Lactation length management

Consequences for fertility and health in dairy cows

Junnan Ma

Propositions

1. Fertility of cows is improved by extending the voluntary waiting period. (this thesis)

2. Extending the voluntary waiting period poses no risk for udder health. (this thesis)

3. Interpretation of scientific results is subjective.

4. Public debate fuels scientific research.

5. The era of social media requires independent thinking.

6. Bad weather makes it even more attractive to take a nap during daytime.

Propositions belonging to the thesis, entitled:

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Lactation length management:

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Chapter 1

General introduction

Background

In most parts of the world, high-yielding dairy cows are milked on average for less than 3 lactations (Seges, 2015), and most cows leave the farm before they are 6 years old (CRV, 2015, De Vries, 2020). The decreasing average time a cow stays in the herd has been of growing concern for dairy industries across the globe (Rushen and de Passillé, 2013). A high replacement rate of dairy cows is attributed to three main challenges in dairy production: fertility problems, mastitis, and locomotion disorders (Langford and Stott, 2012, Dallago et al., 2021). The origin of these disorders lies often in the period around calving, which is characterized by many transitions, including drying-off, calving, and the onset of lactation. During these transitions, large changes in both physiology and management are associated with an increased risk of diseases and disorders, such as clinical mastitis, hypocalcemia, and ketosis (Butler 2000, Fetrow et al. 2006, Friggens et al. 2004, Pinedo et al. 2020) and possibly culling (Olechnowicz et al., 2011).

Health challenges in early lactation

After calving, energy and nutrient requirements increase rapidly due to the onset of lactation, while feed intake at that moment is limited (Ingvartsen and Andersen, 2000). Therefore, cows experience a negative energy balance (**NEB**), which can last from 30 days to 84 days of lactation (Roche et al., 2009, Gross et al., 2011, Bruckmaier and Gross, 2017, Churakov et al., 2021). To cope with the energy deficiency during NEB, cows mobilize body reserves including body fat and muscle protein (Tamminga et al., 1997, Van der Drift et al., 2012). Extensive body fat mobilization and fatty acid oxidation increased plasma non-esterified fatty acids (**NEFA)** (Drackley et al., 2001, Bruckmaier and Gross, 2017). Fatty acids originating from body fat mobilization can be oxidized in the liver, or be esterified into triglycerides and exported from the liver as very low-density lipoproteins towards the mammary gland and secreted in the form of milk fat (Kleppe et al., 1988, Piepenbrink et al., 2003, Churakov et al., 2021). In early lactation, however, fatty acids are strongly increased due to body fat mobilization, while glucose availability is limited and utilized for milk lactose production (reviewed by van Knegsel et al., 2005). As a result, complete hepatic oxidation of fatty acids is limited and fatty acids are incompletely oxidized resulting in elevated ketone body concentrations in plasma, increasing the risk of ketosis (Bobe et al., 2004, Gross et al., 2013). Alternatively, excess available fatty acids can be stored in the liver as triglycerides potentially resulting in fatty liver (Morales-Almaráz et al., 2011). This fat accumulation in the liver is probably not only due to limited fat oxidation capacity, but also to the limited capacity of synthesis and export of hepatic lipoproteins in dairy cows (Overton et al., 1999, Piepenbrink et al, 2003).

Altered metabolic status at the start of lactation, associated with fat mobilization, but also associated with the calving process and mammary adaptations to the new lactation, is a risk period for the occurrence of health problems in dairy cows (Ingvartsen and Moyes, 2013), such as mastitis (Suthar et al., 2013) and metritis (Gross et al., 2019). Severe NEB or direct effects of ketone bodies on neutrophils were associated with the incidence of postpartum mastitis (Hoeben et al., 1999, Suriyasathaporn et al., 1999). Suthar et al. (2013) reported that cows with subclinical ketosis in the first 2 weeks after calving had 9.5 times greater odds of developing clinical mastitis compared with cows without subclinical ketosis. Moreover, cows with ketosis postpartum had more severe mastitis than cows without ketosis, as high β-hydroxybutyrate (**BHB**), NEFA, and low plasma glucose concentration were associated with greater bacterial growth in the quarter inoculated with Escherichia coli in week 3 until 6 after calving (Kremer et al., 1993). Greater NEFA concentration in early lactation could result in cell and tissue damage by stimulating phagocytosis-associated oxidative burst activities of polymorph nuclear leucocytes without changes in phagocytosis itself (Scalia et al., 2006, Moyes et al. 2009, Ingvarsten and Moyes, 2013). The number of leucocytes in plasma is lower during an episode of ketosis (Suriyasathaporn et al., 2000). During NEB, leucocytes also have a slower response in producing chemo attractants, a slower endothelial migration, and lower production of superoxide anions (Suriyasathaporn et al., 2000). Elevated NEFA concentration (Dyk, 1995) and ketone body concentrations (LeBlanc, 2010, Suthar et al., 2013) during the prepartum period were also associated with a high incidence of retained placenta and puerperal metritis (Wathes et al., 2007b).

Besides compromised metabolic status, health problems in early lactation are also related to the calving process and the start of lactation. Calving problems, including dystocia, twins, retained placenta, and stillbirth, are highly related to the occurrence of metritis after calving (Gröhn et al., 1990, Correa et al., 1993). At the start of lactation, loss of protective effect of dry cow antibiotics and new risks of bacterial infection related to milk leakage and open teat canals after milking, which can lead to a high incidence of intramammary infections such as mastitis (Prado-Taranilla et al., 2020). It is important to realize that diseases do not occur independently of each other, instead, they are often strongly interrelated. For example, cows with milk fever are at a 30% higher risk of experiencing ketosis during lactation.

Fertility challenges in early lactation

Fertility in dairy cows has declined over the past 50 years while milk production per cow has increased (Dillon et al., 2006, Macdonald et al., 2008, Rethmeier et al., 2019). Pregnancy rates after insemination have declined by 0.45-1% annually in the UK and Northern American herds (Royal et al., 2000, Butler et al.,2003, Dobson et al., 2007) and the culling rate is 20-35% per year (Smith et al., 2000, Rajala-Schultz and Frazer, 2003). The decline in fertility was related to the increase in milk yield and more severe NEB of high-producing dairy cows (De Vries and Veerkamp, 2000), as also described in earlier reviews (Lucy, 2001, Butler, 2003, Pryce et al., 2004). A more severe NEB has been linked to delayed first ovulation, more irregular ovarian cycles (i.e. short, prolonged, or cessation cycles) (Chen et al., 2015b), and a reduced conception rate after the first insemination (Butler, 2003, Patton et al., 2007).

When cows experience an NEB, low blood glucose and insulin concentrations along with elevated NEFA concentration are related to a delayed increase in gonadotropin (LH and FSH) pulses, which is necessary for stimulation of ovarian follicles (Beam and Butler, 1999). Low blood insulin concentrations resulted in low IGF-I production from the liver, which caused the somatotropic axis of dairy cows in early lactation to be uncoupled (Beam and Butler, 1998) and reduced responsiveness of the ovary to gonadotropins, herewith preventing the dominant follicle from ovulating (Beam and Butler, 1999) and delaying the postpartum resumption of ovarian cyclicity (Gutierrez et al., 1999). Moreover, cows with elevated BHB concentrations during NEB, defined as exceeding 1.1 or 1.6 mmol/L during the first 2 weeks after calving, had a decreased probability of pregnancy and an increased culling risk, respectively (Walsh et al., 2007, Duffield et al., 2009, Roberts et al., 2012).

Inflammation in early lactation

Underlying many physiological and pathological processes, inflammation is a complex conserved response against pathogens, removing injurious agents and initiating the tissue healing process (Sordillo et al., 2009). In early lactation, cows experience events related to calvings, such as parturition and the onset of transition from pregnancy to lactation, including altered permeability of the mammary epithelium, uterine infections, and increased fat oxidation in the liver (Trevisi et al., 2011a, Qin et al., 2018). Systemic inflammations are often accompanied by the acute phase response. The liver secretes acute phase proteins (APP) into the blood, which have a role in the inflammatory response, but also can be used as indicators to assess animal health (Petersen et al., 2004, Gruys et al., 2005). During the acute phase response, the liver produces more α-globulins, known as positive APP; i.e., haptoglobin, and ceruloplasmin (Fleck, 1989, Ceciliani et al., 2012). Conversely, the liver reduces during an acute phase response, the synthesis of albumin, paraoxonase (PON), and lipoproteins, known as negative APP (Schreiber et al., 1982, Bertoni et al., 2008).

Relations between inflammation, metabolic status, health, and fertility

Acute inflammation and chronic inflammation are two distinct processes categorized based on the duration of illness, any changes in body condition, the presence or absence of pain, and the clinical findings on individual organs or systems (Horadagoda et al., 1999, Mayasari et al., 2017). Acute inflammation develops relatively short over minutes or hours and lasts in duration from hours to a few days based on the type and severity of damage to the tissue (Wakefied, 2010). Acute inflammation is characterized by changes in plasma proteins, migration of leukocytes and the vascular endothelium; leukocyte recruitment, and release of proteases and oxidants from phagocytic cells to cope with the injury (Ward, 2010). The inflammation and associated changes play a role in the clinical signal such as redness, fever, pain, and dullness (Horadagoda et al., 1999, Ward, 2010). Chronic inflammation lasts longer in duration (at least a week) compared with acute inflammation and is characterized by recruitment and activation of macrophages (Ward et al., 1996), signed by weight loss and absence of fever in many cases (Horadagoda et al., 1999).

In dairy cows, excessive tissue mobilization and elevated NFFA concentration in plasma during the transition period have been associated with impaired liver function and increased inflammatory responses (Bionaz et al., 2007, Bertoni et al., 2008) which may result in oxidative damage of lipids and other macromolecules (Wathes et al., 2009). Increased NEFA concentrations in plasma are a contributing factor to many pro-inflammatory periparturient diseases in dairy cows including mastitis and metritis (Douglas et al., 2007, Sordillo et al., 2009). Injection of pro-inflammatory cytokines during the transition period stimulated inflammatory responses and decreased feed intake (Dantzer and Kelley, 2007). Lower feed intake could in turn decrease the NEB in early lactation. The severe NEB in early lactation has been related to reduced immune competence and consequently increased susceptibility to disease, especially regarding invading pathogens causing mastitis (Ingvartsen et al., 2003, Sordillo and Aitken, 2009, Trevisi et al., 2014).

Inflammatory responses and a dysfunctional immune system were related to the susceptibility to various health problems (Bernabucci et al., 2005, Sordillo and Aitken, 2009). Ametaj et al.

(2005) reported that plasma concentrations of inflammatory markers, including the positive APP haptoglobin and serum amyloid A, were elevated in cows that developed fatty liver. Similarly, Ohtsuka et al. (2001) observed increased serum TNFα activity in cows with moderate to severe fatty liver. Mayasari et al. (2017) reported that the incidence of clinical health problems (metritis, mastitis, retained placenta, or fever) in the first 2 weeks after calving was associated with high concentrations of ceruloplasmin and reactive oxidative metabolites (ROM), and a low concentration of albumin in plasma.

Relationships between metabolic status, the prevalence of infectious diseases, and immune suppression and inflammatory responses during the transition period are complex (as reviewed by Esposito et al., 2014). Some studies suggested that inflammation plays an important role in the etiology and results in some health disorders in early lactation (Bertoni et al., 2008, Huzzey et al., 2009, Dubuc et al., 2010, Qu et al., 2014), but it is also proposed that subclinical disorders before disease diagnosis could cause an increase in inflammatory mediators, in which case the inflammatory state would be an effect of the disease rather than a cause (Mayasari et al., 2017). Interpretation of these findings is complicated by various routes of administration, doses, and agents. Therefore, questions remain, whether the metabolism changes are a form of inflammation and cause of immune suppression in early lactation, and what is the relation between inflammation and health problems in early lactation is remain to explain.

Traditional lactation system

Traditionally, a 1-year calving interval is considered the optimum lactation cycle for dairy cows, with a 10-month lactation, a 2-month dry period, and a yearly peak in milk yield resulting in the greatest economic results (Auldist et al. 2007, Kolver et al. 2006). The dry period is the nonlactating period in between lactations, which has been commonly applied since the early 1900s (Woodard et al., 1926, Dix Arnold and Becker, 1936). A dry period of 6 until 8 weeks was related to maximal milk yield in the next lactation (Kuhn et al., 2006), possibly explained by maximal regeneration of mammary gland cells (Capuco et al., 1997) of the cow. Originally the need for a DP was also related to restoring the body condition (Woodward et al., 1926). Later, restoration of body condition was not seen as a function of the dry period anymore, due to issues related to high body condition at calving, resulting in a more severe NEB after calving (Morrow, 1976). Nowadays, the DP is also used to treat cows with subclinical mastitis or high somatic cell count (SCC) with antibiotics at drying off (Church et al., 2008). A 6-8 weeks DP is still commonly applied in dairy farms. In the last 2 decades, researchers started re-evaluating the optimal length of the DP as the genetic progress and improved feeding and management systems increased milk production (Schaeffer and Henderson, 1972, Dias and Allaire, 1982), with consequences for metabolic status, health, and fertility (Steeneveld et al., 2013, Chen et al., 2015b, Van Hoeij et al., 2018).

The voluntary waiting period until first insemination (VWP) is the interval after calving during which farmers decide not to inseminate the cow, even if they are seen in oestrus. The VWP is used to give the uterus time to recover from normal calving, recover from any infection, and return to its normal involuted size (DeJarnette et al., 2007). Cows also move through the period of severe NEB and resume normal ovarian cyclicity during this VWP period. Traditionally, most dairy farms apply a VWP of 40 to 60 d, aiming at a 12-month calving interval for high milk production and economic reasons (Österman and Bertilsson, 2003). However, the high production potential with also a high milk yield at dry-off and challenging yearly calving events with an increase for the disease could be reasons not to apply a 12-month calving interval (van Amburgh et al., 1997, Allore and Erb, 2000). In the Netherlands, the interval from calving to insemination and the calving interval varies among farms and cows. On average, the interval of calving to the first insemination is 87 d (Inchaisri et al., 2010a), and the average calving interval increased to 407 d in 2020 (CRV, Arnhem, Netherlands).

Changes in traditional lactation system as solutions for health and fertility problems

Management strategies for transition cows have been researched to find a way to improve the fertility and health of dairy cows. Solutions for improved fertility may be to improve the energy balance in early lactation or to inseminate the cow after the period of NEB. To improve the energy balance in early lactation, cows may be given a short or 0-d DP, which improves the EB through a reduced milk production with similar (Van Knegsel et al., 2014, Chen et al., 2015b) or better feed intake (Jolicoeur et al., 2014). Alternatively, an extended VWP, delaying the period from calving until the first insemination, makes insemination happen at the moment with improved EB and lower milk yield, and herewith improves the reproductive performance of dairy cows (Lehmann et al., 2016). Both strategies could also improve cow health, due to less severe NEB in case of short or 0-d DP, or less frequent calvings in case of an extended VWP. Both strategies will be addressed further in the next sections.

Shortening or omitting the dry period

In recent years, shortening the conventional DP from 6-8 weeks to about 30 d or omitting the DP completely in dairy cows has received considerable attention as the positive effect on NEB and fertility (Gümen et al.,2005, De Feu et al., 2009, Chen et al., 2015a,b). Shortening or omitting the DP improved the EB in early lactation by partially shifting milk yield from post-calving to the pre-calving period (Rastani et al., 2005, Van Knegsel et al., 2014). In a meta-analysis, cows with a short DP produced 1.4 kg/day less milk, and cows with 0-d DP produced 5.9 kg/day less milk postpartum compared with cows with a conventional DP (Van Knegsel et al., 2013). Due to the lower milk yield, cows with a 0-d DP had a more positive EB, which was also greater than the EB of cows with a 30-d DP (Rastani et al., 2005, Van Knegsel et al., 2014). Similarly, cows with a 30-d DP had a better energy balance than cows with a 60-d DP (Rastani et al., 2005, Van Knegsel et al., 2014). Furthermore, cows with an omitted DP had higher plasma glucose, insulin, IGF-1, and lower NEFA, BHBA, and liver TG concentrations compared with cows with 30-d DP or 60-d DP (Andersen et al., 2005, Schlamberger et al., 2010, Van Hoeij, 2017). Plasma metabolites did not differ between cows with a 30-d DP and cows with a 60-d DP. Although cows with 0-d DP or 30-d DP had a better energy balance, cows with a 30-d DP had less effect on plasma metabolites compared with cows with a 60-d DP (Chen et al., 2015a, Pezeshki et al., 2007, Rastani et al., 2005).

Dry period length and fertility

Studies concerning the effects of shortening or omitting the DP on fertility are presented in Table 2.1. Compared with a conventional DP (around 60 d), shortening or omitting the DP was reported to result in an increased first service conception rate and a reduced number of days open (Gümen et al., 2005, Watters et al., 2009, Chen et al., 2015b), no effects of DP length on pregnancy rate and days open were found in other studies (Pezeshki et al., 2007, 2008, De Feu et al., 2009).

It has been suggested that the reduced milk production and improved EB in early lactation when shortening the DP could contribute to enhanced fertility (Grummer, 2007). Indeed, cows with a 0-d DP or 30-d DP had fewer days to resume ovarian cyclicity and to first ovulation compared with cows with a 60-d DP (Gümen et al., 2005, De Feu et al., 2009, Chen et al., 2015b). Earlier resumption of ovarian cyclicity would increase the number of estrous cycles before breeding, which could decrease days open and increase the possibility of becoming pregnant (Darwash et al., 1997, Galvão et al., 2010, Thatcher and Wilcox, 1973). More information about the relation between short or dry DP and days open and ovarian cyclicity will be discussed in this thesis.

Dry period length and udder health

Effects of dry period length on udder health or SCC were contrasting. Studies showed an increase in postpartum SCC in milk (Kuhn et al., 2006, Pezeshki et al., 2007, Steeneveld et al., 2013), a tendency to decrease (Gulay et al., 2003, Pinedo et al., 2011), or no effect (Watters et al., 2008, Bernier-Dodier et al., 2010, van Hoeij et al., 2017) in SCC for cows with a short dry period compared with a conventional dry period. The effect of DP length on mastitis incidence has been examined in only a few studies, which reported no effect on mastitis incidence of dairy cows after a short DP length, compared with a conventional DP (as reviewed by van Knegsel et al., 2013, van Hoeij et al., 2017).

In most studies, cows with a conventional or shortened DP were often preventively treated with dry-cow antibiotics, whereas cows with an omitted DP were not treated with dry-cow antibiotics (Gulay et al., 2003, Rastani et al., 2005, Kuhn et al., 2006, Pezeshki et al., 2007, Church et al., 2008, Watters et al., 2008, Bernier-Dodier et al., 2010, Pinedo et al., 2011, Santschi et al., 2011b, Steeneveld et al., 2013, Shoshani et al., 2014). Thus, DP length and use of dry cow antibiotics were confounded in most studies, and differences in SCC could not be attributed to either DP length or the use of dry cow antibiotics. Except for two studies, where Van Hoeij et al. (2018) reported a 30-d DP without dry cows antibiotics resulted in a lower SCC, and a greater hazard for a case of clinical mastitis in the subsequent lactation of dairy cows compared with 0-d DP. Cows with an 8-week DP had a higher SCC in early lactation than cows with a 4-week DP without the use of antibiotics (O’Hara et al., 2019).

Extending the voluntary waiting period

Extending the voluntary waiting period (VWP) is a management strategy to realize increased calving intervals through a deliberately delayed start of first insemination (Grossman and Koops, 2003). During the transition period, most of the production disorders develop, and cows with extended lactations have a lower frequency of transition periods and herewith a lower frequency of increased risk for disease and impaired reproductive performance (Grummer, 1995, Mulligan and Doherty, 2008). When cows become less diseased, the risk for culling can be expected to reduce and the longevity of cows will increase (Arbel et al., 2001).

Table 1.1 Effect of dry period length on days to first onset of luteal activity (OLA) after calving, pregnancy rate, and calving interval.

Study	Cows (n)	DP (d)	OLA (d)	Pregnancy rate (%)	Days open (d)
Gümen et al.,	58	0	13.2[a7]	31.0[4]	93.8[a]
2005		28	23.8[b]	26.0	121.2[ab]
		56	31.9[b]	31.0	145.4[b]
Pezeshki et al.,	122	35		71.2	91.9
2007[2]		42		50.6	109.5
		56		67.1	85.2
Pezeshki et al.,	61	28		66.9	81.2
2008[2]		49		62.3	91.4
De Feu et al.,	40	6	16.9[a7]	83.3	112.0
2009		62	24.8[b]	64.7	126.0
Watters et al.,	781	34	36[a7]	61.0[5]	113.0[A]
2009[3]		55	42[b]	54.0	133.0[B]
Santschi et al.,	850	35		68.9[5]	134.4
2011[3]		60		66.5	143.3
Chen et al.,	167	0	23.1[A]		
2015		30	28.2		
		60	28.9[B]		
Chen et al.,	130	0 -> 0	27.2	65.0	144.5
2017[1]		30 -> 30	28.8	75.6	147.3
		60 -> 60	27.8	74.0	142.2
Van Hoeij,	130	0		91.0[6]	114.0
2017		30		93.0	113.0

[A,B]Values between dry period length within the study with different subscript letters differ (*P*<0.1).

[a,b]Values between dry period length within the study with different subscript letters differ (*P*<0.05).

[1]Results of two consecutive years with the same dry period length.

[2]Results of multiparous cows.

[3]Results of cows higher or equal to 3[rd] parity.

[4]Results at 150 days in milk (DIM).

[5]Results at week 44 postpartum.

[6]Results at week 44 postpartum.

[7]Results of days to first ovulation postpartum.

Voluntary waiting period and fertility

By extending the VWP, cows are inseminated later in lactation when the EB is improved, which may positively affect fertility. An improved EB was associated with increased plasma insulin concentration (Van Knegsel et al., 2014, Chen et al., 2015a). Increased insulin concentration in turn results in increased concentration of IGF-1 and liver growth hormone, which have beneficial effects on follicle quality and development and stimulate the dominant follicle to ovulate (as reviewed by Lucy, 2008, Walsh et al., 2011). Extending the VWP from 60 to 150 d improved conception rates (41.5% vs 50%), and resulted in fewer treatments for anestrus (28.6% vs. 5.3%) (Ratnayake et al., 1998). This is in line with Larsson and Berglund (2000), who found improved conception rates, lower services per conception, lower hormonal treatments due to anestrus, fewer ovarian cysts, and lower culling rates in cows with calving intervals of 15 months compared with cows with calving intervals of 12 months.

Voluntary waiting period and health

With an extended VWP, the risk for diseases per year can be expected to reduce as there will be fewer calvings and critical transitions per unit time. With a traditional VWP of 50-60 d, a high proportion of cows that were dried off still had a high production level (above 18 kg/d) (Österman and Bertilsson 2003). A high milk yield at dry-off was associated with a high risk of reduced udder health, because the accumulated milk in the udder may result in udder pressure, milk leakage, and increased risk of new intramammary infections (Bertulat et al., 2013, Rajala-Schultz et al., 2005). Extending the VWP may reduce the milk yield at dry-off, which could improve udder health. For every 5-kg increase in milk yield at dry-off above 12.5 kg, the odds of a cow having an intramammary infection (**IMI**) with an environmental pathogen at calving increased at least by 77% (Rajala-Schultz et al., 2005). Extending VWP may reduce the milk yield at dry-off, which could improve udder health. Extending VWP from 40 to 180 days resulted in a greater proportion (34.2 vs. 54.6 %) of cows being dried off at lower milk yields (< 15 kg) (Niozas et al., 2019), and reduced the incidence of udder health problems around calving (Rehn et al., 2000, Rajala-Schultz et al., 2005, Odensten et al., 2007).

Extending the VWP may also have negative consequences on udder health. Cows with an extended CI have more days in late lactation, and cows in late lactation have a greater risk for increased SCC (Singh and Ludri, 2001) related to the decline in milk yield (Hagnestam-Nielsen et al., 2009). Moreover, the risk of clinical mastitis increased with increasing SCC (Steeneveld et al., 2008). Niozas et al. (2019) reported a gradual increase of SCC with increasing days in milk (DIM), but no difference among 3 VWP groups (40, 120, and 180 days) regarding the SCC and incidence of clinical mastitis up to 330 DIM. Additionally, fewer transitions, along with extended VWP, also mean fewer chances to treat the cows with antibiotics at dry-off for subclinical mastitis and high SCC, which may increase the risk of clinical disease in lactation. Therefore, how extended VWP affect the udder health of dairy cows still needs to be clarified.

Aim and outline of this thesis

The overall aim of this thesis was to evaluate the effects of different DP lengths and lactation lengths on fertility and udder health in dairy cows. The specific objectives were: 1) to evaluate relationships between inflammatory biomarkers, clinical health problems, and fertility in the early lactation of dairy cows. 2) to evaluate the effect of 0-d DP on fertility and its relation to metabolic status in early lactation for cows with different dietary energy levels, 3) to evaluate the consequences of extending the VWP on the fertility of dairy cows, 4) to evaluate effects of extending voluntary waiting period on udder health of dairy cows.

