce the risk of metabolic disorders and improve fertility in dairy cows. In the second subsequent lactation after implementation of DP length and dietary treatments, effects of omitting the DP on milk yield reduction and improvements of EB and metabolic status were less pronounced compared with the first lactation, which can be partly explained by the differences in parity and BCS between the first and second lactation. Shortening the DP to 30 d did not influence metabolic status and fertility compared with conventional DP in dairy cows in both lactations. In both lactations, shortening or omitting the DP affected the peak time and peak yield, but did not affect lactation persistency in dairy cows. Furthermore, feeding a glucogenic diet improved EB and metabolic status compared with lipogenic diet, independent of DP length.

Acknowledgements

Acknowledgements

Finally, my four years PhD journey is about to come to an end. Four years ago, it was very difficult to make the decision to do my PhD study in the Netherlands. However, it turns out to be one of my wisest decision I ever made. During the past four years, I learnt and experienced a lot and had many good times. Also, living and studying far away from home gave me some hard time. Fortunately, I have many friends in Wageningen, who help me to get through all these ups and downs. Therefore, I would like to express my sincere appreciation to all people who helped and supported me throughout my PhD period.

First and foremost, I would like to thank my supervisor, Ariette van Knegsel and my promoter, Bas Kemp. Thank you for giving me this chance to do my PhD study at the ADP group, and thank you for all the efforts, time, patience, and hard work to assist me to finish this thesis. Ariette, I am your first PhD candidate and I am really lucky to have you as my supervisor. Thank you for your guidance and sharing your knowledge on almost every aspects: physiology of dairy cows, statistics, scientific writing, presentations, supervision, etc. Thank you for your patience and encouragement during the four year study, especially in the first year when I even could not speak English well. Every time when I got stuck and was frustrated with my project, you were always there to help me out and encourage me to be confident again with my PhD. Thank you! Bas, thank you for all these creative ideas and critical comments about my project. When I got lost in the tons of data, you always can offer a new angle to look at these data and make an attractive story out of these data. Thank you for being available whenever I dropped by for my questions. As a professor, you are very busy, but you always say “PhD come first!”. Ariette and Bas, I could not have started and finished my PhD without your help and supports! Thank you!

I am highly indebted to all the collaborators for their valuable contribution to this thesis. Rupert Bruckmaier and Josef Gross, thank you for your continuing collaboration, comments on the chapters. In addition, thank you for your hospitality and help during my stay in Posieux and I had a great time there. Nicoline Soede, thank you for sharing your knowledge about fertility and offering advices on the manuscripts. I am inspired by your passion and strictness for science. Gerrit Remmelink, thank you for your quick response to my questions all the time. Akke Kok, I could not have accomplished my article about lactation curve without your help. Anette van Dorland, thank you for your inputs and comments on my articles.

Acknowledgements

I would like to express my gratitude to our secretarial team, Nanette van Hapert and Lora van der Kleijn. Thank you for all your help on administrative works and also on my personal life. Both of you are always willing to help me and others whenever we have problems or questions. Because of your supports, our work becomes much easier. Thank you! Ger de Vries Reilingh, thank you for your guidance and help with the lab works.

Many thanks to our “WHY DRY” team: Ariette, Novi Mayasari, Renny van Hoeij, Akke Kok, and Wei Xu. I got a lot of help from you all in the past four years. Novi, my sister, we started our project at more or less the same time. I am so grateful for all your help at my project and your help in the courses that we took together throughout my PhD period. Renny and Akke, thank you for your valuable advices to my work and your encouragement all the time. Wei Xu, it was great to work with you and thank you for all the nice dinners. I will always remember our nice talk during “WHY DRY coffee break”!

Of course, I would like to express my special thanks to my dear roommates in the “Tropical Room”: Carol Souza da Silva! Danny de Koning! Novi Mayasari! Ampai Nangsuay! Sofie van Nieuwamerongen! Conny Maatjens! Tom Berghof! Carolina Lima Alvares da Silva! Wei Xu! We had a lot of fun together!! I will miss all the dinners, drinks, games, and BBQs that we had together!! I am so lucky to have you around! The working atmosphere is so enjoyable because of all of you! I will never forget our plan: travelling together in Asia and reunion in 20 years!! Let’s do it!! I also would like to thank Kristina Simon, Inonge Reimert, Caroline Clouard, Lu Luo, Maarten Hollemans, David Lamot, Carla van der Pol, and Merel Verhoeven. It was great to work with you and thank you all for the nice talks and your supports in the past 4 years!