To study these aims, fertility variables, health problems, and inflammatory biomarkers were measured and analyzed in two experiments. In **Chapter 2**, relations between clinical health problems, metabolites, fertility, and inflammatory biomarkers of cows in early lactation are presented. **Chapter 3** describes the ovarian cyclicity, days open, and pregnancy rate in cows with a 0-d DP or 30-d DP and different dietary energy levels, as compared with cows with a 30 d DP. Moreover, the relationships between ovarian cyclicity and EB and metabolites were assessed. **Chapters 4** and **5** concern experiment 2, in which cows were assigned to 3 VWPs (50 d, 125 d, and 200 d). **Chapter 4** describes the effect of different VWPs on the fertility of dairy cows. Ovarian cyclicity and reproductive performance in different stages of lactation were determined in the first lactation with different VWPs. Moreover, ovarian cyclicity and reproductive performance after different VWPs were related to milk yield, body condition score, and body weight around the end of the VWP. **Chapter 5** focuses on the clinical health problems of cows with different VWPs. **Chapter 5** describes the effect of different VWPs on somatic cell count and clinical mastitis incidence during the complete lactation and the first 6 weeks of the subsequent lactation. In **Chapter 6**, all the results of this thesis are discussed and how to apply different DP and VWP strategies in practice is highlighted.

References

Allore, H. G., Erb, H. N., 2000. Simulated effects on dairy cattle health of extending the voluntary waiting period with recombinant bovine somatotropin. Prev Vet Med. 46, 29-50.

Ametaj, B. N., Bradford, B. J., Bobe, G., Nafikov, R. A., Lu, Y., Young, J. W., Beitz, D. C., 2005. Strong Relationships between Mediators of the Acute Phase Response and Fatty Liver in Dairy Cows. Canadian J. Anim Sci. 85, 165-175.

Andersen, J., Madsen, T., Larsen, T. Ingvartsen, K., Nielsen, M., 2005. The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. J. Dairy Sci. 88, 3530-3541.

Arbel, R., Bigun, Y., Ezra, E., Sturman, H., Hojman, D., 2001. The effect of extended calving intervals in high lactating cows on milk production and profitability. J. Dairy Sci. 84, 600-608.

Austin, E., Mihm, M., Evans, A., Knight, P., Ireland, J., Ireland, J., Roche, J., 2001. Alterations in intrafollicular regulatory factors and apoptosis during selection of follicles in the first follicular wave of the bovine estrous cycle. Biol. Reprod. 64, 839-848.

Beam, S., Butler. W., 1999. Effects of energy balance on follicular development and first ovulation in postpartum. Reprod Fertil. Suppl. 54, 411-424.

Beam, S., Butler, W., 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81, 121-131.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88, 2017-2026.

Bernier-Dodier, P., Delbecchi, L., Wagner, G., Talbot, B., Lacasse, P., 2010. Effect of milking frequency on lactation persistency and mammary gland remodeling in mid-lactation cows. J.Dairy Sci. 93, 555-564.

Bertoni, G., Trevisi, E., Han, X., Bionaz, M., 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91, 3300-3310.

Bertulat, S., Fischer-Tenhagen, C., Suthar, V., Möstl, E., Isaka, N., Heuwieser. W., 2013. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yields. J. Dairy Sci. 96, 3774-3787.

Bionaz, M., Trevisi, E., Calamari, L., Librandi, F., Ferrari, A., Bertoni, G., 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90, 1740-1750.

Bobe, G., Young, J., Beitz, D., 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87, 3105-3124.

Bruckmaier, R., Gross, J. J., 2017. Lactational challenges in transition dairy cows. Animl Prod Sci. 57,1471-1481.

Butler, W. R., 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci. 83, 211-218.

Butler, W., 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci. 60, 449-457.

Capuco, A., Akers, R., Smith, J., 1997. Mammary growth in Holstein cows during the dry period: Quantification of nucleic acids and histology. J. Dairy Sci. 80, 477-487.

Ceciliani, F., Ceron, J., Eckersall, P., Sauerwein, H., 2012. Acute phase proteins in ruminants. J. Prote. 75, 4207-4231.

Chen, J., Gross, J.J., Van Dorland, H., Remmelink, G., Bruckmaier, R., Kemp, B., Van Knegsel, A.T., 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., Remmelink, G., Gross, J.J., Bruckmaier, R., Kemp, B., Van Knegsel, A.T., 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.

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

Churakov, M., Karlsson, J., Rasmussen, A. E., Holtenius, K., 2021. Milk fatty acids as indicators of negative energy balance of dairy cows in early lactation. Animal 15, 100253.

Church, G., Fox, L., Gaskins, C., Hancock, D., Gay, J., 2008. The effect of a shortened dry period on intramammary infections during the subsequent lactation. J. Dairy Sci. 91, 4219-4225.

Correa, M., Erb, H., Scarlett, J., 1993. Path analysis for seven postpartum disorders of Holstein cows. J. Dairy Sci. 76, 1305-1312.

Dallago, G. M., Wade, K. M., Cue, R. I., McClure, J. T., Lacroix, R., Pellerin, D., Vasseur, E., 2021. Erratum: Dallago et al. Keeping dairy cows for longer: A critical literature review on dairy cow longevity in high milk-producing countries. Animals. 11, 2958.

Dantzer, R., Kelley, K. W., 2007. Twenty years of research on cytokine-induced sickness behavior. Brain, behavior, and immunity. 21, 153-160.

Darwash, A., Lamming, G., Wooliams, J., 1997. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. Anim Sci. 65, 9-16.

De Feu, M., Evans, A., Lonergan, P., Butler. S., 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, A., Olson, J., Pinedo, P., 2010. Reproductive risk factors for culling and productive life in large dairy herds in the eastern United States between 2001 and 2006. J. Dairy Sci. 93, 613-623.

De Vries, M., Veerkamp, R., 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83, 62-69.

DeJarnette, J., Sattler, C., Marshall, C., Nebel, R., 2007. Voluntary waiting period management practices in dairy herds participating in a progeny test program. J. Dairy Sci. 90, 1073-1079.

Dias, F., Allaire, F., 1982. Dry period to maximize milk production over two consecutive lactations. J. Dairy Sci. 65, 136-145.

Dillon, P., Berry, D., Evans, R., Buckley, F., Horan, B., 2006. Consequences of genetic selection for increased milk production in European seasonal pasture based systems of milk production. Livest Sci. 99, 141-158.

Douglas, G., Rehage, J., Beaulieu, A., Bahaa, A., Drackley, J., 2007. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. J. Dairy Sci. 90, 2941-2959.

Drackley, J. K., Overton, T. R., Douglas, G. N., 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.

Dubuc, J., Duffield, T., Leslie, K., Walton, J., LeBlanc, S., 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93, 5764-5771.

Duffield, T., Lissemore, K., McBride, B., Leslie, K., 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92, 571-580.

Dyk, P., 1995. Prepartum non-esterified fatty acids in plasma are higher in cows developing periparturient health problems. J. Dairy Sci. 78, 264.

Esposito, G., Irons, P. C., Webb, E. C., Chapwanya, A., 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim Reprod Sci. 144, 60-71.

Fetrow, J., Nordlund, K., Norman, H., 2006. Invited review: Culling: Nomenclature, definitions, and recommendations. J. Dairy Sci. 89, 1896-1905.

Fleck, A., 1989. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proc Nutri Soc. 48, 347-354.

Friggens, N. C., Andersen, J. B., Larsen, T., Aaes, O., Dewhurst, R. J., 2004. Priming the dairy cow for lactation: a review of dry cow feeding strategies. Anim Res. 53, 453-473.

Galvão, K., Frajblat, M., Butler, W., Brittin, S., Guard, C., Gilbert, R., 2010. Effect of early postpartum ovulation on fertility in dairy cows. Reprod Domest Anim. 45, e207-e211.

Gröhn, Y., Bruss, M., 1990. Effect of diseases, production, and season on traumatic reticuloperitonitis and ruminal acidosis in dairy cattle. J. Dairy Sci. 73, 2355-2363.

Gross, J., Bruckmaier, R., 2019. Metabolic challenges in lactating dairy cows and their assessment via established and novel indicators in milk. Animal. 13, s75-s81.

Gross, J., van Dorland, H. A., Bruckmaier, R., Schwarz. F., 2011. Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. Journal of dairy science 94(4):1820-1830.

Grossman, M., Koops, W., 2003. Modeling extended lactation curves of dairy cattle: A biological basis for the multiphasic approach. J. Dairy Sci. 86, 988-998.

Grummer, R., 2007. Strategies to improve fertility of high yielding dairy farms: management of the dry period. Theriogenology. 68, S281-S288.

Gruys, E., Toussaint, M. J., Upragarin, N., Van Ederen, A.M., Adewuyi, A., Candiani, D., Nguyen, T., 2005. Acute phase reactants, challenge in the near future of animal production and veterinary medicine. Journal of Zhejiang University Science. 6, 941-947.

Gulay, M., Hayen, M., Bachman, K., Belloso, T., Liboni, M., Head, H., 2003. Milk production and feed intake of Holstein cows given short (30-d) or normal (60-d) dry periods. J. Dairy Sci. 86, 2030-2038.

Gumen, A., Keskin, A., Yilmazbas-Mecitoglu, G., Karakaya, E., Wiltbank, M., 2011. Dry period management and optimization of post-partum reproductive management in dairy cattle. Reprod Domest Anim. 46, 11-17.

Gümen, A., Rastani, R., Grummer, R., Wiltbank, M., 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. J. Dairy Sci. 88, 2401-2411.

Gutiérrez-Yurrita, P. J., Montes, C., 1999. Bioenergetics and phenology of reproduction of the introduced red swamp crayfish, Procambarus clarkii, in Do· ana National Park, Spain, and implications for species management. Freshwater Biology. 42, 561-574.

Hoeben, D., Burvenich, C., Eppard, P., Hard, D., 1999. Effect of recombinant bovine somatotropin on milk production and composition of cows with Streptococcus uberis mastitis. J. Dairy Sci. 82, 1671-1683.

Horadagoda, N., Knox, K., Gibbs, H., Reid, S., Horadagoda, A., Edwards, S., Eckersall, P., 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Veterinary Record. 144, 437-441.

Huzzey, J., T. Duffield, S. LeBlanc, D. Veira, D. Weary, and M. Von Keyserlingk. 2009. Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92, 621-625.

Inchaisri, C., Jorritsma, R., Vos, P. L., Van der Weijden, G., Hogeveen, H., 2010. Economic consequences of reproductive performance in dairy cattle. Theriogenology 74, 835-846.

Ingvartsen, K., Andersen, J., 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J. Dairy Sci. 83, 1573-1597.

Ingvartsen, K., Dewhurst, R., Friggens, N., 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livest Prod Sci. 83, 277-308.

Ingvartsen, K. L., Moyes, K., 2013. Nutrition, immune function and health of dairy cattle. Animal. 7, 112-122.

Jolicoeur, M., Brito, A., Santschi, D., Pellerin, D., Lefebvre, D., Berthiaume, R., Girard, C., 2014. Short dry period management improves peripartum ruminal adaptation in dairy cows. J. Dairy Sci. 97, 7655-7667.

Kleppe, B. B., Aiello, R. J., Grummer, R. R., Armentano, L. E., 1988. Triglyceride accumulation and very low density lipoprotein secretion by rat and goat hepatocytes in vitro. J. Dairy Sci. 71, 1813-1822.

Kolver, E., Roche, J., Burke, C., Aspin, C., 2006. Effects of genotype and diet on milksolids production, body condition, and reproduction of cows milked continuously for 600 days. Pages 66: 245-251 in Proc. Conference of the New Zealand Society of Animal Production.

Kremer, W., Noordhuizen-Stassen, E., Grommers, F., Schukken, Y., Heeringa, R., Brand, A., Burvenich, C., 1993. Severity of experimental Escherichia coli mastitis in ketonemic and nonketonemic dairy cows. J. Dairy Sci. 76, 3428-3436.

Kuhn, M., Hutchison, J., Norman, H., 2006. Dry period length to maximize production across adjacent lactations and lifetime production. J. Dairy Sci. 89, 1713-1722.

Larsson, B. and B. Berglund. 2000. Reproductive performance in cows with extended calving interval. Reproduction in Domestic Animals 35(6):277-279.

LeBlanc, S., 2010. Monitoring metabolic health of dairy cattle in the transition period. J. ReprodDev. 56, S29-S35.

Lehmann, J., Fadel, J., Mogensen, L., Kristensen, T., Gaillard, C., Kebreab, E., 2016. Effect of calving interval and parity on milk yield per feeding day in Danish commercial dairy herds. J. Dairy Sci. 99, 621-633.

Lucy, M., 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84, 1277-1293.

Lucy, M., 2008. Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: implications for post-partum nutrition and reproduction. Reprod Domest Anim. 43:31-39.

Macdonald, K., Penno, J., Lancaster, J., Roche, J., 2008. Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. J. Dairy Sci. 91, 2151-2163.

Mayasari, N., Chen, J., Ferrari, A., Bruckmaier, R., Kemp, B., Parmentier, H., Van Knegsel, A.,Trevisi, E., 2017. Effects of dry period length and dietary energy source on inflammatory biomarkers and oxidative stress in dairy cows. J. Dairy Sci.100, 4961-4975.

Morales-Almaráz, E., De la Roza-Delgado, B., González, A. , Soldado, A., Rodríguez, M., Peláez, M., Vicente, F., 2011. Effect of feeding system on unsaturated fatty acid level in milk of dairy cows. Renewable Agriculture and Food Systems. 26, 224-229.

Morrow, D. A., 1976. Fat cow syndrome. J. Dairy Sci. 59, 1625-1629.

Moyes, K., Larsen, T., Friggens, N., Drackley, J., Ingvartsen, K., 2009. Identification of potential markers in blood for the development of subclinical and clinical mastitis in dairy cattle at parturition and during early lactation. J. Dairy Sci. 92, 5419-5428.

Mulligan, F., Doherty, M., 2008. Production diseases of the transition cow. Vet J. 176, 3-9.

Niozas, G., Tsousis, G., Malesios, C., Steinhöfel, I., Boscos, C., Bollwein, H., Kaske, M., 2019. Extended lactation in high-yielding dairy cows. II. Effects on milk production, udder health, and body measurements. J. Dairy Sci.102, 811-823.

O'Hara, E. A., Båge, R., Emanuelson, U., Holtenius, K., 2019. Effects of dry period length on metabolic status, fertility, udder health, and colostrum production in 2 cow breeds. J. Dairy Sci. 102, 595-606.

Odensten, M., Berglund, B.,. Waller, K. P., Holtenius, K., 2007. Metabolism and udder health at dry-off in cows of different breeds and production levels. J. Dairy Sci. 90, 1417-1428.

Ohtsuka, H., Koiwa, M., Hatsugaya, A., Kudo, K., Hoshi, F., Itoh, N., Yokota, H., Okada, H., Kawamura, S.I., 2001. Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. VetMed Sci. 63, 1021-1025.

Olechnowicz, J., Jaskowski, J. M., 2011. Reasons for culling, culling due to lameness, and economic losses in dairy cows. Med Wet. 67, 618-621.

Österman, S., Bertilsson, J., 2003. Extended calving interval in combination with milking two or three times per day: effects on milk production and milk composition. Livest Prod Sci. 82, 139-149.

Overton, T. R., Drackley, J. K., Ottemann-Abbamonte, C. J., Beaulieu, A. D., Emmert, L. S., Clark, J. H., 1999. Substrate utilization for hepatic gluconeogenesis is altered by increased glucose demand in ruminants. J. Anim Sci. 77, 1940-1951.

Patton, J., Kenny, D., McNamara, S., Mee, J., O'mara, F., Diskin, M., Murphy, J., 2007. Relationships among milk production, energy balance, plasma analytes, and reproduction in Holstein-Friesian cows. J. Dairy Sci. 90, 649-658.

Petersen, H. H., Nielsen, J. P., Heegaard, P. M., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet Res. 35, 163-187.

Pezeshki, A., Mehrzad, J., Ghorbani, G. R., Rahmani, H. R., Collier, R. J., Burvenich, C., 2007. Effects of short dry periods on performance and metabolic status in Holstein dairy cows. J. Dairy Sci. 90, 5531-5541.

Piepenbrink, M., Overton, T., 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. J. Dairy Sci. 86, 1722-1733.

Pinedo, P., Daniels, A., Shumaker, J., De Vries, A., 2014. Dynamics of culling for Jersey, Holstein, and Jersey× Holstein crossbred cows in large multibreed dairy herds. J. Dairy Sci. 97, 2886-2895.

Pinedo, P., Risco, C., Melendez, P., 2011. A retrospective study on the association between different lengths of the dry period and subclinical mastitis, milk yield, reproductive performance, and culling in Chilean dairy cows. J. Dairy Sci. 94, 106-115.

Pryce, J., Royal, M., Garnsworthy, P., Mao. I., 2004. Fertility in the high-producing dairy cow. Livest Prod Sci. 86, 125-135.

Qin, N., Bayat, A.R., Trevisi, E., Minuti, A., Kairenius, P., Viitala, S., Mutikainen, M., Leskinen, H., Elo, K., Kokkonen, T., 2018. Dietary supplement of conjugated linoleic acids or polyunsaturated fatty acids suppressed the mobilization of body fat reserves in dairy cows at early lactation through different pathways. J. Dairy Sci. 101, 7954-7970.

Qu, Y., Fadden, A., Traber, M., Bobe, G., 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. J. Dairy Sci. 97, 4151-4165.

Rajala-Schultz, P., Frazer, G., 2003. Reproductive performance in Ohio dairy herds in the 1990s. Anim Reprod Sci. 76, 127-142.

Rajala-Schultz, P. J., Hogan, J. S., Smith, K., 2005. Association between milk yield at dry-off and probability of intramammary infections at calving. J. Dairy Sci. 88, 577-579.

Rastani, R., Grummer, R., Bertics, S., Gümen, A., Wiltbank, M., Mashek, D., Schwab, M., 2005. Reducing dry period length to simplify feeding transition cows: Milk production, energy balance, and metabolic profiles. J. Dairy Sci. 88, 1004-1014.

Ratnayake, D., Berglund, B., Bertilsson, J., Forsberg, M., Gustafsson, H., 1998. Fertility in dairy cows managed for calving intervals of 12, 15 or 18 months. Acta Vet Scand. 39, 215-228.

Rehn, H., Berglund, B., Emanuelson, U., Tengroth, G., Philipsson, J., 2000. Milk production in Swedish dairy cows managed for calving intervals of 12 and 15 months. Acta Agriculturae Scandinavica, Section A-Animal Science. 50, 263-271.

Rethmeier, J., Wenzlau, M., Wagner, M., Wiedemann, S., Bachmann, L., 2019. Fertility parameters in German dairy herds: Associations with milk yield and herd size. Czech Journal of Animal Science. 64, 459-464.

Roberts, T., Chapinal, N., LeBlanc, S., Kelton, D., Dubuc, J., Duffield, T., 2012. Metabolic parameters in transition cows as indicators for early-lactation culling risk. J. Dairy Sci. 95, 3057-3063.

Roche, J. R., Friggens, N. C., Kay, J. K., Fisher, M. W., Stafford, K. J., Berry, D. P., 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92, 5769-5801.

Royal, M., Mann, G., Flint, A., 2000. Strategies for reversing the trend towards subfertility in dairy cattle. Vet J. 160, 53-60.

Rushen, J., de Passillé, A., 2013. The importance of improving cow longevity. Pages 28-29 in Proc. Proceedings of the Cow Longevity Conference, Tumba, Sweden.

Scalia, D., Lacetera, N., Bernabucci, U., Demeyere, K., Duchateau, L., Burvenich, C., 2006. In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. J. Dairy Sci. 89, 147-154.

Schaeffer, L., Henderson, C., 1972. Effects of days dry and days open on Holstein milk production. J. Dairy Sci. 55, 107-112.

Schlamberger, G., Wiedemann, S., Viturro, E., Meyer, H., Kaske, M., 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.

Schreiber, J. R., Weinstein, D. B., Hsueh, A. J., 1982. Lipoproteins stimulate androgen production by cultured rat testis cells. J. Steroid Biochem. 16, 39-43.

SEGES, 2018. Månedsstatistik 15, Aarhus, Denmark.

Smith, J., Ely, L., Chapa, A., 2000. Effect of region, herd size, and milk production on reasons cows leave the herd. J. Dairy Sci. 83, 2980-2987.

Sordillo, L. M., Contreras, G., Aitken., S. L., 2009. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Animal health research reviews 10, 53-63.

Steeneveld, W., Schukken, Y., Van Knegsel, A., Hogeveen, H., 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.

Suriyasathaporn, W., Daemen, A., Noordhuizen-Stassen, E., Dieleman, S., Nielen, M., Schukken, Y., 1999. β-hydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. Veterinary immunology and immunopathology. 68, 177-186.

Suriyasathaporn, W., Heuer, C., Noordhuizen-Stassen, E. N., Schukken, Y. H., 2000. Hyperketonemia and the impairment of udder defense: a review. Vet Res. 31, 397-412.

Suthar, V., Canelas-Raposo, J., Deniz, A., Heuwieser, W., 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J. Dairy Sci. 96, 2925-2938.

Tamminga, S., Luteijn, P., Meijer, R., 1997. Changes in composition and energy content of liveweight loss in dairy cows with time after parturition. Livest Prod Sci. 52, 31-38.

Thatcher, W., Wilcox, C., 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 56, 608-610.

Trevisi, E., Amadori, M., Archetti, I., Lacetera, N., Bertoni, G., 2011. Inflammatory response and acute phase proteins in the transition period of high-yielding dairy cows. Acute phase proteins as early non-specific biomarkers of human and veterinary diseases. 15, 355-379.

Trevisi, E., Amadori, M., Riva, F., Bertoni, G., Bani, P., 2014. Evaluation of innate immune responses in bovine forestomachs. Res. Vet Sci. 96, 69-78.

Van Amburgh, M. E., Galton, D. M., Bauman, D. E., Everett, R. W., 1997. Management and economics of extended calving intervals with use of bovine somatotropin. Livest Prod Sci. 50, 15-28.

Van der Drift, S., Houweling, M., Schonewille, J., Tielens, A., Jorritsma, R., 2012. Protein and fat mobilization and associations with serum β-hydroxybutyrate concentrations in dairy cows. J. Dairy Sci. 95, 4911-4920.

Van Hoeij, R., Lam, T., Bruckmaier, R., Dijkstra, J., Remmelink, G., Kemp, B., van Knegsel, A., 2018. Udder health of dairy cows fed different dietary energy levels after a short or no dry period without use of dry cow antibiotics. J. Dairy Sci. 101, 4570-4585.

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(3):1499-1512.

Van Knegsel, A. T., Van den Brand, H., Dijkstra, J., Tamminga, S., Kemp, B., 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reprod Nutri Dev. 45, 665-688.

Van Knegsel, A. T., van der Drift, S. G., Čermáková, J., Kemp, B., 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.

Wakefield, D., Herbort, C.P., Tugal-Tutkun, I., Zierhut, M., 2010. Controversies in ocular inflammation and immunology laser flare photometry. Ocul immunol inflamm. 18, 334-340.

Walsh, C., Mahony, R., Foley, M., Daly, L., O'Herlihy, C., 2007. Recurrence of fetal macrosomia in non-diabetic pregnancies. ObstetGynaecol. 27, 374-378.

Walsh, S., Williams, E., Evans, A., 2011. A review of the causes of poor fertility in high milk producing dairy cows. Anim Reprod Sci. 123, 127-138.

Ward, P. A., 2010. Acute and chronic inflammation. Fundamentals of inflammation:1-16.

Wathes, D. C., Cheng, Z., Bourne, N., Taylor, V. J., Coffey, M. P., Brotherstone, S., 2007. Differences between primiparous and multiparous dairy cows in the interrelationships between metabolic traits, milk yield and body condition score in the periparturient period. Domest. Anim. Endocrin. 33:203-225.

Wathes, D.C., Cheng, Z., Chowdhury, W., Fenwick, M.A., Fitzpatrick, R., Morris, D.G., J. Patton, and J. J. Murphy. 2009. Negative energy balance alters global gene expression and immune responses in the uterus of postpartum dairy cows. Physiol Genom. 39, 1-13.

Watters, R., Guenther, J., Brickner, A., Rastani, R., Crump, P., Clark, P., Grummer, R., 2008. Effects of dry period length on milk production and health of dairy cattle. J. Dairy Sci. 91, 2595-2603.

Chapter 2

Relationships between inflammatory biomarkers, metabolism, and health status of dairy cows in early lactation

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To be submitted

Abstract

The objective of the present study was to determine the relationships between inflammatory biomarkers and oxidative stress with health status and energy balance (**EB**) status in dairy cows in early lactation. Holstein-Friesian dairy cows (N=154) were selected and monitored for health status during week 1 to 6 in lactation. Weekly plasma samples were analyzed for metabolic variables, and inflammatory and oxidative stress markers. First, cows were regrouped into 3 groups based on health status in the first 6 weeks of the lactation (**CHP**: cows with treatment for clinical health problem; **OHP**: cows with no CHP but treatment for other health problem; **NHP**: cows with no treatment for any health problem), where CHP was defined as endometritis, fever, clinical mastitis, or retained placenta and OHP was defined as milk fever, vaginal discharge, cystic ovaries, claw and leg problems, stomach and intestine problems, cobalt deficiency and 3 teats. Second, cows were regrouped into 4 clusters based on EB time series in the first 6 weeks of the lactation (**SP**: stable positive cluster; **MN**: mild negative cluster; **IN**: intermediate negative cluster; **SN**: severe negative cluster). Next, we assessed relationships between health status and metabolic variables, inflammatory biomarkers, relationships between EB clusters and metabolic variables, and inflammatory biomarkers. For health status, the CHP cows had lower plasma albumin ($P < 0.01$), lower paraoxonase concentration ($P < 0.01$), and higher haptoglobin concentration in the first 6 weeks after calving and a lower liver activity index ($P < 0.01$) compared with OHP and NHP cows. The CHP cows tended to have a lower dry matter intake ($P = 0.07$) compared with OHP and NHP cows. In week 1 after calving, the NHP cows had a higher concentration of glucose ($P < 0.01$) compared with OHP cows, but not with CHP cows, and higher insulin concentration ($P < 0.01$) compared with OHP and NHP cows. In addition, the NHP cows had higher IGF-1 concentration ($P < 0.01$) compared with CHP cows in week 1. For EB status, cows in the SN cluster tended to have a higher concentration of creatinine ($P = 0.09$) and a higher concentration of ceruloplasmin ($P = 0.09$) in plasma compared with cows in SP cluster. Cows in SN cluster had high plasma non-esterified fatty acids and β-hydroxybutyrate concentration, and low IGF-1, insulin, and glucose concentration in the first 6 weeks of lactation. Overall, this study demonstrated that health status was related to biomarkers for inflammation (albumin and haptoglobin), paraoxonase, and liver functionality index in dairy cows in early lactation; and that cows can be clustered based on EB time profiles, and the EB time profiles are were associated with metabolic status in early lactation.

Introduction

Early lactation is a high-risk period for health problems in dairy cows, related to the calving process and the initiation of milk synthesis (Vergara et al., 2014). Ribeiro and Carvalho (2017) reported that in the first 3 weeks of lactation, approximately one-third of dairy cows had at least one clinical disease, such as metritis, mastitis, lameness, and respiratory problems. Diseases and disorders in early lactation have a negative effect on milk yield, energy balance (**EB**), and reproductive performance of dairy cows (LeBlanc, 2010, Vergara et al., 2014, Carvalho et al., 2019).

Metabolic changes in early lactation including altered nutrient metabolism, inflammation, and oxidative stress can lead to increased risk for diseases (Sordillo and Raphael, 2013, Wisnieski et al., 2019). Cows that cannot meet energy demands through dry matter intake (**DMI**) experience a negative energy balance (**NEB**) in early lactation (Putman et al., 2018). As a result of NEB, mobilization of body fat increases plasma non-esterified fatty acids (**NEFA**) and beta-hydroxybutyrate (**BHB**) concentrations (Sordillo and Mavangira, 2014). High plasma NEFA and BHB have been associated with an increased risk of health concerns such as ketosis, retained placenta, metritis, and mastitis (Lacetera et al., 2005, Kehrli et al., 2006). Additionally, an elevated NEFA concentration is a contributing factor to many pro-inflammatory periparturient diseases in dairy cows including mastitis and metritis (Douglas et al., 2007, Sordillo et al., 2009).

Acute-phase proteins (**APP**), such as albumin and haptoglobin, are involved in the acute and systemic response to inflammation (Ceciliani et al., 2012). Increased inflammation during NEB is related to pro-inflammatory cytokines (Grimble, 1990, Ametaj et al., 2002), increased synthesis of positive acute-phase proteins (+APP; e.g. haptoglobin and ceruloplasmin), and reduced synthesis of negative acute-phase protein (-APP; e.g. albumin and cholesterol) (Fleck, 1989, Bionaz et al., 2007, LeBlanc, 2012). Earlier studies indicated that increased blood haptoglobin is related to uterine diseases such as clinical metritis (Huzzey et al., 2009, Chan et al., 2010, Dubuc et al., 2010). Quiroz-Rocha (2009) reported that an increased cholesterol concentration (a +APP) in the week before parturition was associated with an increased incidence of retained placenta after calving. Cows affected with ketosis after calving had a higher concentration of ceruloplasmin in plasma than healthy cows (El-Deeb and El-Bahr, 2017). Inflammation plays an important role in the etiology of some health disorders in early lactation (Bertoni et al., 2008, Huzzey et al., 2009, Dubuc et al., 2010), but the relation between inflammation and health problems in early lactation remains unclear. This study aimed to investigate the relationships between metabolism, inflammatory biomarkers, and associated clinical health in early lactation.

Materials And Methods

Experimental design and Animals

The Institutional Animal Care and Use Committee of Wageningen University & Research (Wageningen, Netherlands) approved the experimental protocol, which complies with the Dutch law on Animal Experimentation (protocol number 2016.D-0038.005). The experiment was conducted at Dairy Campus research farm (Leeuwarden, Netherlands) between December 2017 and January 2020.

The experimental design was previously described by Burgers et al. (2020). In summary, 154 Holstein-Friesian dairy (41 primiparous cows, 113 multiparous cows) were selected from the Dairy Campus research herd of 500 lactating cows based on the following criteria: no twin pregnancy, no clinical mastitis or SCC > 250,000 at the final 2 milk test days before dry-off, and expected to finish a complete lactation. In week 6 after calving, cows were blocked for parity, calving date, milk yield in the previous lactation (multiparous cows) or expected milk yield (primiparous cows), and the breeding value for persistency (CRV, Arnhem, Netherlands). First, 50 blocks of 3 cows were formed. After the removal of 2 cows before the end of the voluntary waiting period (VWP) due to culling as a result of diseases, 2 more blocks of 3 cows were added. In total, 154 cows were included (52 blocks×3 cows=156 cows; 2 cows were excluded). Cows were randomly divided into blocks over 3 treatment groups: a voluntary waiting period until the first insemination (VWP) of 50 days (VWP-50), 125 days (VWP-125), or 200 days (VWP-200). Cows in the 3 treatment groups were inseminated after their VWP when estrous was detected. Cows were milked twice daily around 6 am and 6 pm in a 40-cow rotary milking parlor (GEA, Dusseldorf, Germany). For the current study data and samples were used from the first 6 weeks of the first lactation during the experiment, at a time when the VWP contrast was not yet executed. In this study, we included EB, metabolic variables, health problem treatment in weeks 1 until 6, inflammatory biomarkers, and oxidative stress values in weeks 1,2, and 4 in the first lactation.

Rations

Details on diet composition were presented earlier (Burgers et al., 2020). In short, during lactation, a partial mixed ration consisted of grass silage, corn silage, soybean meal, and wheat meal, supporting 22 kg of milk. Concentrate supply started at 1 kg per day from calving and increased stepwise per day from day 21 onwards (to 9 kg for primiparous cows, and 10 kg for multiparous cows).

Milk yield, Dry matter intake, and Energy balance

Milk yield was recorded at every milking, DMI was recorded continuously and reported weekly, and EB was calculated based on the difference between VEM supplied with feed (Van Es,1975, CVB, 2005) and requirements of 42.4 VEM/kg$^{0.75 \cdot d}$ (1,000 VEM=6.9 MJ of net energy). The VEM required for milk production is 442 VEM per kg of fat-and-protein-corrected milk (FPCM) (Van Es, 1975). Milk production was converted to FPCM using the following formula (CVB, 2012):

$$\text{FPCM (kg)} = \text{milk (kg)} \times (0.337 + 0.116 \times \text{fat (\%)} + 0.06 \times \text{protein (\%)})$$

Blood Sampling

Blood samples were taken weekly. In the morning, 3h before feeding, blood samples were collected from the tail vein. Blood was collected in evacuated tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria) containing NaF for glucose, EDTA for insulin, FFA, BHB, and lithium-heparin for IGF-I, albumin, cholesterol, creatinine, total protein, urea, globulin, ceruloplasmin, calcium, glutamic oxaloacetic transaminase (GOT), haptoglobin, paraoxonase, myeloperoxidase (MPO), ferric-reducing antioxidant power (FRAP), reactive oxygen metabolites (ROM), vitamin A, vitamin E, and β-carotene. Samples were kept cold on ice for a maximum of 2h until they were centrifuged at 3,000×g for 15 min at 4°C. Plasma was decanted, aliquoted, and frozen at -20°C until analysis.

Health problems

Treatments for health problems were recorded. Clinical mastitis was diagnosed and recorded by the staff at the Dairy Campus research herd, during the morning or evening milking. A case of clinical mastitis was defined as a case of visibly abnormal milk, visible changes in the udder due to inflammation, or both. Cows that were treated for clinical health problems (CHP), which were defined as endometritis, fever, clinical mastitis, or retained placenta, are diseases that cause an inflammatory response (Mayasari et al., 2017). The OHP was defined as milk fever, vaginal discharge, cystic ovaries, claw and leg problems, stomach and intestine problems, cobalt deficiency, and 3 teats. The NHP cows were defined as cows that were not treated for any health problems. Cows with CHP as well as cows with OHP were treated according to the herd-specific treatment plan based on the type and severity of the disease.

Laboratory Analysis

Metabolite and hormone concentrations were measured at the Veterinary Physiology group of the Vetsuisse Faculty, University of Bern (Bern, Switzerland). The concentration of glucose was measured using commercial kit no. 61269 and no. 61974 from BioMérieux (Marcy l'Étoile, France). Concentrations of NEFA and BHB were measured 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 (RIA).

Inflammatory biomarkers and oxidative stress variables were measured at the Istituto di Zootecnica of the Università Cattolica del Sacro Cuore (Piacenza, Italy), following the procedures previously described by Bionaz et al. (2007), Calamari et al.(2016), Jacometo et al.(2015), and Mayasari et al. (2017) using a clinical auto-analyzer (ILAB 650, Instrumentation Laboratory, Lexington, MA). In short, albumin, cholesterol, total protein, urea, calcium, and creatinine were measured using the IL Test purchased from Instrumentation Laboratory Spa (Werfen Co., Milan, Italy). Globulin was calculated as the difference between total protein and albumin. The haptoglobin was determined with the method described by Skinner et al. (1991) and Owen et al. (1960), this method was based on the peroxidase activity of the methemoglobin-haptoglobin complex measured by the rate of oxidation of guaiacol (hydrogen donor) in the presence of hydrogen peroxide (oxidizing substrate). Ceruloplasmin concentration was determined with the method described by Sunderman and Nomoto (1970). The test is based on the measurement of color, which originates from the oxidation of p-phenylenediamine dihydrochloride induced by the ceruloplasmin. Reactive oxygen metabolites were measured using commercial kits (kit d-ROMs-test MC003; Diacron International s.r.l., Grosseto, Italy). Antioxidant potential was assessed as ferric-reducing antioxidant power (FRAP) using the colorimetric method of Benzie and Strain (1996). Plasma paraoxonase activity was measured by adapting the method of Ferré et al. (2002) to the ILAB 650 conditions. Plasma vitamins A and E were extracted with hexane and analyzed by reverse-phase HPLC using Spherisorb ODS-2, 3 m, in a 150 × 4.6 mm column (Alltech, Deerfield, IL); a UV detector set at 325 nm (for vitamin A) or 290 nm (for vitamin E); using 80:20 methanol:tetrarchy-drofurane as the mobile phase. The liver functionality index (LAI) was calculated according to Trevisi et al., 2010. This aggregate index includes the average blood level (7, 14, and 28 DIM) of some proteins synthesized by the liver: albumin, lipoproteins (indirectly measured as total cholesterol; Bruss, 1997), and retinol-binding protein (RBP) because the retinol released from the liver depends mainly on the synthesis of apo-RBP (Wolf, 1984).

Data analysis

The clustering of the EB time profile in this section was based on Vossebeld et al., 2022, as follows:

Handling of Missing Data and Data Imputation. The EB values at some time points (weeks) in the current study were missing, making it impossible to calculate complete EB time profiles (over the 6 weeks) for all animals. As a result of the conservative approach of discarding animals with three or more missing time points, 1 animal was deleted because of 4 missing values and there were 36 animals (of 153) for which the EB times series had to be imputed. We imputed missing values using the missForest method based on Random Forest (Breiman, 2001; Stekhoven, 2011; Stekhoven and Bühlmann, 2012).

Clustering of Time Profiles. The clustering approach was based on a global alignment kernel (GAK; Cuturi et al., 2007). The elbow approach (Thorndike, 1953) and the silhouette method (Rousseeuw, 1987) were used to select the optimal number of clusters. After applied to the 51 complete (nonimputed) time series, 4 clusters were then used to cluster the imputed time series as described below. We used the missForest approach to generate 1,000 imputed solutions, and the GAK clustering algorithm to obtain 4 clusters of times series.

Statistical Analysis

Clustering of Cows Based on EB Time Profiles. Analysis was performed in R (The R Project; www.r-project.org/). Imputation was performed using the missForest function (options ntree = 1,000, maxiter = 10) from the missForest R package (Stekhoven, 2011). Clustering of time profiles was performed using the tsclust function (distance = ‘gak’) from the dtwclust package (Sarda-Espinosa, 2017). Silhouette values were calculated with the silhouette function from the cluster package (Brigo et al., 2002). Correspondence of the labels via the Hungarian method was obtained using the R function available at https: //www.r-bloggers.com/2012/11/matching-clustering -solutions-using-the-hungarian-method/; clustering consensus was obtained using the majority voting function from the diceR package (Chiu and Talhouk, 2018). The ROC analysis was performed using the R package pROC (Robin et al., 2011).

Relationships between health status and inflammatory biomarkers, metabolic variables. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Cows were grouped into 3 clusters (CHP, OHP, and NHP) based on health status in the first 6 weeks of the lactation. Health status clusters were related to inflammatory biomarkers and oxidative stress values in weeks 1, 2, and 4 and metabolites in week 1 until week 6 in the first lactation of this study.

The MIXED procedure of SAS was used to analyze the relationship between health status and inflammatory biomarkers (**Model 1**). Dependent variables in model 1 were albumin, cholesterol, creatinine, total protein, globulin, urea, calcium, ceruloplasmin, GOT, haptoglobin, FRAP, paraoxonase, MPO, ROM, Vitamin A, Vitamin E, β-carotene, and LAI. The fixed effects were health status clusters (CHP, OHP, or NHP), parity (1 or ≥2), week (1, 2, and 4 postpartum), and their 2-way interactions. Cows were considered the repeated subject.

To analyze the relationship between health status (CHP, OHP, or NHP) and metabolic status, a similar MIXED model of SAS was used (**Model 2**). Dependent variables in model 2 were milk yield, DMI, NEFA, BHB, glucose, insulin, IGF-1, and EB. The fixed effects were health status clusters (CHP, OHP, or NHP), parity (1 or ≥2), and week (1, 2, 3, 4, 5, and 6 postpartum), and their 2-way interactions. Cows were considered the repeated subject.

Relationships between EB clusters and inflammatory biomarkers, metabolic variables. Similar to the health status clusters, the MIXED procedure of SAS was used to analyze the relationships between EB clusters and inflammatory biomarkers (**Model 3**), and metabolic variables (**Model 4**). Dependent variables in model 3 were albumin, cholesterol, creatinine, total protein, globulin, urea, calcium, ceruloplasmin, GOT, haptoglobin, FRAP, paraoxonase, MPO, ROM, Vitamin A, Vitamin E, β-carotene, and LAI. The fixed effects were EB clusters (SP, MN IN, or SN), parity (1 or ≥2), week (1, 2, and 4 postpartum), and their 2-way interactions. Cows were considered the repeated subject. Dependent variables in model 4 were milk yield, DMI, NEFA, BHB, glucose, insulin, and IGF-1. The fixed effects were health status clusters EB clusters (SP, MN IN, or SN), parity (1 or ≥2), and week (1, 2, 3, 4, 5, and 6 postpartum), and their 2-way interactions. Cows were considered the repeated subject.

In all models, a first-order autoregressive covariance matrix [**AR(1)**] was the best fit according to the Akaike information criterion (Aikake, 1997) and was used to account for within-cow variation. In case of a significant interaction effect, multiple comparison adjustments were performed for the post-hoc comparison of groups using the Bonferroni adjustment method. Values are presented as least square means (LSM) and were regarded significant if p-values < 0.05 and as a tendency if $0.05 \leq p$-values < 0.10.

Results

Distribution of health problem treatments

Of the 154 cows that entered the experiment, 66 (43%)cows had a total of 100 health problem treatments in the first 6 weeks postpartum. Of these treatments, 31 treatments happened in the CHP group (24 cows), and 69 treatments were in the OHP group (42 cows) (**Table 1**). The number of cows in the CHP, OHP, and NHP groups were 24, 42, and 88, respectively. Of the CHP treatments, 15 treatments happened in the first week after calving **(Figure 1)**.

Relations between Health status, Inflammatory Biomarkers, and Oxidative stress

Cows with CHP were found to have a lower concentration of albumin, paraoxonase, and a higher concentration of haptoglobin in plasma compared with NHP cows or OHP cows (**Table 2**). The CHP cows had higher creatinine concentration in plasma compared with NHP cows, but not OHP cows. The CHP cows tended to have a lower concentration of β-carotene compared with OHP cows. In week 1 after calving, CHP cows had a higher concentration of urea (P=0.04) compared with NHP cows, whereas no differences were found in week 2 and week 4.

Relations between Health status and Milk Yield, Dry Matter Intake, and Metabolic Status

From week 1 until week 6 after calving, CHP cows tended to have a lower DMI compared with OHP cows and NHP cows (**Table 2; Figure 2A**). Regarding metabolic status, relationships between CHP and concentration of glucose, insulin, and IGF-1 depended on the week in lactation. In week 1, NHP cows had a higher concentration of glucose (P <0.01) compared with OHP cows (**Figure 2B**), a higher concentration of insulin (P <0.01) when compared with OHP cows and CHP cows (**Figure 2C**), and a higher concentration of IGF-1 (P<0.01) compared with CHP cows (**Figure 2D**). No significant relations were found between the health status of cows and milk yield, NEFA, and BHB concentration in plasma (**Table 3**).

Table 1. The number of disease treatments for cows during the first 6 weeks after calving.

Health	Treatments, n	Week1	Week2	Week3	Week4	Week5	Week6	Total
CHP	Fever	0	1	0	0	1	0	2
	Clinical mastitis	1	1	0	0	1	0	3
	Retained placenta	9	1	0	0	0	0	10
	Endometritis	5	1	2	3	2	3	16
OHP	Milk fever	9	0	0	0	0	0	9
	Vaginal discharge	1	1	8	20	7	1	38
	Cystic ovaries	0	0	0	0	0	5	5
	Claw and leg problems	0	0	0	0	1	2	3
	Stomach and intestine problems[1]	0	0	3	0	0	0	3
	Other[2]	1	2	2	4	2	0	11
	Total	26	7	15	27	14	11	**100**

[1] Main stomach and intestine problems: rotavirus, diarrhea, peritonitis.

[2] Main diagnoses in ‘other’: cobalt deficiency, 3 teats.

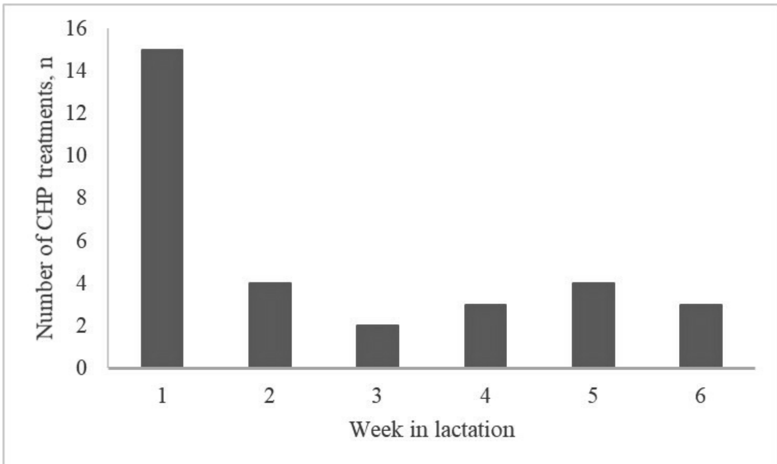

Figure 1. Distribution of clinical health problem (CHP) treatments in the first 6 weeks after calving.

Table 2. Variables regarding inflammatory biomarkers (albumin, cholesterol, total protein, globulin, ceruloplasmin, and haptoglobin), index of liver function (paraoxonase) oxidative stress (GOT, MPO, ROM, and FRAP), creatinine, urea, calcium, vitamin A, vitamin E, β-carotene and LAI in plasma of 154 dairy cows in week 1, 2 and 4 after calving categorized according to the health status in first 6 weeks after calving.

	Health Status[1]				Parity			*P*-value[2]					
	CHP	OHP	NHP	SEM	1	≥2	SEM	HS	Par	Week	HSxPar	HSxWeek	ParxWeek
Cows, n	24	42	88										
Weeks 1, 2 and 4													
Albumin (g/L)	31.9[a]	34.3[b]	34.4[b]	0.62	32.8	34.3	0.46	<0.01	<0.01	0.01	0.94	0.59	0.80
Cholesterol (mmol/L)	2.73	2.90	3.07	0.19	2.75	3.04	0.14	0.17	0.06	<0.01	0.77	0.30	0.14
Creatinine (µmol/L)	84.6[a]	82.4[ab]	81.1[b]	1.67	81.0	84.3	1.25	<0.05	0.02	<0.01	0.41	0.63	0.94
Total protein (g/L)	73.2	72.4	72.8	1.35	70.7	74.9	1.01	0.85	<0.01	<0.01	0.20	0.99	0.18
Globulin (g/L)	41.4	38.0	38.5	1.42	37.9	40.7	1.06	0.12	0.02	<0.01	0.22	0.99	0.03
Urea (mmol/L)	3.42	3.54	3.55	0.18	3.44	3.56	0.13	0.78	0.41	<0.05	0.13	<0.01	0.68
Calcium(mmol/L)	2.40	2.43	2.45	0.04	2.45	2.41	0.03	0.37	0.11	0.16	0.95	0.57	0.60
Ceruloplasmin (µmol/L)	1.84	1.90	1.81	0.11	1.81	1.89	0.08	0.48	0.39	<0.01	0.16	0.20	0.01
GOT(U/L)[3]	77.4	85.4	84.5	5.89	75.4	89.5	4.36	0.49	<0.01	<0.01	0.22	0.30	<0.01
Haptoglobin (g/L)	0.68[a]	0.50[b]	0.43[b]	0.08	0.59	0.48	0.06	<0.01	0.09	<0.01	0.82	0.76	0.15
FRAP(µmol/L)[4]	153	173	158	11.7	169	154	8.72	0.14	0.12	<0.01	0.12	0.30	0.02
Paraoxonase (U/ml)	54.6[a]	74.2[b]	73.4[b]	4.97	70.4	64.4	3.68	<0.01	0.14	<0.01	0.60	0.06	0.17
MPO (U/L)[5]	461	437	434	17.4	451	436	13.0	0.34	0.30	<0.01	0.80	0.39	0.02
ROM (mg H_2O_2/100mL)[6]	15.3	13.7	13.7	0.80	14.4	14.0	0.60	0.14	0.56	<0.01	0.96	0.13	0.04
Vitamin A (mg/100mL)	29.1	33.1	33.8	2.43	31.4	32.5	1.82	0.20	0.58	<0.01	0.08	0.60	0.57
Vitamin E (mg/mL)	2.03	2.48	2.48	0.26	2.11	2.55	0.20	0.27	0.04	<0.01	0.83	0.66	0.80
β-carotene (mg/100mL)	0.44	0.62	0.54	0.06	0.52	0.55	0.55	0.05	0.55	<0.01	0.44	0.43	0.51
LAI[7]	-0.54[a]	-0.03[b]	0.04[b]	0.16	-0.33	-0.02	0.12	<0.01	0.02		0.44		

[1]CHP: clinical health problem; OHP: other health problem; NHP: no health problem in the first 6 weeks after calving.

[2] HS= health status; Par= parity. [3]GOT= glutamic oxaloacetic transaminase. [4]FRAP= ferric-reducing antioxidant power. [5]MPO= myeloperoxidase. [6]ROM= Reactive oxygen metabolites

[7]LAI= Liver activity index

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Table 3. Variables regarding milk yield, dry matter intake, and metabolic status after calving for cows were categorized according to the health status of 154 dairy cows during the first 6 weeks after calving.

	Health status[1]				Parity			P-value[2]					
	CHP	OHP	NHP	SEM	1	≥2	SEM	HS	Par	Week	HSxPar	HSxWeek	ParxWeek
Cows, n	24	42	88										
Week 1- 6													
Milk yield (kg/d)	28.4	31.9	32.2	1.72	24.5	37.1	1.29	0.12	<0.01	<0.01	0.11	0.14	<0.01
DMI (kg/d)[3]	16.1	17.6	17.4	0.58	14.3	19.7	0.43	0.07	<0.01	<0.01	0.17	0.75	<0.01
NEFA (mmol/L)[4]	0.31	0.31	0.28	0.02	0.28	0.32	0.03	0.50	0.24	<0.01	0.26	0.07	0.72
BHB (mmol/L)[5]	0.77	0.74	0.74	0.08	0.74	0.76	0.06	0.93	0.83	<0.01	0.46	0.91	0.06
Glucose (mmol/L)	3.43	3.32	3.38	0.09	3.54	3.22	0.07	0.46	<0.01	<0.01	0.80	<0.01	0.45
Insulin (µIU/mL)	14.3	11.8	12.7	1.30	13.1	12.8	0.95	0.24	0.81	<0.01	0.26	<0.01	0.22
IGF-1 (ng/mL)[6]	99.0	112	117	12.0	127	91.9	8.85	0.34	<0.01	<0.01	0.09	0.02	0.03
EB (kJ/$kg^{0.75}$·d)[7]	-158	-183	-171	43.6	-120	-222	32.7	0.87	<0.01	<0.01	0.29	0.55	0.84

[1]CHP: clinical health problem; OHP: other health problem; NHP: no health problem in the first 6 weeks after calving.