In Zodiac, there are not many Chinese, but we are like a big family: Yanyan Sun, Li Meng, Qingyuan Li, Tieshan Xu, Lihong Gu, Wenjuan Mu, Mandy Bao, Fubiao Niu, Wenbiao Shi, Yuan He, Lei Mao, Wei Xu, and Lu Luo. We had dinner together from time to time and help each other all the time. I am lucky to have you around and thank you for all your helps and the wonderful time together.

I still remember my first birthday party in Wageningen. It was less than one month after I came to Netherlands and I suffered a lot from homesick. Thank you, my “CSC” friends, for giving me a very warm birthday party and helping to

Acknowledgements

get over the hard time! Yue Lu, Yuanyuan Zhang, Yiqian Fu, Yin Song, Huchen Li, Guozhi Bi, Lihe Xu, Yongfu Tang, and Jiawei Chen, thank you! In addition, I would like to thank our “三国杀” group: Xu Chen, Wenbiao Shi, Jing Tang, Yongfu Tang, Fei Xue, Huchen Li, Yuan He, and Yang Zhang. Thank you for these pleasant and relaxing time together!

Special thanks to Xu Chen, Zhengyi Lin, Jiahui Yu, Dong Hu, Qiqi Lu, Jingwen Deng, Yutian Lu, Lu Luo, and Zhichun Yan! Thank you for your being always supportive and being with me, especially in the last period of my PhD. We hung out together, we sang together, we had dinner together, we got drunk together, all these cherished memories will be with me for the rest of my life.

Thanks the Chinese Scholarship Council for financing my stay in the Netherlands and the financers of the WHYDRY project (Dutch Dairy Board, Product Board Animal Feed and CRV) for financing the WHYDRY project, which was the basis of the current PhD study.

Last but not least, I would like to thank my wife, Tingting. Long-distance marriage is a challenge, but we made it! Thank you for your supports, encouragement, and understanding all these years, which allow me to focus on my PhD study and work full of enthusiasm. Also, I am so grateful to my family! 首先感谢给予我生命的父母，谢谢你们这么多年来养育之恩，谢谢你们给予我的理解和支持，谢谢你们这一路以来对我的鞭策和鼓励！正是由于你们多年来的言传身教，我才能站在博士答辩台上，谢谢你们！谢谢我的岳父岳母，感谢你们一直的关心和支持，感谢在我不在的这四年里对婷婷的照顾！还要感谢我的哥哥和嫂子长期以来对我的关心和照顾！希望我们这个大家庭的所有人健康，幸福，我爱你们！

About the Author

About the Author

Curriculum Vitae

Juncai Chen was born on 29 September 1987 in Dujiangyan, Sichuan, China. In September 2005, he started BSc at College of Animal Science and Technology, Sichuan Agriculture University (SICAU), Ya'an. In 2009, he started MSc in Animal Nutrition Institute, SICAU, under the supervision of Prof. Dr. Zhisheng Wang. In 2012, he obtained his MSc degree with the award of Outstanding Graduate in Ya'an. In the same year, he started his PhD entitled "The effects of dry period length on milk production, metabolism, and fertility in dairy cows over two subsequent lactations" at the Adaptation Physiology Group of Wageningen University, the Netherlands. The outcomes of his research are presented in this thesis.

Publications

Refereed Scientific Journals

J. Chen, J. J. Gross, H. A. van Dorland, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, A. T. M. van Kneysel. 2014. Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation. *Journal of dairy science*, 98(2): 1033-1045.

J. Chen, N. Soede, H. A. van Dorland, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, A. T. M. van Kneysel. 2015. Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths. *Theriogenology*.84:1387-1396.

J. Chen, G. J. Remmelink, J. J. Gross, R. M. Bruckmaier, B. Kemp, A. T. M. van Kneysel. 2016. Effects of dry period length and dietary energy source on milk yield, energy balance, and metabolic status of dairy cows over two consecutive years: effects in the second year. *Journal of dairy science*, 99(6): 4826-4838.

J. Chen, A. Kok, G. J. Remmelink, J. J. Gross, R. M. Bruckmaier, B. Kemp, A. T. M. van Kneysel. 2016. Effects of dry period length and dietary energy source on lactation curve characteristics over 2 consecutive years. *Journal of dairy science*, 99(11): 9287-9299.

L. Mao, J. Chen, Q. Peng, A. Zhou, Z. Wang. 2013. Effects of different sources and levels of zinc on H₂O₂-induced apoptosis in IEC-6 cells. *Biological Trace Element Research*, 155(1): 132-41.