[2] HS= health status; Par= parity. [3]DMI= days in milk. [4]NEFA= non-esterified fatty acids. [5]BHB= beta-hydroxybutyrate. [6]IGF-1= Insulin-like growth factor 1. [7]EB= energy balance.

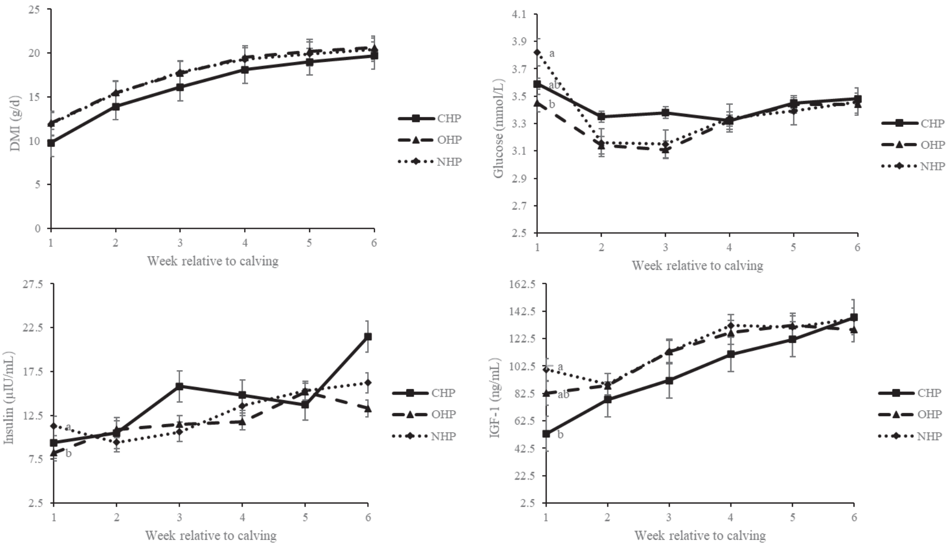

Figure 2. (A) Dry matter intake (DMI), (B) Glucose, (C) Insulin, and (D) IGF-1 of cows in different health status clusters (CHP, OHP, and NHP) in the first 6 weeks after calving. CHP: cows with clinical health problems treatment; OHP: healthy cows with mild health problems treatments; NHP: cows with no treatment for health problems in the first 6 weeks after calving. Values represent LSMEANS±SEM.

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Energy Balance Time Profiles Clustering

Based on their similarity in EB profile in the first 6 weeks after calving, 153 cows were clustered into 4 clusters (**Figure 3A**). We assigned descriptive names to each cluster based on the characteristics of the EB dynamics in postpartum weeks:

(1) SP cluster: includes cows with a stable positive EB time profile over the 6-week period. The animals do not experience NEB (EB > 0).

(2) MN cluster: includes cows with a moderate NEB (-100 $kJ/kg^{0.75}$ < EB < 0) after calving.

(3) IN cluster: includes cows with an intermediate NEB (-400 $kJ/kg^{0.75}$ < EB < -200 $kJ/kg^{0.75}$) after calving.

(4) SN cluster: includes cows with severe NEB (-600 $kJ/kg^{0.75}$ < EB <-200 $kJ/kg^{0.75}$) after calving.

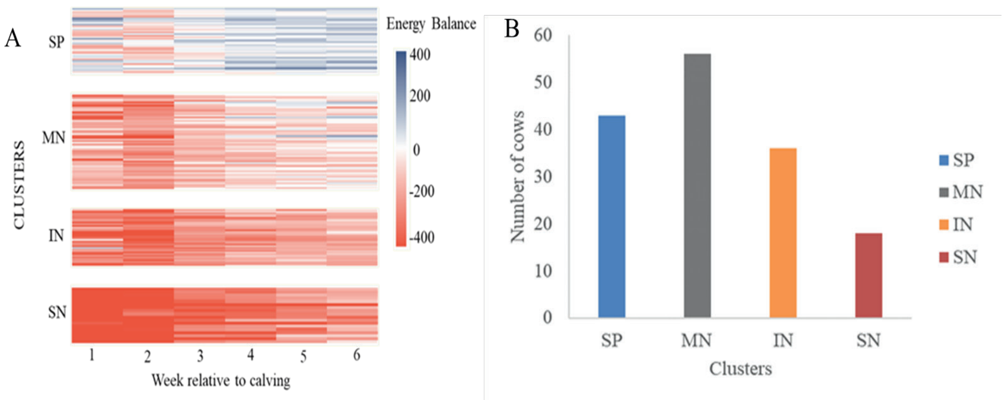

Figure 3. (A) Clustering of energy balance time profiles. Each line represents the time series of the energy balance of an individual cow. Energy balance is expressed in $kJ/kg^{0.75*d}$. SP = stable positive cow cluster; MN = mild negative cow cluster; IN = intermediate negative cow cluster, SN = severe negative cow cluster. (B) The number of cows in each cluster of time series of energy balance using imputed data.

Relationships between EB clusters, Inflammatory Biomarkers, and oxidative stress

Cows in the SN cluster tended to have a higher concentration of creatinine and a lower concentration of ceruloplasmin in plasma compared with cows in the SP cluster (**Table 4**). In addition, cows in the SN cluster tended to have a higher concentration of urea compared with cows in the IN cluster. No difference between EB clusters was found in other inflammatory biomarkers.

Relation between EB status and Milk Yield, Dry Matter Intake, and Metabolic Status

From week 1 until week 6 after calving, cows in the SP cluster had a higher concentration of glucose, insulin, and IGF-1 in plasma compared with cows in other EB clusters (**Table 5**). In addition, cows in the MN cluster had a higher concentration of glucose and IGF-1 in plasma compared with cows in the SN cluster. Relations between EB status and milk yield and DMI, as well as the concentration of NEFA and BHB, depended on the week in lactation. Cows in the SN cluster had the highest milk yield and cows in the SP cluster had the lowest milk yield (**Figure 4A**). With the largest significant difference in week 1, cows in the SN cluster had a significantly lower DMI ($P<0.01$) compared with cows in other clusters (**Figure 4B**). Cows in the SN cluster had the highest level of NEFA and BHB, and cows in the SP cluster had the lowest level of NEFA and BHB in plasma (**Figure 4C, Figure 4D**).

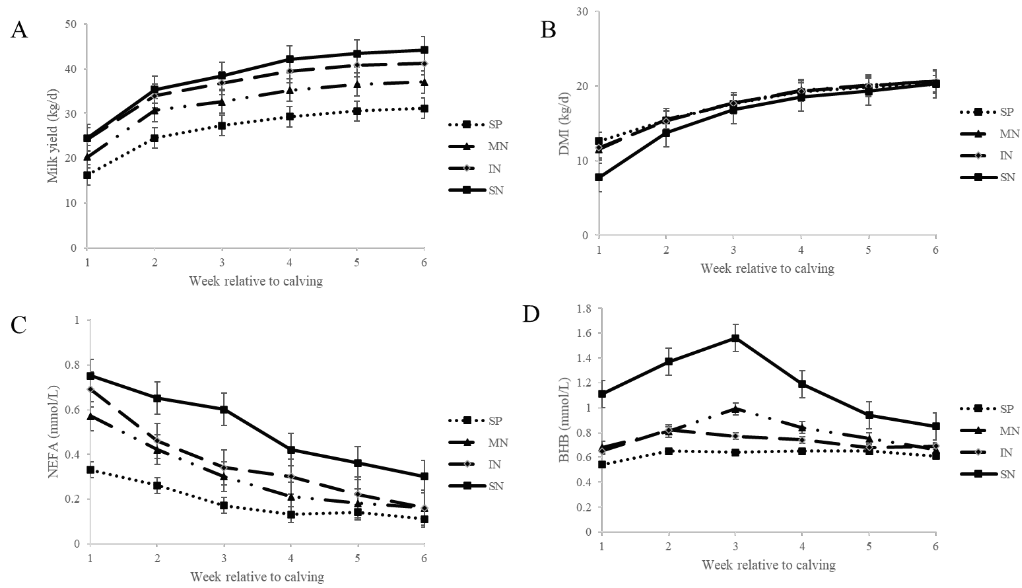

Figure 4. (A) Milk yield, (B) Dry matter intake, (C) NEFA, and (D) BHB of cows in different energy balance clusters (SP = stable positive cluster, MN = mild negative cluster, IN = intermediate negative cluster, SN = severe negative cluster) in the first 6 weeks after calving. Values represent LSMEANS±SEM.

Table 4. Variables regarding inflammatory biomarkers (albumin, cholesterol, total protein, globulin, ceruloplasmin, and haptoglobin), index of liver function (paraoxonase) oxidative stress (GOT, MPO, ROM, and FRAP), creatinine, urea, calcium, vitamin A, vitamin E, β-carotene and LFI in plasma of 153 dairy cows in week 1, 2 and 4 after calving categorized according to energy balance (EB) clusters in first 6 weeks after calving.

	Energy Balance cluster[1]					Parity			P-value[2]					
	SP	MN	IN	SN	SEM	1	≥2	SEM	EB	Par	Week	NEBxPar	NEBxWeek	ParxWeek
Cows, n	43	56	36	18										
Weeks 1, 2 and 4														
Albumin (g/L)	33.6	34.3	34.0	35.1	1.09	33.8	34.7	0.62	0.33	0.19	0.01	0.95	0.88	0.95
Cholesterol (mmol/L)	2.96	2.99	3.04	2.86	0.17	2.83	3.09	0.19	0.96	0.19	<0.01	0.96	0.50	0.63
Creatinine (µmol/L)	82.2	84.6	83.6	87.4	2.73	84.1	84.8	1.55	0.09	0.66	<0.01	0.84	0.97	0.83
Total protein (g/L)	73.1	72.6	72.7	74.6	2.27	71.9	74.6	1.29	0.84	0.05	<0.01	0.96	0.36	0.13
Globulin (g/L)	39.5	38.3	38.7	39.4	2.47	38.0	39.9	1.40	0.70	0.22	<0.01	0.99	0.15	0.02
Urea (mmol/L)	3.50	3.71	3.30	3.74	0.29	3.56	3.57	0.16	0.08	0.95	0.02	0.36	0.53	0.32
Calcium(mmol/L)	2.43	2.45	2.44	2.41	0.06	2.46	2.41	0.03	0.86	0.16	0.09	0.96	0.52	0.48
Ceruloplasmin (µmol/L)	1.73	1.85	1.80	2.13	0.17	1.89	1.87	0.10	0.09	0.83	0.11	0.27	0.22	0.11
GOT(U/L)[3]	78.2	84.9	84.8	89.9	9.02	78.5	90.4	5.12	0.24	0.03	<0.01	0.06	0.09	0.04
Haptoglobin (g/L)	0.48	0.47	0.44	0.60	0.07	0.54	0.45	0.07	0.76	0.24	<0.01	0.94	0.15	0.55
FRAP(µmol/L)[4]	161	166	149	129	19.0	148	154	10.8	0.16	0.64	0.04	0.14	0.82	0.13
Paraoxonase (U/ml)	69.5	75.7	67.1	67.8	8.14	73.0	67.0	4.62	0.17	0.22	<0.01	0.28	0.55	0.25
MPO (U/L)[5]	442	434	411	458	28.5	438	434	16.3	0.28	0.82	<0.01	0.12	0.34	0.07
ROM (mg H_2O_2/100mL)[6]	13.6	13.6	13.3	14.8	1.32	13.8	13.8	0.75	0.81	0.99	0.04	0.25	0.40	0.17
Vitamin A (mg/100mL)	33.2	34.6	31.6	29.6	4.09	31.4	33.1	2.33	0.46	0.50	<0.01	0.81	0.53	0.46
Vitamin E (mg/mL)	2.42	2.50	2.60	1.77	0.44	2.09	2.55	0.25	0.37	0.08	<0.01	0.75	0.77	0.80
β-carotene (mg/100mL)	0.57	0.55	0.56	0.46	0.11	0.51	0.55	0.06	0.80	0.53	<0.01	0.06	0.56	0.82
LAI[3]	-0.07	0.08	0.02	0.06	0.18	0.07	-0.02	0.11	0.71	0.47		0.96		

[1] Energy balance clusters: SP = stable positive cow cluster; MN = mild negative cow cluster; IN = intermediate negative cow cluster, SN = severe negative cow cluster. [2] Par: parity

[3]GOT= glutamic oxaloacetic transaminase. [4]FRAP= ferric-reducing antioxidant power. [5]MPO= myeloperoxidase. [6]ROM= Reactive oxygen metabolites

[7]LAI= Liver activity index.

2

Table 5. Variables regarding milk yield, dry matter intake, and metabolic status after calving for cows were categorized according to the energy balance (EB) clusters of 153 dairy cows during the first 6 weeks after calving.

	Energy Balance cluster[1]					Parity			*P*-value[2]					
	SP	MN	IN	SN	SEM	1	≥2	SEM	EB	Par	Week	NEBxPar	NEBxWeek	ParxWeek
Cows, n	43	56	36	18										
Week 1- 6														
Milk yield (kg/d)	26.5	32.1	36.1	38.0	1.95	21.3	31.1	0.63	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
DMI (kg/d)[3]	17.5	17.5	17.4	16.1	0.95	14.5	19.7	0.54	0.56	<0.01	<0.01	0.24	<0.01	0.02
NEFA (mmol/L)[4]	0.19	0.31	0.36	0.51	0.05	0.37	0.32	0.03	<0.01	0.12	<0.01	0.58	<0.01	0.24
BHB (mmol/L)[5]	0.62	0.79	0.73	1.17	0.13	0.91	0.75	0.07	<0.01	0.04	<0.01	0.11	<0.01	0.01
Glucose (mmol/L)	3.57[a]	3.31[b]	3.23[bc]	2.96[c]	0.12	3.33	3.20	0.07	<0.01	009	<0.01	0.20	0.93	0.39
Insulin (µIU/mL)	15.1[a]	12.1[b]	11.0[b]	9.39[b]	1.78	11.3	12.5	1.02	<0.01	0.27	<0.01	0.80	0.93	0.37
IGF-1 (ng/mL)[6]	139[a]	109[b]	85.8[bc]	76.0[c]	17.2	110	95.1	9.84	<0.01	0.16	<0.01	0.59	0.28	0.06

[1] Energy balance clusters: SP = stable positive cow cluster; MN = mild negative cow cluster; IN = intermediate negative cow cluster, SN = severe negative cow cluster. [2] Par: parity.

[3]DMI= days in milk. [4]NEFA= non-esterified fatty acids. [5]BHB= beta-hydroxybutyrate. [6]IGF-1= Insulin-like growth factor 1. [7]EB= energy balance.

Discussion

In the current study, the CHP cows had lower concentrations of albumin, lower LAI, and a higher concentration of haptoglobin in plasma compared with OHP and NHP cows. Our findings are in line with Tóthová et al. (2017), who reported that cows with post-partum metritis, as well as mastitis had lower plasma albumin concentrations compared with healthy cows. Cows with retained placenta and metritis had lower concentrations of plasma albumin compared with healthy cows (Green et al., 2009, Burke et al., 2010). Several studies have observed lower serum concentrations of albumin in cows with clinical health problems in early lactation, especially mastitis, compared with healthy cows (Katolm et al., 1992, Risvanli et al., 1999). This general decrease in albumin concentrations in sick animals may be attributed to the role of albumin as a negative APP (Gruys et al., 1994). Serum haptoglobin has been stated as a valid biomarker that can distinguish diseased animals from healthy animals (Eckersall and Bell, 2010) and is especially effective in the diagnosis and prognosis of mastitis, enteritis, and endometritis (Murata et al., 2004; Petersen et al., 2004). Additionally, it has been reported that healthy cows have plasma haptoglobin concentrations < 20 mg/L, which can increase to > 2 g/L within 2 days after the occurrence of infection (Eckersall and Bell, 2010). The concentrations of haptoglobin in healthy cows in the current study were much higher than 20 mg/L. In contrast to the cows in the Eckersall and Bell (2010) study that were not undergoing any known inflammatory processes; however, the early lactation stage may explain the higher concentrations during the current study. Acute-phase proteins of liver origin serve as useful biomarkers to evaluate chronic inflammation mainly due to the decrease of circulating concentrations of negative APP (e.g., albumin) and the contemporary increase of positive APP (e.g., haptoglobin; Bertoni et al., 2008, Ceciliani et al., 2012). The substantially reduced LAI in CHP cows in the current study was due to the lower plasma concentration of albumin and higher concentration of haptoglobin compared with OHP and NHP cows. A low concentration of LAI in plasma was confirmed to be related to a high frequency of inflammatory conditions and serious clinical health problems (Trevisi et al., 2008), and cows with low LAI, which are more susceptible to metabolic and infectious diseases, should be closely monitored so that diseases can be identified at an early stage (Trevisi et al., 2010).

In the current study, the CHP cows had lower concentrations of paraoxonase in plasma compared with OHP and NHP cows. Paraoxonase is mainly synthesized in the liver, Feingold et al. (1998) concluded that paraoxonase is a negative APP and a marker for liver functionality. In this case, a cause for decreased paraoxonase could be an inflammatory condition; i.e. any serious disease. Kovačić et al. (2019) demonstrated decreased paraoxonase in cows with subclinical and clinical mastitis indicating oxidative stress and inflammatory response in the mastitis development. Low plasma paraoxonase concentration was associated with increased occurrence of metritis in early lactation (Bionaz et al., 2007).

In the present study, cows with CHP had higher creatinine concentrations in plasma compared with cows with NHP, but not compared with OHP cows. High creatinine concentrations in plasma indicate a prolonged active-tissue protein catabolism (Carlotti et al., 2008), and this protein deficiency impairs animals' humoral and cellular immunity, making them more susceptible to diseases (Titgemeyer and Loest, 2001). There was a 2.2-fold greater risk of metritis in cows with a serum creatinine concentration > 2.0 mg dL^{-1} postpartum than in those with a lower concentration of this metabolite (Torres et al., 2020). Cows with endometritis had higher creatinine in plasma compared with healthy cows (Sattler and Fürll, 2004, Kaya et al., 2016).

The results or our study indicated that the CHP cows tended to have a lower DMI in early lactation compared with OHP cows and NHP cows. Lower DMI is related to a stronger NEB, resulting in lower concentrations of glucose, insulin, and IGF-1, and an increased risk of health problems (Mair et al., 2016). The CHP cows had lower insulin and IGF-1 concentration concentrations in plasma compared with NHP cows in the first week after calving. The OHP cows had lower glucose concentrations in plasma compared with NHP cows, but not with CHP cows in week 1. In the current study, within CHP cows, 48% (15/31) of CHP treatments happened in week 1 (Figure 1), which could explain the significant metabolites changes in week 1. In addition, health disorders showed high initial effects on feed intake decrease, and the largest decrease in total feed intake was observed during the first occurrence of mastitis (Bareille et al 2003). Therefore, early diagnosis and treatment of health problems in early lactation are essential for the success of the adaptation to the new lactation.

In the current study, cows in the SN cluster tended to have higher concentrations of creatinine and ceruloplasmin in plasma compared with cows in the SP cluster. This is in line with Xu et al., (2020), who reported that EB was negatively correlated with plasma creatinine (r=-0.51), and this was explained by the mobilization of muscle protein in dairy cows in NEB. Mobilization of the muscle protein is related to higher concentrations of plasma creatine and creatinine in cows with low energy balance (Castillo et al., 2006). Ceruloplasmin, a positive APP, determination could provide information about the health status of cows (Skinner et al., 2001), the inflammation increases during NEB by stimulating the hepatic synthesis of positive APP (e.g. ceruloplasmin) (Ametaj et al., 2005, Bionaz et al., 2007). Thus, the relatively high ceruloplasmin concentration of cows in the SN cluster may be related to inflammation.

In our current study, cows in different EB clusters had different metabolic patterns postpartum. Cows in clusters with a large decrease in EB postpartum also had high NEFA and BHB concentrations, and low IGF-1, insulin, and glucose concentrations, which is in agreement with previous studies (Reist et al., 2002; Friggens et al., 2007; Van et al., 2020). The lowest EB, resulting from the highest milk yield but lowest DMI, is characterized in the SN cluster. During a severe NEB, cows have a low concentration of glucose in plasma. This leads to a low insulin concentration, resulting in an increased concentration of NEFA as a consequence of body fat mobilization. To compensate for the energy deficit in early lactation, dairy cows mobilize body reserves (Collard et al., 2000) such as body fat (van der Drift et al., 2012), which is related to a high concentration of NEFA and BHB. Increased plasma NEFA and BHB are known to increase the risk of negative health effects (Adewuyi et al., 2005; Ospina et al., 2010).

Conclusions

The findings of this study showed that incidence of clinical health problem in early lactation is related to lower concentrations of albumin, paraoxonase, a lower LAI level, and a higher concentration of haptoglobin in plasma. High concentrations of glucose, insulin, and IGF-1 in plasma in week 1 were associated with the occurrence of clinical health problem in the first 6 weeks after calving. This study also identified that dairy cows can be clustered based on their EB time profiles. Clusters of EB profiles were associated with metabolic status, milk yield, and DMI. Overall, concentration changes of inflammatory biomarkers (albumin and haptoglobin), paraoxonase, and LAI in plasma may help indicate future health problem treatments. And in future studies, maybe we can explore the value to cluster cows based on indicators for EB, e.g. body weight, or body condition.

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References

Adewuyi, A., Gruys, E., Van Eerdenburg. F., 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet Q. 27, 117-126.

Ametaj, B. N., Bradford, B. J., Bobe, G., Nafikov, R. A., Lu, Y., Young, J. W., Beitz, D. C., 2005. Strong Relationships between Mediators of the Acute Phase Response and Fatty Liver in Dairy Cows. Canadian J. Anim Sci. 85, 165-175.

Ametaj, B., Bradford, B. J., Bobe, G., Lu, Y., Nafikov, R., Sonon, R., Young, J,. Beitz. D., 2002. Acute phase response indicates inflammatory conditions may play a role in the pathogenesis of fatty liver in dairy cows. J. Dairy Sci. 85, 189.

Bareille, N., Beaudeau, F., Billon, S., Robert, A., Faverdin, P., 2003. Effects of health disorders on feed intake and milk production in dairy cows. Livest Prod Sci. 83, 53-62.

Benzie, I. F., Strain, J. J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochem. 239, 70-76.

Bertoni, G., Trevisi, E., Han, X., Bionaz, M., 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91, 3300-3310.

Bionaz, M., Trevisi, E., Calamari, L., Librandi, F., Ferrari, A., Bertoni, G., 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90, 1740-1750.

Breiman, L., 2001. Random forests. Mach. Learn. 45, 5-32.

Brigo, D., Mercurio, F., Rapisarda, F., 2002. Parametrizing correlations: A geometric interpretation. Milan: Banca IMI Working Paper.

Bruss, M. L., 1997. Lipids and ketones. Clinical biochemistry of domestic animals. 83-115.

Burke, C. R., Meier, S., McDougall, S., Compton, C., Mitchell, M., Roche, J. R., 2010. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J. Dairy Sci. 93, 5363-5373.

Calamari, L., Ferrari, A., Minuti, A., Trevisi, E., 2016. Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on Fourier Transform mid-infrared spectroscopy: preliminary results. BMC veterinary research. 12, 1-10.

Carlotti, A., Bohn, D., Matsuno, A., Pasti, D., Gowrishankar, M., Halperin, M., 2008. Indicators of lean body mass catabolism: emphasis on the creatinine excretion rate. QJM: An International Journal of Medicine 101, 197-205.

Carvalho, M., Peñagaricano, F., Santos, J., DeVries, T., McBride, B., Ribeiro, E., 2019. Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. J. Dairy Sci.102, 11701-11717.

Castillo, C., Hernandez, J., Valverde, I., Pereira, V., Sotillo, J., Alonso, M. L., Benedito, J., 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res Vet Sci. 80, 133-139.

Ceciliani, F., Ceron, J., Eckersall, P., Sauerwein, H., 2012. Acute phase proteins in ruminants. J. Proteom. 75, 4207-4231.

Chan, E. K., Nagaraj, S. H., Reverter. A., 2010. The evolution of tropical adaptation: comparing taurine and zebu cattle. Anim Genet. 41, 467-477.

Chiu, D. S., Talhouk, A., 2018. diceR: An R package for class discovery using an ensemble driven approach. BMC Bioinformatics. 19:11.

Collard, B. L., Boettcher, P. J., Dekkers, J. C., Petitclerc, D., Schaeffer, L. R., 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. J. Dairy Sci. 83, 2683-2690.