Conference Book of Abstract

J. Chen, H. A. van Dorland, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, A. T. M. van Kneysel. 2013. Effects of dry period length and dietary energy source on

About the Author

metabolic status of dairy cows in early lactation. In Proceedings of the 15th International Conference on Production Diseases in Farm Animals, 24-28 June 2013, Uppsala, Sweden, pp. 69.

J. Chen, N. Soede, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2015. Effects of dry period length on onset of ovarian activity and ovarian cyclicity in the subsequent lactation. In Proceedings of the Joint Annual Meeting 2015 ADSA-ASAS, 12-16 July 2015, Orlando, Florida, USA, pp. 69.

J. Chen, N. Soede, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2016. Relationships of uterine health with metabolism in dairy cows with different dry period lengths. In Proceedings of the 15th International Conference on Production Diseases in Farm Animals, 20-23 June 2016, Wageningen, the Netherlands, pp. 105.

WIAS Training and Supervision Plan¹

Description	Year
The Basic Package (3.0 ECTS)	
Ethics and Philosophy in Life Sciences	2012
WIAS Introduction Course	2012
International Conferences (4.5 ECTS)	
15th International Conference on Production Diseases in farm animals (ICPD), Sweden	2013
2015 ADSA-ASAS Joint Annual Meetings, Orlando, USA	2015
16th International Conference on Production Diseases in farm animals (ICPD), The Netherlands	2016
Seminars and Workshops (3.9 ECTS)	
Dairy Nutrition Symposium, Wageningen, the Netherlands	2012
WIAS Science Day, Wageningen, The Netherlands	2013-2016
International Symposium on Dairy Cattle Nutrition 2012 "Nutritional management in early lactation "	2012
International Symposium on Dairy Cattle Nutrition 2014 "Strategies Towards a Quota-Free Dairy Production "	2014
Animal nutrition research forum in Ghent university	2015
Phd workshop Carousel, 17 April	2015
2nd PhD symposium	2015
Animal nutrition research forum in Ghent university	2015
Animal nutrition research forum in Wageningen university	2016
BASF workshop:Health and Nutrition in High-Yielding Dairy Cows	2016
Presentations (7.0 ECTS)	
ICPD, Oral Presentation, Sweden	2013
WIAS Science Day, Poster Presentation, Wageningen, The Netherlands	2014
WIAS Science Day, Oral Presentation, Wageningen, The Netherlands	2015
Animal nutrition research forum, Oral Presentation, Ghent, Belgium	2015

About the Author

ADSA-ASAS Joint Annual Meetings, Oral Presentation, Orlando, USA	2015
ICPD, Oral Presentation, Wageningen, The Netherlands	2016
ICPD, Poster Presentation, Wageningen, The Netherlands	2016

Disciplinary and Interdisciplinary Courses (5.9 ECTS)

Fatty acids in dairy cattle in relation to product quality and health	2012
Tropical farming systems with livestock	2013
Adaptative animals and livestock farming systems to meet global changes	2014
Epigenesis and Epigenetics	2014

Advanced Statistics Courses (3.0 ECTS)

Advanced Statistics: Design of Experiments	2013
Statistics for the Life Sciences	2015

Professional Skills Support Courses (4.3 ECTS)

Course Techniques for Scientific Writing	2014
Project and Time Management	2014
Career Perspectives	2016

Research Skills Training (3.0 ECTS)

Preparing own PhD Research Proposal	2012
External training period, Switzerland, 2 months	2013

Didactic Skills Training (4.0 ECTS)

Lecture in Adaptation Physiology	2015
Lecture in Infections and Disorders	2015-16
Supervision of 2 MSc Thesis Students	2015

Management Skills Training (3.0 ECTS)

Organisation Committee WIAS Science Day	2016
---	------

Education and Training Total	41.0 ECTS
-------------------------------------	------------------

¹1 ECTS credit equals a study load of approximately 28 hours

● Colophon

This research described in this thesis was financially supported by Dutch Dairy Board (PZ, the Netherlands), Product Board Animal Feed (PDV, the Netherlands), and CRV (the Netherlands). Juncai Chen was sponsored by a Chinese Scholarship Council (CSC) Fellowship.

Cover design:

Tingting Wen & Jiahui Yu

Thesis design and layout:

Juncai Chen

Cover formatting and thesis printed by:

GVO drukkers & vormgevers

B.V. | Ponsen & Looijen, Ede,
the Netherlands