Cuturi, M., Vert, J. P., Birkenes, O., Matsui, T., 2007. A kernel for time series based on global alignments. Pages II-413–II-416 in Proc. 2007 IEEE International Conference on Acoustics, Speech and Signal Processing-ICASSP'07. IEEE.

Douglas, G., Rehage, J., Beaulieu, A., Bahaa, A., Drackley, J., 2007. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. J. Dairy Sci. 90, 2941-2959.

Dubuc, J., Duffield, T., Leslie, K., Walton, J., LeBlanc, S., 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93, 5764-5771.

Eckersall, P. D., Bell, R., 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The Vet. J. 185:23-27.

El-Deeb, W. M., El-Bahr, S. M., 2017. Biomarkers of ketosis in dairy cows at postparturient period: acute phase proteins and pro-inflammatory cytokines. Veterinarski arhiv. 87, 431-440.

Feingold, K. R., Memon, R. A., Moser, A. H., Grunfeld, C., 1998. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. Atherosclerosis. 139, 307-315.

Ferré, N., Camps, J., Prats, E., Vilella, E., Paul, A., Figuera, L., Joven. J., 2002. Serum paraoxonase activity: a new additional test for the improved evaluation of chronic liver damage. Clinical Chem. 48, 261-268.

Fleck, A., 1989. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proc Nutr Soc. 48, 347-354.

Friggens, N., Berg, P., Theilgaard, P., Korsgaard, I., Ingvartsen, K., Løvendahl, P., Jensen, J., 2007. Breed and parity effects on energy balance profiles through lactation: Evidence of genetically driven body energy change. J. Dairy Sci. 90, 5291-5305.

Green, M. P., Ledgard, A. M., Berg, M. C., Peterson, A. J., Back, P. J., 2009. Prevalence and identification of systemic markers of sub-clinical endometritis in postpartum dairy cows. Pages 37-42 in Proc of the New Zealand Society of Animal Production. New Zealand Society of Animal Production.

Grimble, R., 1990. Nutrition and cytokine action. Nutr Res Rev. 3, 193-210.

Gruys, E., Obwolo, M.J., Toussaint, M.J., 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. Veterinary Bulletin, 64, 1009-1018.

Hammon, D., Evjen, I., Dhiman, T., Goff, J., Walters, J., 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. Immunopathol. 113, 21-29.

Huzzey, J., Duffield, T., LeBlanc, S., Veira, D., Weary, D., Von Keyserlingk, M., 2009. Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92, 621-625.

Jacometo, C. B., Osorio, J. S., Socha, M., Corrêa, M. N., Piccioli-Cappelli, F., Trevisi, E., Loor, J. J., 2015. Maternal consumption of organic trace minerals alters calf systemic and neutrophil mRNA and microRNA indicators of inflammation and oxidative stress. J. Dairy Sci. 98, 7717-7729.

Kaewlamun, W., Grimard, B., Duvaux-Ponter, C., Ponter, A., 2020. Kick-starting ovarian cyclicity by using dietary glucogenic precursors in post-partum dairy cows: a review. Int. J. Vet. Sci. 8, 39-48.

Kaya, S., Merhan, O., Kacar, C., Colak, A., Bozukluhan, K., 2016. Determination of ceruloplasmin, some other acute phase proteins, and biochemical parameters in cows with endometritis. Vet. World 9,1056-1062.

Kehrli, M., Neill, J., Burvenich, C., Goff, J., Lippolis, J., Reinhardt, T., Nonnecke, B., 2006. Energy and protein effects on the immune system. Ruminant physiology. Digestion, metabolism and impact of nutrition on gene expression, immunology and stress. 455-471.

Kovacic, J. C., Dimmeler, S., Harvey, R. P., Finkel, T., Aikawa, E., Krenning, G., Baker, A. H., 2019. Endothelial to mesenchymal transition in cardiovascular disease: JACC state-of-the-art review. Journal of the American College of Cardiology. 73, 190-209.

Lacetera, N., Scalia, D., Bernabucci, U., Ronchi, B., Pirazzi, D., Nardone, A., 2005. Lymphocyte functions in overconditioned cows around parturition. J. Dairy Sci. 88, 2010-2016.

LeBlanc, S., 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod Dev. 56, S29-S35.

LeBlanc, S., 2012. Interactions of metabolism, inflammation, and reproductive tract health in the postpartum period in dairy cattle. Reprod Domest Anim. 47, 18-30.

Mayasari, N., Chen, J., Ferrari, A., Bruckmaier, R., Kemp, B., Parmentier, H., Van Knegsel, A., Trevisi. E., 2017. Effects of dry period length and dietary energy source on inflammatory biomarkers and oxidative stress in dairy cows. J. Dairy Sci. 100, 4961-4975.

Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. The Veterinary Journal. 168, 28-40.

Ospina, P. A., Nydam, D. V., Stokol, T., Overton, T. R., 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.

Owen, J. A., Better, F. C., Hoban, J., 1960. A simple method for the determination of serum haptoglobins. J. Clin. Pathol. 13, 163-164.

Petersen, H. H., Nielsen, J. P., Heegaard, P., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35, 163-187.

Pinedo, P., Santos, J., Chebel, R., Galvão, K., Schuenemann, G., Bicalho, R., Gilbert, R., Zas, S. R., Seabury, C., Rosa, G., 2020. Early-lactation diseases and fertility in 2 seasons of calving across US dairy herds. J. Dairy Sci. 103, 10560-10576.

Putman, A., Brown, J., Gandy, J., Wisnieski, L., Sordillo, L., 2018. Changes in biomarkers of nutrient metabolism, inflammation, and oxidative stress in dairy cows during the transition into the early dry period. J. Dairy Sci. 101, 9350-9359.

Quiroz-Rocha, G.F., LeBlanc, S. J.,. Duffield, T. F., Wood, D., Leslie, K. E., Jacobs, R. M., 2009. Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. The Canadian Veterinary Journal. 50, 383.

Reist, M., Erdin, D., Von Euw, D., Tschuemperlin, K., Leuenberger, H., Chilliard, Y., Hammon, H., Morel, C., Philipona, C., Zbinden, Y., 2002. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. J. Dairy Sci. 85, 3314-3327.

Ribeiro, E.S., Carvalho, M.R., 2017. Impact and mechanisms of inflammatory diseases on embryonic development and fertility in cattle. Anim Reprod. 14, 589-600.

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J.C., Müller, M., 2011. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 12, 77.

Rousseeuw, P.J., 1987. Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. J. Comput. Appl. Math. 20, 53-65.

Sarda-Espinosa, A., 2017. Package ‘dtwclust’: Time series clustering along with optimizations for the dynamic time warping distance.

Sattler, T., Fürll, M., 2004. Creatine kinase and aspartate aminotransferase in cows as indicators for endometritis. Transboundary and Emerging Diseases. 5, 132-137.

Skinner, J. G., 2001. International standardization of acute phase proteins. Veterinary Clinical Pathology 30, 2-7.

Skinner, J.G., Brown, R. A., Roberts, L., 1991. Bovine haptoglobin response in clinically defined field conditions. Vet. Rec. 128, 147-149.

Sordillo, L., Mavangira, V., 2014. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim Prod Sci. 54, 1204-1214.

Sordillo, L.M., Raphael, W., 2013. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. Veterinary Clinics: Food Animal Practice. 29, 267-278.

Steeneveld, W., Schukken, Y., Van Knegsel, A., Hogeveen, H., 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.

Stekhoven, D. J., 2011. Using the missForest package. R package, 1-11.

Stekhoven, D. J., Bühlmann, P., 2012. MissForest-Non-parametric missing value imputation for mixed-type data. Bioinformatics. 28, 112-118.

Sunderman, F. W., Nomoto, S., 1970. Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. Clinical Chem. 16, 903-910.

Thorndike, R. L., 1953. Who belongs in the family? Psychometrika. 18, 267-276.

Titgemeyer, E. and C. Löest. 2001. Amino acid nutrition: Demand and supply in forage-fed ruminants. J. Anim Sci. 79, E180-E189.

Torres, E., Mellado, M., Leyva, C., García, J. E., Véliz, F. G., Hernández-Bustamante, J., 2020. Serum metabolites and body condition score associated with metritis, endometritis, ketosis, and mastitis in Holstein cows. Pesquisa Agropecuária Brasileira. 55.

Tóthová, C., Mudroň, P., Nagy, O., 2017. The electrophoretic pattern of serum proteins in dairy cows with inflammatory diseases. Acta veterinaria. 67(2).

Trevisi, E., Zecconi, A., Bertoni, G., Piccinini, R., 2010. Blood and milk immune and inflammatory profiles in periparturient dairy cows showing a different liver activity index. J. Dairy Res.77, 310-317.

Trevisi, E., Amadori, M., Archetti, I., Lacetera, N., Bertoni, G., 2011. Inflammatory response and acute phase proteins in the transition period of high-yielding dairy cows. Acute phase proteins as early non-specific biomarkers of human and veterinary diseases. 15, 355-379.

Trevisi, E., Ferrari, A. R., Bertoni, G., 2008. Productive and metabolic consequences induced by the retained placenta in dairy cows. Vet. Res. Com. 32, 363-366.

Turk, R., Juretic, D., Geres, D., Turk, N., Rekic, B., Simeon-Rudolf, V., Svetina, A., 2004. Serum paraoxonase activity and lipid parameters in the early postpartum period of dairy cows. Res. Vet Sci. 76, 57-61.

Van der Drift, S., Houweling, M., Schonewille, J., Tielens, A., Jorritsma, R., 2012. Protein and fat mobilization and associations with serum β-hydroxybutyrate concentrations in dairy cows. J. Dairy Sci. 95, 4911-4920.

Vergara, C., Döpfer, D., Cook, N., Nordlund, K., McArt, J., Nydam, D., Oetzel, G., 2014. Risk factors for postpartum problems in dairy cows: Explanatory and predictive modeling. J. Dairy Sci. 97, 4127-4140.

Wisnieski, L., Norby, B., Pierce, S., Becker, T., Gandy, J., Sordillo, L., 2019. Predictive models for early lactation diseases in transition dairy cattle at dry-off. Prev Vet Med. 163, 68-78.

Xu, W., Vervoort, J., Saccenti, E., Kemp, B., van Hoeij, R. J., van Knegsel, A., 2020. Relationship between energy balance and metabolic profiles in plasma and milk of dairy cows in early lactation. J. Dairy Sci. 103, 4795-4805.

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Chapter 3

Consequences of transition treatments on fertility and associated metabolic status for dairy cows in early lactation

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Abstract

This study aimed to (1) investigate effects of reducing postpartum dietary energy level for cows after a 0-d dry period (DP) on resumption of ovarian cyclicity and reproductive performance, (2) relate days open with other reproductive measures, and (3) relate onset of luteal activity (OLA) and days open with metabolic status in early lactation. Holstein-Friesian dairy cows were randomly assigned to 1 of 3 transition treatments: 0-d DP and low postpartum dietary energy level from 22 days in milk(DIM)onwards (0-d DP (LOW)) (n = 43), 0-d DP and standard postpartum dietary energy level (0-d DP (STD)) (n = 43), and a short DP and standard postpartum dietary energy level (30-d DP (STD)) (n = 42). Milk progesterone concentration was determined three times per week until 100 DIM. Plasma metabolite and hormone concentrations were measured weekly until week 7 postpartum. Reducing postpartum dietary energy level in older cows (parity ≥ 3) after 0-d DP and 22 DIM did not affect milk production but prevented a positive energy balance and shortened the interval from calving to OLA. In addition, services per pregnancy and days open were reduced in cows of parity ≥ 3 on 0-d DP (LOW), compared with cows of parity ≥ 3 with 0-d DP (STD), but not in cows of parity.

Introduction

Shortening or omitting the dry period (**DP**) length was reported to improve fertility, indicated by an earlier onset of first ovulation postpartum (Watters et al., 2009) and overall improved resumption of ovarian cyclicity (Chen et al.,2015; Santschi et al., 2011; Watters et al., 2009). Consistent with the concept that earlier first ovulation may improve reproductive performance, omitting the DP was associated with an increased percentage of cows pregnant to first artificial insemination (**AI**) (55, 26, and 20% for 0-d, 28-d, and 56-d DP, respectively) and decreased days open (94, 121, and 145 d for 0-d, 28-d, and 56-d DP, respectively) (Gümen et al., 2005). In some other studies, no effects of DP on pregnancy rate and days open were found after a short DP (Pezeshki et al., 2008; Chen et al.,2015) or an omitted DP (Chen et al.,2015), even the number of days open was somewhat lower in cows with a 56-d DP than with a 42-d DP, but first-service pregnancy rate was greater in multiparous cows with a 35-d DP vs. longer DP (Pezeshki et al., 2007). In all these studies, however, the dietary energy level was not adjusted to the lower expected milk yield for cows with 0-d DP or a short DP, compared with cows with a conventional DP.

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Unfavorable consequences of omitting the DP can be a reduction in milk yield and fattening of cows in the subsequent lactation (Chen et al., 2016). Adjusting dietary energy levels in early lactation could be a strategy to limit these negative consequences (Van Hoeij et al., 2017a). Earlier we reported that reducing dietary energy level for cows with 0-d DP did not affect milk yield or milk composition, but resulted in a less positive energy balance (**EB**) and less body weight gain in the subsequent lactation, compared with cows fed a standard energy level (**STD**) after 0-d DP (Van Hoeij et al., 2017). Moreover, cows with 0-d DP fed a low energy level (**LOW**) had less days open than cows with 30-d DP(STD) or 0-d DP(STD). Days open is influenced by both the interval between calving to insemination and the success of an insemination (Britt et al., 1974). Resumption of ovarian cyclicity, which is a combined trait consisting of onset of ovarian activity and regularity of the ovarian cycles, are related with the timing of the first insemination and success of insemination (Pushpakumara et al., 2003). It can be hypothesized that the reduced days open for cows with 0-d DP(LOW) is related with an improved resumption of ovarian cyclicity or an increased conception rate after insemination or both.

Improvement in reproductive performance related with a reduction in length of the DP can be associated with an improved EB and metabolic status (Chen et al.,2015; Gümen et al., 2005; Watters et al., 2009). Concentrations of plasma glucose, IGF-I, and insulin were greater, but milk yields, plasma concentrations of non-esterified fatty acids (**NEFA**), and liver tri-acyl glycerides (**TAG**) concentrations were lower for cows with a 0-d DP than for cows with a 30-d or 60-d DP (Chen et al., 2015). These endocrine and metabolic factors are associated with ovarian activity postpartum (Butler, 2000; Armstrong et al., 2003; Lucy, 2003). Elevated concentrations of NEFA and β-hydroxybutyrate (**BHB**) in plasma reduced oocyte and blastocyst quality in vitro (Fouladi-Nashta et al., 2007, Garnsworthy et al., 2009) and were associated with a later onset of luteal activity (OLA) in vivo (Chen et al., 2015, Van Knegsel et al., 2014). In particular, IGF-1 and insulin are key factors for the ovarian function because they both stimulate estradiol-17β production in granulosa cells (Gutiérrez et al., 1997; Glister et al., 2001) and proliferation of follicular cells (Spicer et al., 1993; Spicer and Stewart, 1996) in vitro. Contradicting, high plasma insulin concentration had negative effects on oocyte maturation in vitro (Fouladi-Nashta et al., 2007) and insulin stimulating diets are possibly beneficial to establish ovarian activity postpartum, but not to establish pregnancy (Garnsworthy et al., 2009). Therefore, it can be hypothesized that for reproductive performance, with days open being the net result of resumption of ovarian cyclicity and pregnancy rates, an optimal energy balance and metabolic status would be essential rather than a maximal EB or insulin concentration.

Earlier, we reported consequences of reducing postpartum dietary energy level for cows after 0-d DP on energy balance, metabolic status and days open, as compared with cows after 0-d DP or a short DP with standard dietary energy level (Van Hoeij et al., 2017a). The overall aim of the current study was to unravel the effect of reducing postpartum dietary energy level on days open for cows after 0-d DP. The first objective of the current study was to investigate the effects of reducing postpartum dietary energy level for cows after 0-d DP on resumption of ovarian cyclicity, and reproductive performance, as compared with cows after 0-dDP or 30-d DP with standard postpartum dietary energy level. The second objective of this study was to relate days open with other reproductive measures. The third objective of this study was to relate OLA and days open with metabolic status in early lactation.

Materials and Methods

Animals and Housing

The Institutional Animal Care and Use Committee of Wageningen University & Research approved the experimental protocol in compliance with the Dutch law on Animal Experimentation as described earlier (protocol number 2014125; Van Hoeij et al., 2017). The experiment was performed from 27 January 2014 until 9 May 2016. Holstein-Friesian dairy cows (n = 128) at Dairy Campus research farm (Lelystad, The Netherlands) were selected based on (1) expected calving interval < 490 days, (2) daily milk yield > 16 kg at 90 days before the expected calving date and (3) no clinical mastitis and SCC < 250,000 cells/mL at 2 final test days before conventional drying off day. Cows pregnant with twins were excluded from the study. Cows were housed in a free stall barn with a slatted floor and cubicles and were milked twice daily (6:00 and 18:00 h). Cows with a 30-d DP were fed a dry cow ration from 7 days before drying off and milked once daily from 4 days before drying off. 0-d dry period cow antibiotics were used in any of the cows in this study.

Experimental Design

The experiment was originally designed to study the effects of DP length, dietary energy level, and mid-lactation ration (glucogenic or lipogenic ration) on milk production, milk composition, EB, plasma metabolites, and lactation persistency during a complete lactation (Van Hoeij et al., 2017a). Cows were blocked by expected calving date, milk yield in previous lactation and parity. We aimed for 40 cows per transition treatment, but because of the long-term characteristic of this experiment we included 2 spare blocks and when cows had to be omitted before calving they were replaced. Within each block of 6 cows, 4 cows were assigned randomly to 0-d dry period (0-d DP) and 2 cows to a short dry period of 30 days (30-d DP). Cows with a 0-d DP were assigned randomly to either a low postpartum dietary energy level (LOW) or a standard postpartum dietary energy level (STD) in early lactation. In the first 3 weeks after calving, dietary energy level was the same for all 3 transition treatments: all cows received 1 kg of concentrate from 10 days before the expected calving date and from 4 DIM concentrate supply increased stepwise for all transition treatments with 0.3 kg/d until 6.7 kg/d at 22 DIM (Hoeij et al., 2017). For cows fed the STD diet, concentrate supply was increased further until 8.5 kg/d at 28 DIM, resulting in a dietary energy level contrast (LOW vs. STD) from 22 DIM onwards (Figure 1). A standard dietary energy level was based on the energy requirement for expected milk yield of 30-d DP cows (Spicer et al., 1996). All 30-d DP cows were fed an STD. A low dietary energy level was based on the energy requirement for expected milk yield of cows with 0-d DP (Spicer et al., 1996). Thus, all cows were assigned to one of 3 transition treatments: 0-d DP (LOW) (n = 43), 0-d DP (STD) (n = 43), 30-d DP (STD) (n = 42) (Table 1). From week 8 postpartum onwards, cows received either a glucogenic or lipogenic basal ration. Preliminary analysis showed that mid-lactation ration (glucogenic vs. lipogenic ration) did not affect days open or ovarian cyclicity in the first 100 days in milk (DIM) and was therefore not included in the analysis of this study.

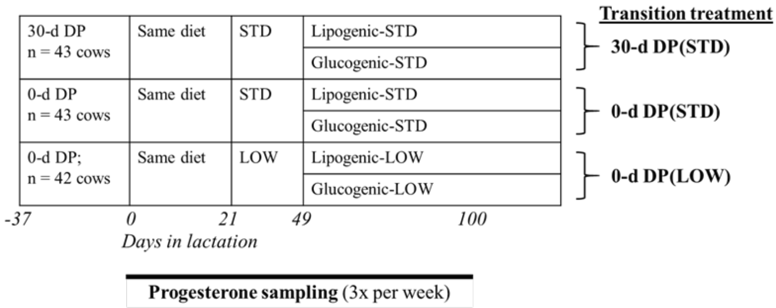

Figure 1. Overview of experimental design and sampling protocol for cows with different transition treatments. 30-d DP = 30 days dry period; 0-d DP= 0 day dry period; STD = standard energy level; LOW = low energy level.

Table 1. Distribution of cows with 0 (0-d DP) or 30 days (30-d DP) of dry period and fed a low (LOW) or standard (STD) dietary energy level.

Cows, n	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	Total
Cows in experiment	42	43	43	128
Cows with OLA activity within 100 DIM[1]	42	42	43	127
Cows with complete 1st ovarian cycle	41	39	39	119
Cows with complete 2nd ovarian cycle	32	26	29	87

Measurements

Feed intake and energy balance.

Daily concentrate intake was recorded by a computerized feeder (Manus VC5, DeLaval, Steenwijk, the Netherlands). Daily ration intake was recorded individually using roughage intake control (RIC) troughs and averaged per week (Insentec, Marknesse, the Netherlands).

Energy balance was calculated as net energy (NE) intake minus NE for maintenance, milk yield and pregnancy per week with Dutch NE system for lactation (VEM system; Van Es et al., 1975). According to the Dutch NE system, the daily requirement for maintenance is 42.4 VEM/kg $^{0.75}$ BW per day, milk yield is 442 VEM/kg fat- and protein-corrected milk (FPCM). 1000 VEM is equal to 6.9 MJ NE.

Milk sampling and progesterone assay.

Milk samples were collected three times a week (Monday, Wednesday and Friday) during morning milking from the day of parturition until 100 days in milk (DIM). Samples were stored at -20 °C until analysis of progesterone (P4) concentration. Milk P4 concentration was measured by enzyme immunoassay (Ridgeway Science Ltd., Gloucestershire, UK). The intra-assay and inter-assay coefficients of variation were 4.4% and 16.7%, respectively.

Blood Sampling and Analysis

Blood was collected weekly from calving until 7 weeks postpartum as described earlier (Hoeij et al., 2017). In short, blood samples were collected after the morning milking and between 3 and 1 h before the morning feeding from the coccygeal vein or artery into evacuated EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria). Concentrations of NEFA and BHB were measured enzymatically using kit no. 994–75409 from Wako Chemicals (Neuss, Germany) and kit no. RB1007 from Randox Laboratories (Ibach, Switzerland,) respectively (Graber et al., 2011). The plasma glucose concentration was measured using kit no. 61,269 from BioMerieux (Marcy l'Etoile, France) (Graber et al., 2011). The plasma insulin concentration was measured using kit no. PI-12K from EMD Millipore Corporation (Billerica, MA, USA). The plasma IGF-1 concentration was measured using kit no. A15729 from Beckman Coulter (Fullerton, CA, USA).

Definitions of ovarian cyclicity.

First OLA postpartum was defined within 100 DIM as the moment P_4 was 4 ng/ml or higher for 2 or more consecutive milk samples. Ovarian cycle length was defined as the number of days between OLA in one ovarian cycle and the OLA in next ovarian cycle. Regularity of ovarian cyclicity of cows was classified into one of 3 groups according to P_4 profile from parturition until 100 DIM (adapted from Chen et al., 2005):

- Normal ovarian cycle: cycles with 18 - 24 days in length.
- Prolonged ovarian cycle: cycles with more than 24 days in length.
- Short ovarian cycle: cycles with less than 18 days in length.

The percentages of the different type of cycles per cow were calculated within 100 DIM.

Reproduction protocol.

Cows were inseminated after a voluntary waiting period (**VWP**) of 50 days until at least 170 DIM. Artificial insemination was performed 12 hours after oestrous detection by Lely Qwes-HR Activity Tags (Lely, Maassluis, the Netherlands). Pregnancy of cows that were inseminated more than 30 days ago was checked by ultrasound.

Statistical analysis

Data of 128 cows until 100 DIM were collected, among which 6 cows which entered twice. The numbers of cows per treatment, cows with OLA, cows with complete 1st ovarian cycle and complete 2nd ovarian cycle within 100 DIM are presented in table 1.

First, regularity of ovarian cyclicity (percentages of normal, prolonged and short cycles per cow within 100 DIM) was analysed with the GLIMMIX procedure of SAS (Version 9.2; SAS institute, Inc., Cary, NC, USA). Fixed effects in the model were dry period strategy (0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)) and parity class (2 or ≥ 3).

Second, days open, days to first OLA, first length of luteal phase, length of follicular phase and length of the complete postpartum ovarian cycle, were analysed with MIXED procedure of SAS (Version 9.4; SAS Institute, Inc., Cary, NC, USA). Fixed effects in the model were dry period strategy (0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)) and parity class (2 or ≥ 3).

Third, cows were classified on number of days open (< 80, 80 to 130, > 130 d) or days to OLA (< 21, 21 to 30, > 30 DIM). Weekly plasma concentrations of glucose, NEFA, BHBA, IGF-1, insulin, and EB in week 1 till 7 postpartum were averaged. Subsequently, the MIXED procedure of SAS was used to analyse differences in plasma metabolites, hormones and EB among classes for days open or days to OLA. Fixed effects in the model were either days open class or OLA class, and always DP strategy (0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)) and parity class (2 or ≥ 3) and time (week relative to calving). Cow was considered as the repeated subject. Model assumptions were evaluated by examining the distribution of residuals. Values are presented as the least square mean with their standard errors of the mean. Differences were regarded as significant if $P < 0.05$, and trends were discussed if $P < 0.10$.When present ($P < 0.05$), interactions were clarified in a figure.

Results

The actual dry period length (DP) of cows with 30-d DP (STD) was 30 ± 6 d, the DP of cows with 0-d DP (LOW) or 0-d DP (STD) was 0 d. Results on EB and metabolic status after different transition treatments in the current experiment were reported earlier (Van Hoeij et al., 2017a). In short, reducing the level of energy in early lactation for cows after 0-d DP reduced EB both from week 4 till 7 and from week 8 till 44 (Van Hoeij et al., 2017b) postpartum, compared with a standard energy level after 0-DP. Postpartum, EB of cows with a 30-DP was more negative than of cow with a 0-d DP, the more negative EB was reflected in a greater plasma NEFA and BHB concentration, and a lower plasma glucose, insulin and IGF-1 concentration during weeks 4 and 7, compared with cows with a 0-d DP.

Effect of dry period strategy on days open and ovarian activity.

The effect of dry period strategy on days open was dependent on parity (Table 2; Figure 2a). Young cows (parity 2) with 0-d DP(LOW) or 0-d DP(STD) had less days open, compared with young cows with 30-d DP (89.9, 96.6 vs. 119.9 d for 0-d DP(LOW), 0-d DP(STD) vs. 30-d DP(STD); $P = 0.03$).

Older cows (parity ≥ 3), however, had less days open with 0-d DP(LOW), compared with older cows with 0-d DP (STD) or older cows with 30-d DP (STD) (105.2 vs. 182.3, 137.8 d for 0-d DP(LOW) vs. 0-d DP(STD), 30-d DP(STD); $P < 0.01$).

For young cows, there was no effect of different dry period strategies on services to conception. For older cows, cows with 0-d DP(LOW) or 30-d DP(STD) had less services to conception compared with older cows with 0-d DP(STD) (2.68, 2.71 vs. 4.31 for 0-d DP(LOW), 30-d DP(STD) vs. 0-d DP(STD); $P < 0.01$ respectively) (Figure 2b).

Cows with 0-d DP(LOW) had the greatest percentage of cows pregnant within 100 DIM compared with cows with 0-d DP(STD) or 30-d DP(STD), but there was no effect on the percentage of cows pregnant within 44 weeks. Cows with 0-d DP(LOW) had less days to first OLA compared with cows with 30-d DP(STD) ($P < 0.01$). Within 100 DIM, cows with a 0-d DP had more ovarian cycles compared with cows with 30-d DP length ($P = 0.01$). Among these cycles, cows with 0-d DP(LOW) had a lower percentage of prolonged cycles compared with cows with 0-d DP(STD) or 30-d DP(STD). For the first ovarian cycle, cows with 0-d DP(LOW) had shorter luteal phase and longer follicular phase compared with cows in group 0-d DP(STD).

Relationships between days open and ovarian activity.

Cows with short (< 80 d) and medium (80-130 d) days open had less days from calving till first AI compared with cows with long days open (>130 d) ($P<0.01$) (Table 3). Cows with short (< 80 d) and medium (80-130 d) days open had less services per conception compared with cows with long days open (>130 d) ($P<0.01$).

Relations between days open and OLA, percentage of normal, short and prolonged cycles and cycle length were depended on DP strategy. Cows with medium days open (80-130 d) with a 0-d DP had less days to OLA compared with cows with medium days open with 30-d DP (18.64 vs 18.10, 28.62 d for 0-d DP(LOW) vs. 0-d DP(STD), 30-d DP(STD) ($P = 0.01$)) (Figure A1a). Also, cows with medium (80-130 d) days open in group 0-d DP(LOW) had a greater percentage of normal regular cycles compared with cows with medium days open with 0-d DP(STD) (48.50 vs 25.75%; $P = 0.04$) (Figure A1b). Cows with days open less than 130 d (short and medium) had a lower percentage of prolonged ovarian cycles compared with cows with long days open (>130 d) and cows that did not get pregnant at all ($P<0.01$). Cows that did not get pregnant had lower percentage of short cycles compared with cows in other groups ($P<0.01$).

Table 2. Reproduction measures and incidence of normal and abnormal ovarian cyclicity within 100 days in milk (DIM) of cows with different transition treatments[1].

	Transition Treatments						SEM	*P*-Value[2]		
	0-d DP (LOW)		0-d DP (STD)		30-d DP (STD)			TT	P	TT × P
Parity	2	≥3	2	≥3	2	≥3				
Cows (n)	21	21	22	21	21	22				
Days open (days)	99.9^{a}	110.7^{a}	96.6^{a}	159.3^{b}	$114.9^{a,b}$	$127.5^{a,b}$	15.0	<0.01	<0.01	<0.01
Calving to first AI[3] (days)	73.5	66.9	66.1	71.9	67.7	69.0	5.0	0.97	0.48	0.50
Services per conception	2.3^{b}	2.7^{b}	2.1^{b}	4.3^{a}	$2.9^{a,b}$	2.7^{b}	0.4	0.27	0.02	<0.01
Pregnant within 44 weeks (%)	95.2	95.2	95.4	87.1	85.2	95.5	7.1	0.96	0.90	0.99
Pregnant within 100 DIM (%)	66.7^{a}	$57.1^{a,b}$	$59.1^{a,b}$	23.8^{b}	$42.9^{a,b}$	22.7^{b}	10.4	0.02	0.04	0.63
Days to 1st OLA[4]	17.0^{a}	$21.9^{a,b}$	$20.2^{a,b}$	27.6^{b}	$26.3^{a,b}$	28.3^{b}	2.6	<0.01	<0.01	0.31
Cycle number per cow within 100 DIM	$2.1^{a,b}$	$2.0^{a,b}$	2.2^{a}	$1.8^{a,b}$	$1.9^{a,b}$	1.7^{b}	0.2	<0.01	<0.01	0.07
Normal cycles (per cow within 100 DIM) (%)	48.6	39.6	41.7	39.7	42.0	42.1	8.4	0.48	0.44	0.29
Short cycles (per cow within 100 DIM) (%)	$16.5^{a,b}$	$18.1^{a,b}$	$18.5^{a,b}$	5.9^{a}	23.2^{b}	$7.0^{a,b}$	5.6	0.06	<0.01	<0.01
Prolonged cycles (per cow within 100 DIM) (%)	34.9^{a}	$42.4^{a,b}$	$40.9^{a,b}$	54.4^{b}	$34.8^{a,b}$	$50.9^{a,b}$	8.6	0.02	0.09	0.45
1st ovarian cycle postpartum (days)										
Luteal phase length (days)	$18.0^{a,b}$	$18.9^{a,b}$	$21.0^{a,b}$	21.8^{a}	13.9^{b}	$17.9^{a,b}$	2.7	<0.01	0.10	0.15
Follicular phase length (days)	$6.9^{a,b}$	10.4^{a}	$7.4^{a,b}$	6.5^{b}	$8.2^{a,b}$	$9.4^{a,b}$	1.3	<0.01	<0.01	<0.01
Cycle length (days)	24.9	28.3	28.4	28.3	22.2	27.3	2.9	0.02	<0.01	0.09

[a, b]Values with different superscripts differ ($P < 0.05$).

[1]Dry period strategies were: 0-d dry fed low energy level postpartum (0-d(LOW)); 0-d dry fed a standard energy level postpartum (0-d(STD)); 30-d dry fed a standard energy level postpartum (30-d DP(STD)).

[2]DP = Dry period strategy, P = Parity ; [3]AI = Artificial Insemination; [4]DIM = Days in milk; [5]OLA = Onset of luteal activity.

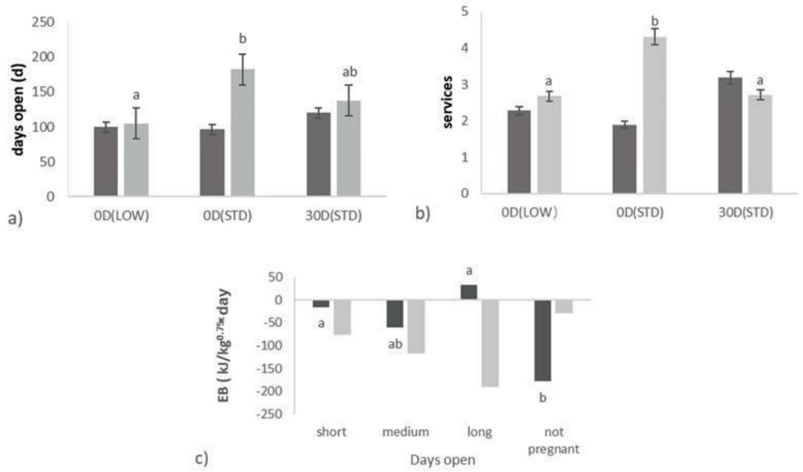

Figure 2. The effect of transition treatments (0-d DP (LOW), 0-d DP (STD), 30-d DP (STD) for cows of different parity classes (parity = 2 in dark grey or parity ≥ 3 in light grey) on days open (a), services per pregnancy (b); The relation of days open class (short:<80 d, medium: 80–130 d, long:>130 d and not pregnant) for cows of different parity classes (parity = 2 in dark grey or parity ≥ 3 in light grey) with energy balance (EB) in week 1 till 7 of lactation (c); [a,b]Values within parity class in the same row with different superscripts differ ($p < 0.05$).

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For the first ovarian cycle postpartum, cows with short (< 80 d) and medium (80 - 130 d) days open had shorter luteal phase and shorter cycle length, compared with cows with long days open (> 130 d) ($P = 0.01$). There was a tendency that cows with short (< 80 d) and medium (80 - 130 d) days open had shorter follicular phase compared with cows with long days open (> 130 d) ($P = 0.07$).

Relationships between Onset of Luteal Activity and Metabolic Status

Cows with OLA at less than 21 DIM had greater glucose, IGF-1 and insulin concentration, better EB and lower NEFA concentration compared with cows with OLA at 21 or more than 21 DIM ($P < 0.05$) (Table 4). For cows with OLA at less than 21 DIM, cows with 0-d DP (LOW) or 0-d DP (STD) had greater insulin concentration than cows with 30-d DP (STD) (16.69, 18.93 vs. 11.81 µIU/mL for 0-d DP (LOW), 0-d DP (STD) vs. 30-d DP (STD); $p < 0.01$)

Relations between days open and metabolic status.

Relations of days open with plasma concentration of insulin and EB in the first 7 weeks of lactation were dependent on parity (table 5). Young cows with long (>130 d) and short (<80 d) days open had a more positive EB compared with young cows with a medium days open (80–130 d) and cows that did not get pregnant at all (p = 0.01) (32.89, −15.69 vs. −60.47, −178.09 kJ/kg$^{0.75}$·day for long, short vs. medium days open, not pregnant) (Figure 2c). There was a trend that young cows with long days open (>130 d) had a greater insulin concentration in week 1 till 7 of lactation compared with young cows that did not get pregnant at all (17.47 vs. 10.22 μIU/mL) (p = 0.07). Cows with short and medium days open (<130 d) had a higher plasma insulin concentration, compared with cows that did not get pregnant at all.

Although EB in the first 7 weeks of lactation was negatively related with days open, this relationship was not so clear when the EB in the first 14 weeks was evaluated in relation with days open class. There was a tendency for the most negative EB in the first 14 weeks for cows with medium days open, compared with cows with short and long days open, which is also illustrated by Figure 3.

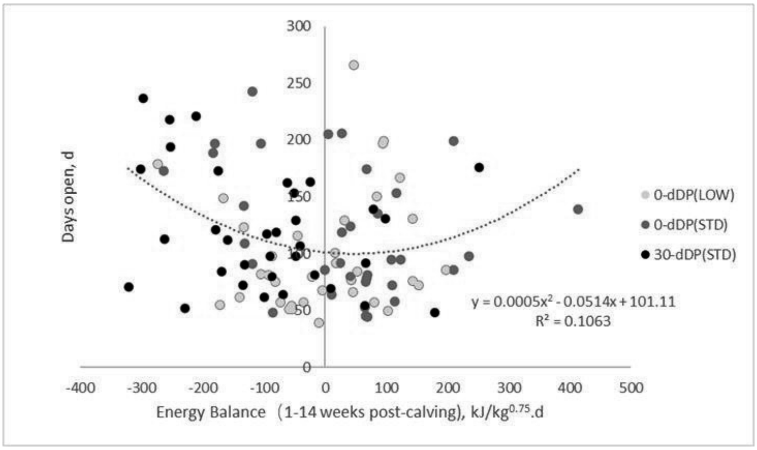

Figure 3. Energy balance for cows with different transition treatments in 1–14 weeks post-calving. Transition treatments were: 0-d dry fed low energy level postpartum (0-d (LOW)); 0-d dry fed a standard energy level postpartum (0-d (STD)); 30-d dry fed a standard energy level postpartum (30-d (STD)).

Table 3. Relationship between days open and characteristics of ovarian cycles of dairy cows with different transition treatments[1].

Variable	Days Open: Short (<80 d)	Days Open: Mid (80–130 d)	Days Open: Long (>130 d)	Days Open: Not Pregnant [3]	SEM	Transition Treatments: 0-d DP (LOW)	Transition Treatments: 0-d DP (STD)	Transition Treatments: 30-d DP (STD)	SEM	*P*-Value [2]: Days Open	*P*-Value [2]: P	*P*-Value [2]: TT	*P*-Value [2]: TT × Days Open
Cows, n	37	37	37	17		42	43	43					
Calving to first AI [4] (days)	60.42^{a}	68.85^{a}	80.16^{b}	68.07^{a}	5.55	71.02	67.44	69.66	6.03	<0.01	0.48	0.97	0.83
Services per pregnancy	1.44^{a}	2.71^{b}	4.09^{c}		0.36	2.36	3.31	2.57	0.16	<0.01	0.35	0.79	0.79
Days to first OLA [5]	24.73^{b}	22.20^{a}	23.76^{c}	24.68^{c}	1.36	19.26	25.53	26.73	2.84	<0.01	<0.01	<0.01	<0.01
Cycle number (per cow within 100 DIM)	1.62^{a}	2.49^{c}	2.00^{b}	1.74^{a}	0.06	2.32	1.77	1.80	0.20	<0.01	<0.01	<0.01	0.22
Normal cycles (per cow within 100 DIM) (%)	47.96^{a}	43.98^{a}	30.47^{b}	38.83^{ab}	4.66	42.28	40.02	38.62	6.41	<0.01	0.41	0.82	<0.01
Short cycles (per cow within 100 DIM) (%)	18.01^{a}	14.93^{a}	13.74^{a}	6.76^{b}	3.06	14.40	10.65	15.04	6.45	<0.01	<0.01	0.19	<0.01
Prolonged cycles (per cow within 100 DIM) (%)	35.03^{a}	40.92^{a}	55.31^{b}	52.90^{b}	4.88	42.17	49.26	46.68	9.75	<0.01	0.10	0.66	<0.01
1st ovarian cycle postpartum (days)													
Luteal phase length	17.94^{a}	18.22^{a}	22.03^{b}	18.29^{b}	0.74	20.18	21.01	16.16	2.92	<0.01	0.82	<0.01	0.05
Follicular phase length	7.02	7.33	9.72	7.73	0.77	8.83	6.59	8.43	1.38	0.07	0.01	<0.01	0.31
Cycle length	25.00^{a}	25.28^{a}	31.89^{b}	25.56^{a}	1.59	28.58	27.56	24.66	3.19	<0.01	0.30	<0.01	<0.01

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

[1]Transition treatments were: 0-d dry fed low energy level postpartum (0-d (LOW)); 0-d dry fed a standard energy level postpartum (0-d (STD)); 30-d dry fed a standard energy level postpartum (30-d (STD)); [2] TT = Transition treatment; *P* = Parity. [3]Cows did not get pregnant through the lactation. [4]AI = Artificial Insemination; [5]OLA = Onset of luteal activity. None of the variables had an interaction between Days open class and parity class, treatments and parity class.

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Table 4. Relationship between days to onset of luteal activity (OLA) and postpartum[1] plasma metabolites and metabolic hormones of cows after different dry period strategies[2].

	Days to OLA		SEM	Transition Treatments			SEM	P-Value[3]						
	<21 d	≥21 d		0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)		OLA	TT	P	W	OLA × TT	OLA × P	OLA × W
Cows, *n*	66	53												
Glucose (mmol/L)[4]	3.92[a]	3.71[b]	0.05	3.89	3.90	3.66	0.04	<0.01	<0.01	<0.01	<0.01	0.21	0.15	0.65
NEFA (mmol/L)[4]	0.11[a]	0.20[b]		0.12	0.12	0.23		<0.01	<0.01	<0.01	<0.01	0.47	0.01	0.24
	(0.10–0.13)	(0.17–0.22)		(0.11–0.14)	(0.10–0.14)	(0.20–0.26)								
BHB (mmol/L)[4]	0.63[a]	0.73[b]		0.66	0.64	0.74		<0.01	0.02	<0.01	0.01	0.01	0.07	0.36
	(0.59–0.67)	(0.68–0.79)		(0.60–0.72)	(0.59–0.69)	(0.69–0.80)								
IGF-1 (ng/mL)[4]	129.11[a]	95.31[b]	6.48	122.63	121.82	92.17	5.39	<0.01	<0.01	<0.01	<0.01	0.29	0.43	0.01
Insulin (μIU/mL)[4]	15.86[a]	11.41[b]	0.82	14.58	15.51	10.81	0.68	<0.01	<0.01	0.86	<0.01	0.10	0.71	0.08
EB ($kJ/kg^{0.75}\cdot day$) (week 1 till 7)[5]	−15.52[a]	−153.05[b]	26.61	−55.59	−18.33	−178.94	22.11	<0.01	<0.01	0.06	<0.01	0.22	<0.01	0.19

[a,b]Values with different superscripts differ ($p < 0.05$). [1]Postpartum = weeks 1 to 7 after calving.

[2]Transition treatments were: 0-d dry fed low energy level postpartum (0-d(LOW)); 0-d dry fed a standard energy level postpartum (0-d (STD)); 30-d dry fed a standard energy level postpartum (30-d (STD)).

[3]TT= Transition treatment; p = Parity; W = Week relative to calving.

[4]Concentration in plasma was measured weekly between weeks 1-7 post-calving. Non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) were log transformed for analysis, but are shown as actual values with confidence interval.

[5]EB = Energy balance. None of the variables had an interaction between treatments and parity class.

Table 5. Relationship between days open classes and postpartum[1] plasma metabolites and metabolic hormones of cows after different transition treatments[2].

	Days Open					Transition Treatments				P-Value[2]						
	<80 d	80-130 d	>130 d	Not Pregnant[5]	SEM	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)	SEM	Days Open	TT	P	W	Days Open × P	Days Open × TT	Days Open × W
	34	34	37	13												
Glucose (mmol/L)[3]	3.86	3.81	3.81	3.71	0.62	3.87	3.91	3.64	0.05	0.89	<0.01	0.04	<0.01	0.73	0.88	0.28
NEFA (mmol/L)[3]	0.12	0.15	0.15	0.16		0.13	0.10	0.22		0.12	<0.01	<0.01	<0.01	<0.01	0.44	0.53
	(0.09–0.14)	(0.13–0.18)	(0.12–0.18)	(0.12–0.20)		(0.11–0.16)	(0.08–0.12)	(0.19–0.26)								
BHB (mmol/L)[3]	0.66	0.67	0.73	0.66		0.69	0.62	0.73		0.53	0.03	0.12	<0.01	0.43	0.85	0.53
	(0.59–0.73)	(0.61–0.74)	(0.66–0.80)	(0.58–0.76)		(0.62–0.76)	(0.56–0.68)	(0.67–0.80)								
IGF-1 (ng/mL)[3]	122.26	116.49	111.48	105.99	7.24	122.04	128.34	91.79	6.61	0.72	0.73	0.37	<0.01	0.42	0.87	0.10
Insulin (µIU/mL)[3]	14.88[a]	14.07[a]	13.75[ab]	12.12[b]	0.98	14.04	16.42	10.65	0.78	<0.01	<0.01	0.42	<0.01	<0.01	0.92	0.57
EB ($kJ/kg^{0.75}\cdot day$) (week 1 till 7)[4]	−48.34[a]	93.09[b]	92.98[b]	−118.71[b]	31.64	−87.88	2.54	−179.51	38.84	<0.01	0.09	0.90	<0.01	<0.01	0.58	0.21
EB ($kJ/kg^{0.75}\cdot day$) (week 1 till 14)	−15.86	−26.47	−10.98	−33.50	21.53	−23.32	49.36	−91.15	20.60	0.05	0.02	0.02	0.36	0.05	0.72	0.37

[a,b]Values with different superscripts differ ($p < 0.05$).

[1]Postpartum = weeks 1 to 7 after calving, unless otherwise stated.

[2]Transition treatments were: 0-d dry fed low energy level postpartum (0-d(LOW)); 0-d dry fed a standard energy level postpartum (0-d (STD)); 30-d dry fed a standard energy level postpartum (30-d (STD)).

[3]TT = Transition treatments; *P* = Parity; W = Week relative to calving. [4]Concentration in plasma was measured weekly between weeks 1-7 post-calving. NEFA and BHB were log transformed for analysis, but are shown as actual values with confidence interval.

[5]EB = Energy balance.

[6]Cows did not get pregnant through the lactation. None of the variables had an interaction between treatments and parity class.

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Discussion

Reducing dietary energy level for older cows (parity ≥ 3) with 0-d DP(LOW) reduced days open with 77.1 and 32.5 days compared with older cows with 0-d DP(STD) or 30-d DP(STD). This reduction in days open was partly related with less days postpartum to OLA, partly to less services per conception, and possibly to less prolonged cycles for cows with 0-d DP(LOW), compared with cows with 0-d DP(STD) or cows with 30-d DP(STD). Also, in earlier studies, omitting of the DP resulted in a reduced interval from calving to first ovulation and less days open compared with 28 or 56 d dry period (Gümen et al., 2005; De Feu et al., 2009). Our results also shows that cows with 0-d DP had less days to OLA and more cycle numbers within 100 DIM compared with cows with 30-d DP. 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 ovulatory cycles before first service compared with cows with late or no ovulation (Galvão et al., 2010). Minimizing the interval to first ovulation provides ample time for conception of multiple ovarian cycles prior to insemination, which in turn improves conception rate and reduces less inseminations per pregnancy (Butler and Smith, 1989). In our study, cows with 0-d DP (LOW) had a 4.4 days earlier OLA than cows with 0-d DP (STD). Moreover, also the older cows (parity ≥ 3) with 0-d DP (LOW) had 1.6 less services to conception than cows with a 0-d DP (STD), which contributed more to a reduction in days open of older cows with 0-d DP (LOW). Gumen et al. (2005) also found that the number of services to conception was lower for cows with 0-d DP (1.75) than for cows with a standard DP (3.00), with cows with a short DP being intermediate (2.44).

No improvement in days open was found in young cows with different DP strategies in our study. Watters et al. (2009) also reported that reduction in days open (20 d) and increased pregnancies per insemination (8%) after 34-d DP were only observed in older cows (parity >2) and not in young cows (cows going from first to second lactation) when compared with cows with 55-d DP. Smith and Wallace (1998) reported that ovulation before 21 DIM was associated with reduced pregnancy rates, increased services per pregnancy, and a prolonged calving to pregnancy interval for multiparous, but not primiparous dairy cows. In our results, both younger and older cows had fewer days to OLA, but only older cows had shorter days open. Differences in response of older vs. younger cows to shortening or omitting the DP to fertility measures could be due to the relative priority of young cows for growth, as observed in another study on shortening of the DP (Watters et al. 2009) and a study on improving metabolic status by a more glucogenic diet (Van Knegsel et al., 2007).

In the current study, cows with 0-d DP had more ovarian cycles within 100 DIM compared with cows with 30-d DP. In addition, cows with 0-d DP(LOW) had a lower incidence of prolonged cycles compared with cows with 0-d DP(STD) or 30-d DP(STD), which can be related to shorter luteal phase length and follicular phase length. The prolonged luteal phase is one of the most common ovarian disturbances in dairy cows (Shrestha et al., 2004a). Studies reported that cows with prolonged luteal phase had lower first AI conception rate, more services to conception and more days open compared with cows with normal ovarian cycles (Lamming and Darwash, 1998; Royal et al., 2000, Shrestha et al., 2004b). The most important risk factors for developing prolonged luteal phases are puerperal problems, such as metritis and mastitis. The ratio of prostaglandin E_2 (**PGE_2**) to prostaglandin F2α (**$PGF_{2\alpha}$**) decides the fate of the corpus luteum, with persistence if PGE_2 dominates or luteolysis if $PGF_{2\alpha}$ dominates (Kaneko and Kawakami, 2009). Lipopolysaccharide stimulates the secretion of prostaglandins and particularly PGE2 (Herath et al., 2006). Metritis and mastitis could compromise release of prostaglandin F2α (**$PGF_{2\alpha}$**) (Opsomer et al., 2000; Shrestha et al., 2004b; Ranasinghe et al., 2011) by increasing lipopolysaccharide in postcalving cows (Mateus et al., 2003), thereby delaying luteolysis, which results in a prolonged luteal phase (Opsomer et al., 1998). In our study, however, no significant differences were found in the incidence of metritis and mastitis between the different study groups, the incidence of metritis in two groups were 7.14% and 4.65%, though the incidence of mastitis in group 0-d DP(LOW) was 30.95%, which was numerically lower than 39.53% in group 0-d DP(STD). A high milk yield is also one of the major risk factors for a prolonged luteal phase in high-producing dairy cows (Kafi et al., 2012). Also in the current study, the milk yield of cows with 30-d DP(STD) was greater than that of cows with 0-d DP(LOW) or 0-d DP(STD) (30.3 vs 24,.2, 24.7 kg/d) ($P < 0.01$).

The second objective of the current study was to relate days open of dairy cows after different transition treatments to underlying reproductive measures. Short (< 80 d) and medium (80 - 130 d) days open was related to a short interval form calving to first AI, compared with long days open. This is in accordance with Harrison (1975), who observed that average days open was positively related ($r^2 = 0.99$) to the interval to first AI in a field study in 12 commercial dairy herds in Michigan. In that study, the first insemination was directly decided by the time of the first ovulation after calving. Additionally, prolonged days open was related with a high number of services to conception (Gonzalez-Recio and Alenda, 2005). In our study, short (< 80 d) days

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open was related to less days to OLA, which can also partly reveal the occurrence of delayed resumption of ovarian cyclicity (Opsomer et al., 2000; Shrestha et al., 2004). Gautam et al. (2010) reported that delayed resumption of ovarian activity adversely influenced the hazard of pregnancy, which was partly driven by a delay in first AI as well as by a substantial reduction in first AI conception rate. In our study, more days open was not only related to delayed OLA, but to an increased number of services per pregnancy.

The third objective of the current study was to relate days open and OLA of cows after different transition treatments to their metabolic status in early lactation. Independent of DP strategy in current study, cows with OLA at less than 21 DIM had a better EB during week 1 to 7 and the concentration of IGF-1 and insulin were greater compared with cows with OLA between 21 and 30 DIM or longer than 30 DIM. The more negative EB in cows with OLA between 21 and 30 DIM or at longer than 30 DIM is possibly related with a compromised ovarian follicular development by suppressing plasma IGF-1 concentration and pituitary luteinizing hormone (LH) pulsatility (Lucy, 2000). This negatively impacts reproduction as IGF-1 is unable to synergise with the gonadotrophins on ovarian cells preventing the dominant follicle from ovulating (Beam and Butler, 1999) and delaying the resumption of cyclicity (Gutierrez et al., 1999), at last leading to a prolonged interval from calving to first ovulation (Shrestha et al., 2004a). We also noticed that cows with OLA at more than 30 DIM had a lower concentration of glucose and tended to have a greater NEFA concentration compared with cows with OLA at less than 21 DIM. Greater concentrations of circulating NEFAs are associated with lower follicular estradiol concentrations, impairing ovulation of the dominant follicle (Garverick., 2013). 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). Like discussed above, also, in the current study, cows with more prolonged cycles (0-d DP(STD) had numerically greatest incidence of mastitis in early lactation.

In the current study, cows with long days open (>130 d) or cows that did not get pregnant at all had greater plasma concentration of NEFA as well as a more severe NEB than cows with short (<80 d) days open. Multiple studies have reported a negative relationship of NEFA with reproduction (Jorritsma et al., 2003; Rutter et al., 1987; Rutter et al., 1988). Increased NEFA concentrations during the transition period were associated with decreased pregnancy rate at first AI (Garverick et al., 2013) or at 70 d after the voluntary waiting period (Ospina et al., 2010a), whereas another study in 60 freestall herds found that high circulating NEFA was associated with a reduced 21-d pregnancy rate after voluntary waiting period (Ospina et al., 2010b). All these studies found positive relations between plasma NEFA and days to OLA and days open, as reported in the current study. Greater concentration of NEFA during NEB may prevent follicle development, interrupt the complicated endocrine system, and advance the formation of ovarian cysts (Behrooz et al., 2019). In contrast, A low plasma NEFA concentration, combined with a positive EB, was maybe related to an earlier postovulatory increase of progesterone, a greater follicle development, and a better resumption of ovarian activity, resulting in fewer days open. In addition, long days open in young cows could be related with a too positive EB and high insulin concentration, while the cows with short days open had the medium insulin concentration and EB. As shown by Armstrong (2003), high energy intake leading to higher insulin concentrations increased the growth rate of the dominant follicle but impaired oocyte quality. Energy balance is positively correlated with the number of large follicles in ovary, and negatively correlated with the numbers of small and medium follicles (Lucy et al., 1991). However, cows with medium-sized follicles (between 14.5 and 17.5 mm), had a greater pregnancy rate than cows with follicles of other sizes (Keskin et al., 2016).

Remarkably, multiparous cows after 0-d DP and fed a standard energy level did not have a reduction in days open, as we saw for cows after 0-d DP and fed a low energy level. Cows fed a standard energy level after 0-d DP were characterized by more days open, lower pregnancy rates within 100 DIM, more services to conception and more prolonged cycles, compared with cows fed a low energy level after 0-d DP. This is in line with Watters (2009), that when cows fed low energy level with short DP, both younger and older cows had fewer days to first ovulation. Still in our study, only the older cows had earlier time to pregnancy when dietary energy level was reduced after 0-d DP. It is unknown why cows with 0-d DP(STD) treatment had a lower fertility (more days open, lower pregnancy rates within 100 DIM) compared with cows with 0-d DP(LOW) treatment. It can be speculated, however, that the energy balance is possibly too positive in early lactation in this group (-2 vs 55 kJ/ $kg^{0.75}$ ·day for 0-d DP(LOW) vs 0-d DP(STD), respectively; Van Hoeij et al., 2016). As discussed above, too high plasma insulin concentration had negative effects on oocyte maturition in vitro (Foulahdi-Nashta et al., 2007) and insulin stimulating diets are possibly beneficial to establish ovarian activity postpartum, but not to establish pregnancy (Garnsworthy et al., 2009). Diets designed to increase plasma insulin concentration had negative effects on blastocyst rate in heifers (Adamiak et al., 2005; 2006) and in lactating dairy cows (Fouladi-Nashta et al., 2006). This is in line with our findings, where on the one hand early OLA was related to a greater plasma insulin concentration, while on the other hand days open was not related to plasma insulin concentration in early lactation. Moreover, the relation between OLA and plasma insulin was different for the 3 dry period strategies. It can be speculated that, cows with 0-d DP(LOW) had a more optimal EB and insulin concentration than cows with 0-d DP(STD) or 30-d DP (STD) to support fertility.

Conclusion

Reducing postpartum dietary energy level for older cows (parity ≥ 3) after 0-d DP (0-d DP (LOW)) improved fertility by reducing the interval from calving to OLA, reducing services per conception and consequently reducing days open compared with a standard dietary energy level after 0-d DP (0-d DP (STD)) or after 30 d (30-d DP (STD)). Less days to OLA (<21 d) was associated with a better metabolic status, indicated by a greater concentration of glucose, IGF-1, and insulin and a lower concentration of NEFA and BHBA during weeks 1 through 7 postpartum. A low number of days open (< 80-d) was associated with less days to OLA, less services per conception, better EB in week 1 till 7 of lactation and better metabolic status. Energy balance in week 1 till 14 of lactation, however, was not linearly related with days open. This might indicate that cows with an intermediate EB in week 1 till 14 of lactation (0-d DP (LOW)) had a more optimal EB to support fertility than cows with a positive EB (0-d DP (STD)) or cows with most negative EB (30-d DP (STD)) in week 1 till 14 of lactation.

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Appendix A

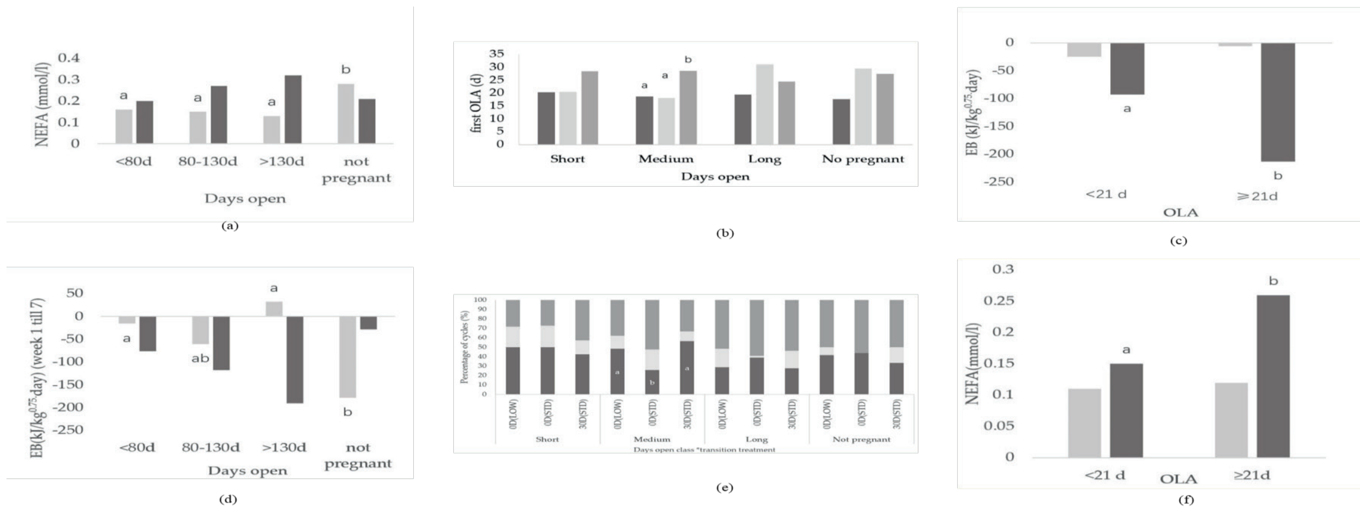

Figure A1. (**a**) interactions of different days open (short:<80 d, medium:80–130 d, long:>130 d and not pregnant) with different transition treatment (0-d DP (LOW) in dark grey, 0-d (STD) in light grey, 30-d DP (STD) in grey) for days to first OLA**;** (**b**) interactions of different days open (short:<80 d, medium:80–130 d, long:>130 d and not pregnant) with different transition treatment (0-d DP (LOW), 0-d (STD), 30-d DP (STD)) for different ovarian cycles (normal cycles (in dark grey), short cycles (in light grey) and prolonged cycles (in grey)); (**c**) interactions of different OLA (<21 d, ≥21d) with different parity class (parity = 2 in light grey, parity ≥ 3 in dark grey) for plasma non-esterified fatty acid (NEFA) concentration**;** (**d**) interactions of different OLA (<21 d, ≥21d) with different parity class (parity = 2 in light grey, parity ≥ 3 in dark grey) for energy balance (EB)**;** (**e**) interactions of different days open (short:<80 d, medium:80–130 d, long:>130 d and not pregnant) with different parity class

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References

Adamiak, S., Mackie, K., Watt, R., Webb, R., Sinclair, K.D., 2005. Impact of Nutrition on Oocyte Quality: Cumulative Effects of Body Composition and Diet Leading to Hyperinsulinemia in Cattle1. Boil. Reprod. 73, 918–926.

Adamiak, S.J., Powell, K., Rooke, J.A., Webb, R., Sinclair, K.D., 2006. Body composition, dietary carbohydrates and fatty acids determine post-fertilisation development of bovine oocytes in vitro. Reproduction. 131, 247–258.

Armstrong, D.G., Gong, J.G., Webb, R., 2003. Interactions between nutrition and ovarian activity in cattle: Physiological, cellular and molecular mechanisms. Reprod. (Cambridge, England) Suppl. 61, 403–414.

Beam, S.W., Butler, W., 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. Suppl. 54, 411–424.

Britt, J.H., Kittok, R.J., Harrison, D.S., 1974. Ovulation, Estrus and Endocrine Response after GnRH in Early Postpartum Cows. J. Anim. Sci. 39, 915–919.

Butler, W., Smith, R., 1989. Interrelationships Between Energy Balance and Postpartum Reproductive Function in Dairy Cattle. J. Dairy Sci. 72, 767–783.

Butler, W., 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 60, 449–457.

Chen, J., Gross, J.J., Van Dorland, H., Remmelink, G., Bruckmaier, R., Kemp, B., Van Knegsel, A.T., 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., Remmelink, G., Gross, J.J., Bruckmaier, R., Kemp, B., Van Knegsel, A.T., 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.

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

Darwash, A.O., Lamming, G.E., Wooliams, J.A., 1997. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. Anim. Sci. 65, 9–16.

De Feu, M., Evans, A., Lonergan, P., Butler, S., 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.

Fouladi-Nashta, A.A., Campbell, K.H.S., 2006. Dissociation of oocyte nuclear and cytoplasmic maturation by the addition of insulin in cultured bovine antral follicles. Reproduction. 131, 449–460.

Fouladi-Nashta, A.A., Gutierrez, C.G., Gong, J.G., Garnsworthy, P., Webb, R., 2007. Impact of Dietary Fatty Acids on Oocyte Quality and Development in Lactating Dairy Cows1. Boil. Reprod. 77, 9–17.

Garnsworthy, P., Fouladi-Nashta, A.A., Mann, G., Sinclair, K.D., Webb, R., 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.

Garverick, H.A., Harris, M., Vogel-Bluel, R., Sampson, J., Bader, J., Lamberson, W., Spain, J., Lucy, M., Youngquist, R., 2013. Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. J. Dairy Sci. 96, 181–188.

Galvão, K., Frajblat, M., Butler, W., Brittin, S., Guard, C., Gilbert, R., Gilbert, R., 2009. Effect of Early Postpartum Ovulation on Fertility in Dairy Cows. Reprod. Domest. Anim. 45, 207–211.

Gautam, G., Nakao, T., Yamada, K., Yoshida, C., 2010. Defining delayed resumption of ovarian activity postpartum and its impact on subsequent reproductive performance in Holstein cows. Theriogenology. 73, 180–189.

Glister, C., Tannetta, D., Groome, N.P., Knight, P.G., 2001. Interactions between follicle-stimulating hormone and growth factors in modulating secretion of steroids and inhibin-related peptides by nonluteinized bovine granulosa cells. Boil. Reprod. 65, 1020–1028.

González-Recio, O., Alenda, R., 2005. Genetic Parameters for Female Fertility Traits and a Fertility Index in Spanish Dairy Cattle. J. Dairy Sci. 88, 3282–3289.

Graber, M., Kohler, S., Müller, A., Burgermeister, K., Kaufmann, T., Bruckmaier, R., Van Dorland, H., 2011. Identification of plasma and hepatic parameters related to metabolic robustness in dairy cows. J. Anim. Physiol. Anim. Nutr. 96, 75–84.

Gümen, A., Rastani, R., Grummer, R., Wiltbank, M.C., 2005. Reduced Dry Periods and Varying Prepartum Diets Alter Postpartum Ovulation and Reproductive Measures. J. Dairy Sci. 88, 2401-2411.

Gutierrez, C.G., Dobrinski, I., Smith, T.T., Suarez, S.S., Ball, B.A., 1997. Development of a long-term bovine granulosa cell culture system: Induction and maintenance of estradiol production, response to follicle- stimulating hormone, and morphological characteristics. Boil. Reprod. 56, 608–616.

Gutierrez, C.G., Gong, J., Bramley, T., Webb, R., 1999. Effects of genetic selection for milk yield on metabolic hormones and follicular development in postpartum dairy cattle. J. Reprod. Fertil. Abst. Ser. 24, 32–36.

Harrison, D.S., 1975. Reproduction, Milk Production and Culling in Dairy Cows Inseminated at First Estrus after 40 or 60 Days Postpartum, Thesis (MS)-Michigan State University. Department of Dairy Science: East Lansing, USA.

Herath, S., Fischer, D.P., Werling, D., Williams, E., Lilly, S.T., Dobson, H., Bryant, C.E., Sheldon, I.M., 2005. Expression and function of Toll-like receptor 4 in the endometrial cells of the uterus. Endocrinology. 147, 562–570.

Huszenicza, G., Jánosi, S., Kulcsár, M., Kóródi, P., Reiczigel, J., Kátai, L., Peters, A., De Rensis, F., 2005. Effects of Clinical Mastitis on Ovarian Function in Post-partum Dairy Cows. Reprod. Domest. Anim. 40, 199–204.

Jorritsma, R., Wensing, T., Kruip, T.A., Vos, P.L., Noordhuizen, J.P., 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Veter- Res. 2003, 34, 11–26.

Kafi, M., Mirzaei, A., Tamadon, A., Saeb, M., 2012. Factors affecting the occurrence of postpartum prolonged luteal activity in clinically healthy high-producing dairy cows. Theriogenology. 77, 421–429.

Kaneko, K., Kawakami, S., 2009. The roles of PGF2α and PGE2 in regression of the corpus luteum after intrauterine infusion of Arcanobacterium pyogenes in cows. Theriogenology. 71, 858–863.

Keskin, A., Mecitoğlu, G., Bilen, E., Güner, B., Orman, A., Okut, H., Gumen, A., 2016. The effect of ovulatory follicle size at the time of insemination onpregnancy rate in lactating dairy cows. Turk. J. Veter- Anim. Sci. 40, 68–74.

Lamming, G.E., Darwash, A.O., 1998. The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. Anim. Reprod. Sci. 52, 175–190.

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., 2003. Mechanisms linking nutrition and reproduction in postpartum cows. Reprod. (Cambridge, England) Suppl. 61, 415–427.

Lucy, M., Staples, C., Michel, F., Thatcher, W., Bolt, D., 1991. Effect of Feeding Calcium Soaps to Early Postpartum Dairy Cows on Plasma Prostaglandin F2α, Luteinizing Hormone, and Follicular Growth. J. Dairy Sci. 74, 483–489.

Lucy, M., Staples, C.R., Thatcher, W.W., Erickson, P., Cleale, R.M., Firkins, J.L., Clark, J.H., Murphy, M.R., Brodie, B.O., 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. Sci. 54, 323–331.

Mateus, L., Lopes-Da-Costa, L., Diniz, P., Ziecik, A.J., 2003. Relationship between endotoxin and prostaglandin (PGE2 and PGFM) concentrations and ovarian function in dairy cows with puerperal endometritis. Anim. Reprod. Sci. 2003, 76, 143–154.

Mihandoost, B., Mogheiseh, A., Nazifi, S., Ahmadi, M.R., Ansari-Lari, M., 2019. Metabolic status and ultrasound traits of reproductive tract of estrus dairy cows in early postpartum period. Comp. Haematol. Int. 28, 689–693.

Opsomer, G., Coryn, M., Deluyker, H., Kruif, A., 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., Gröhn, Y.T., Hertl, J., Coryn, M., Deluyker, H., De Kruif, A., 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: A field study. Theriogenology. 53, 841–857.

Ospina, P., Nydam, D., Stokol, T., Overton, T., 2010a. 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.

Ospina, P., Nydam, D., Stokol, T., Overton, T., 2010b. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and β-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J. Dairy Sci. 93, 3595–3601.

Petersson, K.-J., Strandberg, E., Gustafsson, H., Berglund, B., 2006. Environmental effects on progesterone profile measures of dairy cow fertility. Anim. Reprod. Sci. 91, 201–214.

Pezeshki, A., Mehrzad, J., Ghorbani, G., Rahmani, H., Collier, R., Burvenich, C., 2007. Effects of Short Dry Periods on Performance and Metabolic Status in Holstein Dairy Cows. J. Dairy Sci. 90, 5531–5541.

Pezeshki, A., Mehrzad, J., Ghorbani, G.R., De Spiegeleer, B., Collier, R., Burvenich, C., 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., Gardner, N., Reynolds, C., Beever, D., Wathes, D., 2003. Relationships between transition period diet, metabolic parameters and fertility in lactating dairy cows. Theriogenology. 60, 1165–1185.

Ranasinghe, R., Nakao, T., Yamada, K., Koike, K., Hayashi, A., Dematawewa, C., 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.

Royal, M., Mann, G., Flint, A., 2000. Strategies for Reversing the Trend Towards Subfertility in Dairy Cattle. Veter- J. 160, 53–60.

Rutter, S., Jackson, D., Johnson, C., Forbes, J., 1987. Automatically recorded competitive feeding behaviour as a measure of social dominance in dairy cows. Appl. Anim. Behav. Sci. 1987, 17, 41–50.

Santschi, D.E., Lefebvre, D., Cue, R., Girard, C., Pellerin, D., 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.K., Nakao, T., Suzuki, T., Higaki, T., Akita, M., 2004. Effects of abnormal ovarian cycles during pre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. Theriogenology. 61, 1559–1571.

Smith, M.C.A., Wallace, J.M., 1998. Influence of early post partum ovulation on the re-establishment of pregnancy in multiparous and primiparous dairy cattle. Reprod. Fertil. Dev. 10, 207–216.

Spicer, L.J., Alpizar, E., Echternkamp, S.E., 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 vitro1. J. Anim. Sci. 71, 1232–1241.

Spicer, L.J., Stewart, R.E., 1996. Interactions among basic fibroblast growth factor, epidermal growth factor, insulin, and insulin-like growth factor-I (IGF-I) on cell numbers and steroidogenesis of bovine thecal cells: Role of IGF-I receptors. Boil. Reprod. 54, 255–263.

Van Hoeij, R., Dijkstra, J., Bruckmaier, R., Gross, J.J., Lam, T., Remmelink, G., Kemp, B., Van Knegsel, A., Van Knegsel, A.T., 2017a. The effect of dry period length and postpartum level of concentrate on milk production, energy balance, and plasma metabolites of dairy cows across the dry period and in early lactation. J. Dairy Sci. 100, 5863–5879.

Van Hoeij, R., Dijkstra, J., Bruckmaier, R., Gross, J.J., Lam, T., Remmelink, G., Kemp, B., Van Knegsel, A., 2017b. Consequences of dietary energy source and energy level on energy balance, lactogenic hormones, and lactation curve characteristics of cows after a short or omitted dry period. J. Dairy Sci. 100, 8544–8564.

Van Knegsel, A., Remmelink, G., Jorjong, S., Fievez, V., Kemp, B., Van Knegsel, A.T., 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., Wiltbank, M.C., Guenther, J., Brickner, A., Rastani, R., Fricke, P., Grummer, R., 2009. Effect of dry period length on reproduction during the subsequent lactation. J. Dairy Sci. 92, 3081-3090.

Chapter 4

Consequences of extending the voluntary waiting period for insemination on reproductive performance in dairy cows

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Abstract

The aim of the study was to evaluate the effect of extended voluntary waiting period (**VWP**) on ovarian cyclicity and reproductive performance of dairy cows. Holstein-Friesian dairy cows (N=154) were blocked and randomly assigned to one of 3 groups with different VWP (50, 125 or 200 d: **VWP-50**, **VWP-125** or **VWP-200**). Milk samples were collected 3 times a week and analysed for progesterone concentration. Ovarian cycles were classified as: normal (18 to 24 days), short (<18 days) or prolonged (>24 days). For cows that became pregnant within 100 days after VWP, a VWP-200 d was related with fewer days until pregnancy after end of the VWP (19.4 d) compared with VWP-50 or VWP-125 (35.5, 37.3 d respectively). During 100 days (-50 until 50 d) around the end of VWP, cows in VWP-200 had a greater percentage of normal cycles (91.9 vs 58.0%, P <0.01) and a lower percentage of prolonged cycles (6.0 vs 32.7%, P =0.01) compared with cows in VWP-50. In the 4 weeks around the end of the VWP, cows in VWP-125 and VWP-200 had a lower milk yield compared with cows in VWP-50 (32.0, 27.5 vs 37.4 kg/d, P<0.01). Inseminations continued until 300 days in milk, resulting in fewer pregnant cows for longer VWPs. In conclusion, extending the VWP from 50 to 125 or 200 days resulted in a greater percentage of normal ovarian cycles and a lower milk yield around the end of VWP. Moreover, VWP-200 reduced days open after the end of the VWP, compared with VWP-50.

Introduction

The voluntary waiting period until first insemination (**VWP**) is the postpartum period during which cows are deliberately not inseminated, to give cows time to recover from negative energy balance (**NEB**) and resume normal ovarian cyclicity during this period (Chen et al., 2015). Traditionally, most dairy farms apply a VWP of 40 to 60 d, aiming at a 12-month calving interval for high milk production and economic reasons (Österman and Bertilsson, 2003). Using this traditional VWP, however, artificial insemination (**AI**) starts during a period of high milk yield (Ancker et al., 2006) when most of the cows are in NEB and mobilizing body reserves. After the traditional VWP, not all cows are immediately in estrous (Kawashima et al., 2012, Cheong et al., 2016) and first-service conception rates can vary from 26.7% to 50.7% (Tillard et al., 2008, Siddiqui et al., 2013). Starting AI during peak milk yield is associated with high reproductive failure (Santos et al., 2009), indicated by low conception rates after first insemination, more inseminations per pregnancy, and more days open (Lucy, 2001, Pryce et al., 2004, Inskeep and Dailey, 2005).

Extending the VWP by deliberately postponing the first insemination postpartum allows cows more time to recover from calving and the NEB. It can be hypothesized that extending the VWP will result in more regular ovarian cyclicity at the end of the VWP, which can be expected to be beneficial for reproductive performance. In an earlier study, extending the VWP from 60 to 150 d resulted in increased calving interval from 12 to 15 months, improved conception rate (50% vs 41.5%), reduced veterinary treatments for anestrus (5.3% vs. 28.6%) (Ratnayake et al., 1998), and decreased the number of inseminations per conception (1.9 vs 1.6) (Larsson and Berglund, 2000). In a more recent study, increasing the VWP from 40 to 120 or 180 days reduced the number of days open after the end of the VWP, and increased pregnancy rates for both extended VWP groups (Niozas et al., 2019). In contrast, Arbel et al. (2001) observed no effect of extending VWP from 93 d to 154 d on days open after the end of VWP, the differences between the groups for days open (61 d) and days in milk (59 d) closely followed the difference in VWP. Furthermore, it has been discussed that extension of the VWP might increase the risk of development of ovarian cysts as well as weaker heat symptoms, making heat detection more difficult (Larsson and Berglund, 2000). It can be hypothesized that an improved reproductive performance after an extended VWP may be due to a lower milk yield at the time of insemination, more time to recover from NEB and the calving process, and a better body condition at first AI, compared with a traditional VWP. Ambiguous consequences of a deliberately extended VWP on reproduction, as reported in earlier studies, might be related to limited contrast between study groups in VWP, in milk yield or in body condition after extending the VWP. Therefore, the objective of this study was to investigate the consequences of a VWP of 50, 125 or 200 d on ovarian cyclicity and reproductive performance, and to relate the ovarian cyclicity and reproductive performance after different VWP to milk yield, body condition score and body weight around the end of the VWP.

Materials and methods

Animals and housing

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University & Research (the Netherlands) and complies with the Dutch law on Animal Experimentation (protocol number 2016.D-0038.005). This experiment was described earlier (Burgers et al., 2021). In short, the experiment was conducted at Dairy Campus research farm (WUR Livestock Research, Leeuwarden, the Netherlands) and included 154 Holstein-Friesian dairy cows which were followed from December 2017 until January 2020. Of the cows in the experiment, 32% were primiparous and 68% were multiparous. These cows were selected based on: being pregnant with a Holstein-Friesian calf (no twin gestation), no clinical mastitis or high somatic cell count (**SCC**>250.000 cells) during the last 2 test days before dry off, and expected to be able to accomplish a full lactation. Cows were milked twice daily around 6 am and 6 pm in a 40-cow rotary milking parlor (GEA, Dusseldorf, Germany).

Experimental design

The experiment started with drying-off at 45 days before the expected calving date and cows were monitored for a complete subsequent lactation. Animals that were culled were followed until they were culled.

Cows were blocked for parity, expected calving date, calving interval, breeding value for persistency (**CRV**, Arnhem, the Netherlands) and expected fat and protein corrected milk (**FPCM**) based on their previous lactation in multiparous cows and breeding value for milk production in primiparous cows. The experiment included 154 cows in total, each block consisted of 3 cows. First, 50 blocks of 3 cows were formed. After removal of 2 cows before the end of VWP because of culling, 2 more blocks of 3 cows were added. Cows in each block were randomly assigned to one of the 3 treatments: a VWP of 50 d (**VWP-50**), 125 d (**VWP-125**), or 200 d (**VWP-200**).

Cows were artificially inseminated as soon as heat was detected after the end of VWP of 50, 125 or 200 days. Heat was detected by either the Nedap Smarttag (Nedap, Groenlo, the

Netherlands) or visually by the animal caretaker. Heat attentions were noted by the animal caretakers and reported to AI service (CRV, Arnhem, the Netherlands) the same evening. The day after (around 7.00 am), all cows were rechecked to see whether new cows in oestrus could also be inseminated that morning. All cows in heat were inseminated between 7.30-8.30 am. At 35-49 days after insemination, a veterinarian checked for pregnancy using ultrasound scanning, according to the standard protocol of Dairy Campus. Cows in all 3 groups were inseminated until 300 days in milk, thus cows in VWP-50, VWP-125, and VWP-200 were insemination during a period of 250, 175, and 100 days after the end of the VWP, respectively.

Rations

Ration composition was described earlier in detail (Burgers et al., 2021). In short, dry cows received one ration over the entire dry period (45 d) with 5.66 MJ/kg dry matter and 13% protein. The roughage in the dry period ration consisted of grass silage and corn silage (ratio 70:30) and was supplemented with wheat straw (30-40% of the total ration). Cows received 1 kg concentrate per day from 10 days before the expected calving date onwards. Lactating cows received a partial mixed ration for their expected milk production (at 60 DIM: 36 kg/d; and at 305 DIM: 22 kg/d). The partial mixed ration consisted of grass silage, corn silage, and soybean meal supplemented with wheat straw for 22 kg milk, including 1 kg concentrate in the milking parlour. In addition, concentrate was supplied separate from the partial mixed ration in concentrate feeders (Manus VC5, DeLaval, Steenwijk, the Netherlands). Individual concentrate allowance was built up in 21 days to 9 kg for primiparous cows or 10 kg for multiparous cows. from 100 DIM onwards, concentrate allowance depended on milk production of the last 5 days. The ration was switched to the dry period ration a week before next dry-off.

Measurements

Milk sampling and progesterone assay

Each Monday, Wednesday and Friday from calving until confirmed pregnancy (as checked by the veterinarian), milk samples were collected in 10 mL tubes with bronopol as a preservative and stored at -20°C until analysis. Milk samples were used to determine ovarian cyclicity from calving until pregnancy using progesterone (**P4**) levels in milk analysed with a commercial ELISA kit (Ridgeway Science, Gloucester, UK). Analysis of milk progesterone was carried out according to the protocol of the manufacturer (Ridgeway Science as described earlier (Roelofs et al., 2006)). The intra-assay and inter-assay coefficients of variance were 9.2% and 11.6%, respectively.

Onset of luteal activity (**OLA**) was defined as two or more successive milk samples with P4 concentration of 2 ng/mL or higher. Ovarian cycle length was defined as the interval between OLA in one ovarian cycle and OLA in the next ovarian cycle. Based on the P4 profile, the luteal activity intervals for each cow were classified into 1 of 3 categories (Chen et al., 2015): short (<18 days), normal (18-24 days) or prolonged (>24 days). For statistical analyses, the number of cycles of each category were counted per cow over two different periods: in the 100 days around the end of the VWP (i.e. from -50 until 50 days relative to the end of the VWP), and from calving until pregnancy.

Milk yield, body condition and body weight

Milk yield was recorded at every milking, from day of calving until dry-off. Milk yield and FPCM yield per day of CI were calculated per week and averaged over the 4 weeks around the end of VWP, i.e. week -2 until 2 relative to the end of VWP. Milk was converted to FPCM using the following formula (CVB, 2012):

FPCM (kg) = milk (kg) * (0.337 + 0.116 * fat (%) + 0.06 * protein (%)).

Body condition was scored (**BCS**) by the same technician every month on a 1-5 scale (Ferguson et al., 1994). Body weight was recorded twice daily after each milking and averaged over the 4 weeks around the end of VWP, i.e. week -2 until 2 relative to the end of VWP. Body weight development was computed as the change in weight from week -2 to 2 relative to the end of the VWP.

Statistical analyses

Six cows were not inseminated due to early culling (4 cows due to lameness, 2 cows due to accidents), and were excluded, resulting in 148 cows in total for the statistical analysis. Analyses were performed for all 148 cows and for the subgroups of cows that became pregnant within 100 days after the end of the VWP or within 300 days in milk. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). A Pearson chi-square was used to assess whether the percentage of cows that became pregnant within 100 days after the end of the VWP depended on VWP treatment (PROC FREQ).

Effects of the fixed factors VWP (50 d, 125 d, or 200 d), parity class (1, or ≥2) and their interaction on fertility variables were assessed using different models, depending on the variable distribution. Regarding variables for ovarian cyclicity, OLA was analysed using a linear model (PROC MIXED); the number of cycles (in the 100 days around the end of the VWP or from calving to pregnancy) using a generalized linear model with a negative binomial regression (PROC GLIMMIX); and the percentage of normal, prolonged, or short cycles each with a logistic regression (PROC LOGISTIC). Regarding reproductive performance, days open, days until pregnancy after the end of the VWP, and number of inseminations per conception were analyzed using a linear model. First-service conception rate was analyzed using a generalized linear model with a binary distribution and the default logit link function (PROC GLIMMIX). To evaluate time to pregnancy or time to the first AI from calving or from end of VWP, a survival analysis (PROC LIFETEST) was used to obtain Kaplan-Meier curves. To evaluate statistical differences of Kaplan-Meier curves among the VWP treatments, a Cox proportional hazards model (PROC PHREG) was used. Last, milk yield, body weight, body weight development and body condition score in the 4 weeks around the end of the VWP were analysed using linear models. Values were regarded significant if $P < 0.05$ and as a tendency if $0.05 \leq P < 0.10$. LSMEANS were presented and a Bonferroni correction was used for post-hoc pairwise comparisons between VWP treatments.

Results

Milk production per day of calving interval

Results about lactation yield were already published by Burgers et al., 2021. In short, VWP did not affect the milk yield per day of calving interval. For cows in parity=1, milk production per day of calving interval was 23.3, 22.7 and 23.5 kg/d for cows with VWP-50, VWP-125 and VWP-200, respectively. For cows in parity≥2, milk production per day of calving interval was 29.5, 28.0 and 25.6 kg/d for cows with VWP-50, VWP-125 and VWP-200, respectively. Effect of VWP on FPCM depended on parity, the VWP did not affect FPCM yield per day of calving interval for cows in parity=1, whereas FPCM yield per day of calving interval was higher in VWP-50 compared with VWP-200 for cows in parity≥2 (30.4 vs 27.4 kg/d for VWP-50 vs VWP-200, respectively, $P<0.01$).

Ovarian cyclicity of all cows within 100 days around the end of the VWP

In the 100 days around the end of the VWP, cows in VWP-125 had more ovarian cycles than cows in VWP-50 (Table 1). Cows in VWP-125 and VWP-200 had a greater percentage of normal ovarian cycles than cows in VWP-50 ($P<0.01$). Cows in VWP-200 had a lower percentage of prolonged cycles than cows in VWP-50 and VWP-125 ($P = 0.01$).

Fertility characteristics of cows that became pregnant within 100 days after the VWP

Of cows that became pregnant within 100 days after the end of the VWP, cows in VWP-125 and VWP-200 had more ovarian cycles from calving until pregnancy than cows in VWP-50

(P<0.01). Cows in VWP-200 tended to have a greater percentage of normal cycles from calving until pregnancy than cows in VWP-50 or VWP-125 (P=0.09) (Table 1).

Due to experimental treatment, cows in VWP-200 had the longest days open, followed by cows in VWP-125 and VWP-50 (P<0.01; Table 2). In contrast, cows in VWP-200 had fewer days open after end of the VWP than cows in VWP-50 or VWP-125 (P=0.03). Survival curves showed cows in VWP-200 spent longer time after calving to get first AI than cows in VWP-50 and VWP-125 because of the deliberately VWP lengths (Figure 1a), but there was no difference in time to get first AI after the end of VWP among three treatments (P>0.1) (Figure 1b). Cows in VWP-200 spent shorter time to get pregnant after the end of the VWP compared with cows in VWP-50 (Hazard Ratio 0.43, confidence interval 0.27-0.68, P<0.01) and cows in VWP-125 (Hazard Ratio 0.46, confidence interval 0.29-0.73, P<0.01) (Figure 1d).

Milk production, body condition and body weight around the end of the VWP

Extending the VWP from 50 d to 125 d or 200 d reduced milk yield in the 4 weeks (-2 to 2 weeks) around the end of the VWP by 5.4 kg/d or 9.9 kg/d, respectively, and FPCM yield by 4.5 kg/d or 7.6 kg/d, respectively (Table 3). Moreover, cows in VWP-50 were still losing body weight in the 4 weeks around the end of the VWP, while cows in VWP-125 were gaining body weight in this period. In the 4 weeks around the end of VWP, multiparous cows had a higher milk yield and FPCM yield than primiparous cows in VWP-50 or VWP-125, but not in VWP-200. For multiparous cows, milk yield in the 4 weeks around the end of the VWP decreased when VWP was extended from 50 to 125 or 200 d, but not for primiparous cows (Figure 2). Days open after the end of the VWP was weakly correlated with milk yield (r=0.31, P<0.01) and FPCM yield (r= 0.23, P=0.01), but not with body weight and body weight development around end of the VWP (scatter plots shown in Figure 3). When correlations were performed separately for the three VWP treatments or the six VWP × parity classes, none were significant.

Fertility characteristics of cows that were pregnant within 300 DIM after different VWP

Cows in this experiment were inseminated until 300 DIM, irrespective of VWP length. In this period, 48 of 52 inseminated cows were pregnant in VWP-50, 42 of 49 in VWP-125, and 38 of 47 in VWP-200 (Table 4). Including all cows that were pregnant within 300 DIM, extending the VWP from 50 d to 200 d, but not from 50 to 125 d, increased conception rate after first AI (P=0.03). Cows in VWP-125 and VWP-200 had 4.8 d and 35 d fewer days until pregnancy after the end of the VWP than cows in VWP-50 (P<0.01). Within all cycles until pregnancy for pregnant cows or until 100 days after the end of VWP for cows that were not pregnant, cows in

VWP-125 or VWP-200 had a higher percentage of normal cycles and lower percentage of prolonged cycles than cows in VWP-50.

Table 1. Ovarian cyclicity of all cows around the end of the voluntary waiting period (VWP), and ovarian cyclicity from calving until pregnancy for cows that became pregnant within 100 days after the end of the VWP. Cows had a VWP of 50, 125 or 200 days. Values represent LSMEANS and maximal SEM.

	Voluntary Waiting Period			SEM	*P*-value		
	50 d	125 d	200		VWP	Parity (P)	VWP×P
All Cows, *n*	52	49	47				
OLA[1] (d)	24.3	24.8	28.3	2.9	0.32	0.73	0.28
Cycles per cow (100 days[2] around the end of VWP)	1.6[a]	2.3[b]	2.1[ab]	0.2	0.02	0.51	0.41
Normal cycle (100 days[2] around the end of VWP in %)	58.0[a]	77.3[b]	91.9[b]	0.1	<0.01	<0.01	0.82
Prolonged cycle (100 days[2] around the end of VWP in %)	32.7[a]	19.0[a]	6.0[b]	0.1	0.01	0.04	0.43
Short cycle (100 days[2] around the end of VWP in %)	9.3	3.7	2.1	0.0	0.33	0.06	0.16
Cows pregnant within 100 days after VWP, *n*	39	36	38				
OLA[2] (d)	23.2	26.6	27.2	2.8	0.38	0.99	0.49
Cycles per cow (calving until pregnancy in %)	1.9[a]	4.8[b]	7.0[c]	0.4	<0.01	0.87	0.15
Normal cycle (calving until pregnancy in %)	53.8	64.6	71.4	0.2	0.09	0.05	0.26
Prolonged cycle (calving until pregnancy in %)	37.5	28.2	22.9	0.1	0.19	0.30	0.01
Short cycle (calving until pregnancy in %)	8.6	7.2	5.7	0.1	0.85	0.14	0.14

[a,b]Values within VWP within a row with different superscript letters differ ($P < 0.05$)

[1]OLA: Days to onset of luteal activity after calving;

[2]100 days: -50 until 50 days around the end of VWP;

Table 2. Fertility characteristics of all cows and cows that became pregnant within 100 days after the end of the voluntary waiting period of 50, 125 or 200 days. Values represent LSMEANS and maximal SEM.

	Voluntary Waiting Period			SEM	*P*-value		
	50 d	125 d	200 d		VWP	Parity (P)	VWP×P
Cows (all), *n*	52	49	47				
First-service conception rate	43.8	42.0	63.3		0.17	0.59	0.52
Inseminated cows that were pregnant within 100 days after VWP	39	36	38				
Days open	85.5^{a}	162^{b}	219^{c}	7.2	<0.01	0.55	0.15
Days until pregnancy after end VWP	35.5^{a}	37.3^{a}	19.4^{b}	7.2	0.03	0.55	0.15

^{a,b}Values within VWP within a row with different superscript letters differ ($P < 0.05$).

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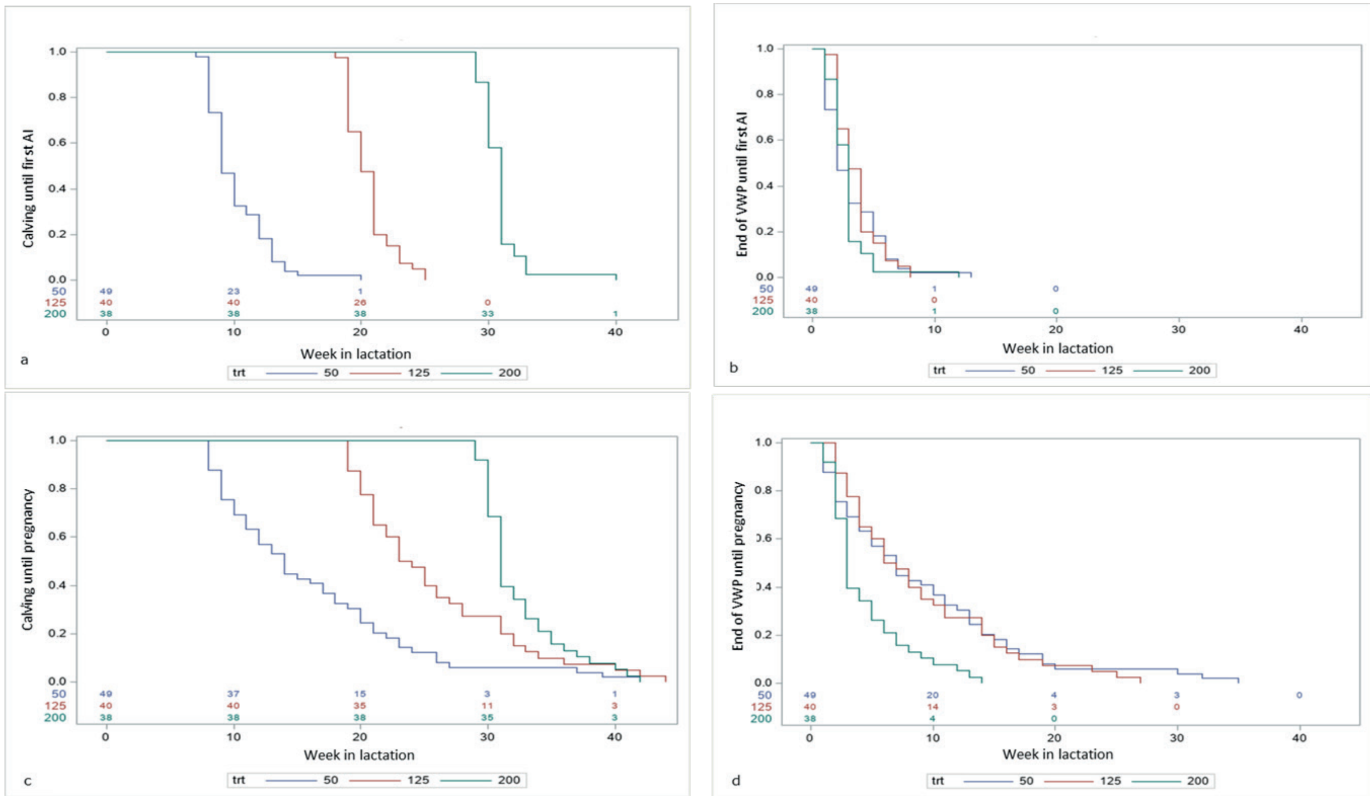

Figure 1. a-d: Kaplan-Meier survival curves (with number of subjects at risk). The survival function is (a) the time of survival for no AI after calving, (b) the time of survival for no AI after the end of VWP, (c) the time of survival for no pregnancy after calving, (d) the time of survival for no pregnancy after the end of voluntary waiting period (VWP).

Table 3. Milk production, FPCM[1], body condition and body weight around the end of the voluntary waiting period (VWP) for all cows with a VWP of 50, 125 or 200 days. Values represent LSMEANS and maximal SEM.

	Voluntary Waiting Period			SEM	*P*-value		
	50 d	125 d	200		VWP	Parity (P)	VWP×P
Cows, *n*	52	49	47				
Milk production (-2 to 2 weeks around the end of VWP), kg/d	37.4^a	32.0^b	27.5^c	1.5	<0.01	<0.01	<0.01
FPCM[1] (-2 to 2 weeks[2] around the end of VWP), kg/d	36.8^a	32.3^b	29.2^c	1.4	<0.01	<0.01	<0.01
Body weight (-2 to 2 weeks[2] around the end of VWP), kg	621	628	645	14	0.14	<0.01	0.83
Body weight change (-2 to 2 weeks[2] around the end of VWP), kg	-3.3^b	5.3^a	1.9^{ab}	3.6	0.04	0.06	0.32
Body condition score (-2 to 2 weeks[2] around the end of VWP)	2.5	2.5	2.4	0.2	0.61	0.05	0.32

^{a,b}Values within VWP within a row with different superscript letters differ ($P < 0.05$)

[1]FPCM: Fat and protein corrected milk production.

[2]Weeks around end of VWP (week -2, -1, 1, 2 relative to end of the VWP).

4

Table 4. Fertility characteristics of all cows[1] until pregnancy or until 100 days after the voluntary waiting period of 50, 125 or 200 days[3], and of pregnant cows[4]. Values represent LSMEANS and maximal SEM.

	Voluntary Waiting Period			SEM	*P*-value		
	50 d	125 d	200		VWP	Parity (P)	VWP×P
Cows (all)[1], *n*	52	49	47				
OLA (d)[2,3]	23.8	25.5	27.2	2.9	0.51	0.89	0.56
Number of ovulations[3]	3.3^{a}	6.4^{b}	8.1^{c}	0.5	<0.01	0.94	0.18
Cycles per cow[3]	2.2^{a}	5.4^{b}	7.0^{c}	0.5	<0.01	0.80	0.11
Normal cycles[3]	49.8^{a}	65.4^{b}	71.4^{b}	0.2	0.02	0.03	0.36
Short cycles[3]	6.8	6.9	5.7	0.0	0.95	0.21	0.15
Pregnant cows[4], *n*	48	42	38				
Days open	104.4^{a}	174.6^{b}	219.	15.0	<0.01	0.17	0.74
Days open after VWP	54.4^{a}	49.6^{b}	19.4^{c}	15.0	<0.01	0.17	0.74
Inseminations per conception	2.1^{ab}	2.2^{a}	1.4^{b}	0.3	0.05	0.76	0.36

[a,b] Values within VWP within a row with different superscript letters differ ($P < 0.05$)

[1]Number of cows in the complete experiment;

[2]Days to onset of luteal activity after calving;

[3]Variables related with ovarian cyclicity were determined until 100 days after the end of the voluntary waiting period or until pregnancy (whichever came first).

[4]Cows which became pregnant in the complete period where experimental treatments were applied (until 300 DIM).

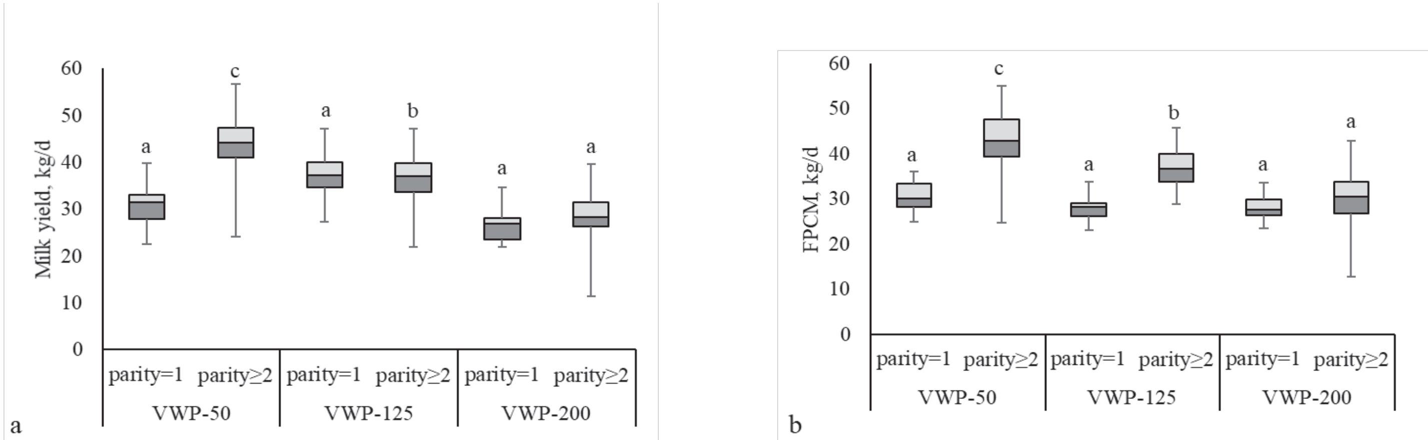

Figure 2. a, b: Milk yield (a) and FPCM (fat- and protein-corrected milk production) (b) during -2 to 2 weeks around the end of VWP for cows of parity 1 or ≥2 and a VWP of 50 d, 125 d, or 200 d. Letters a and b above box plots indicate differences between parities within VWP class.

4

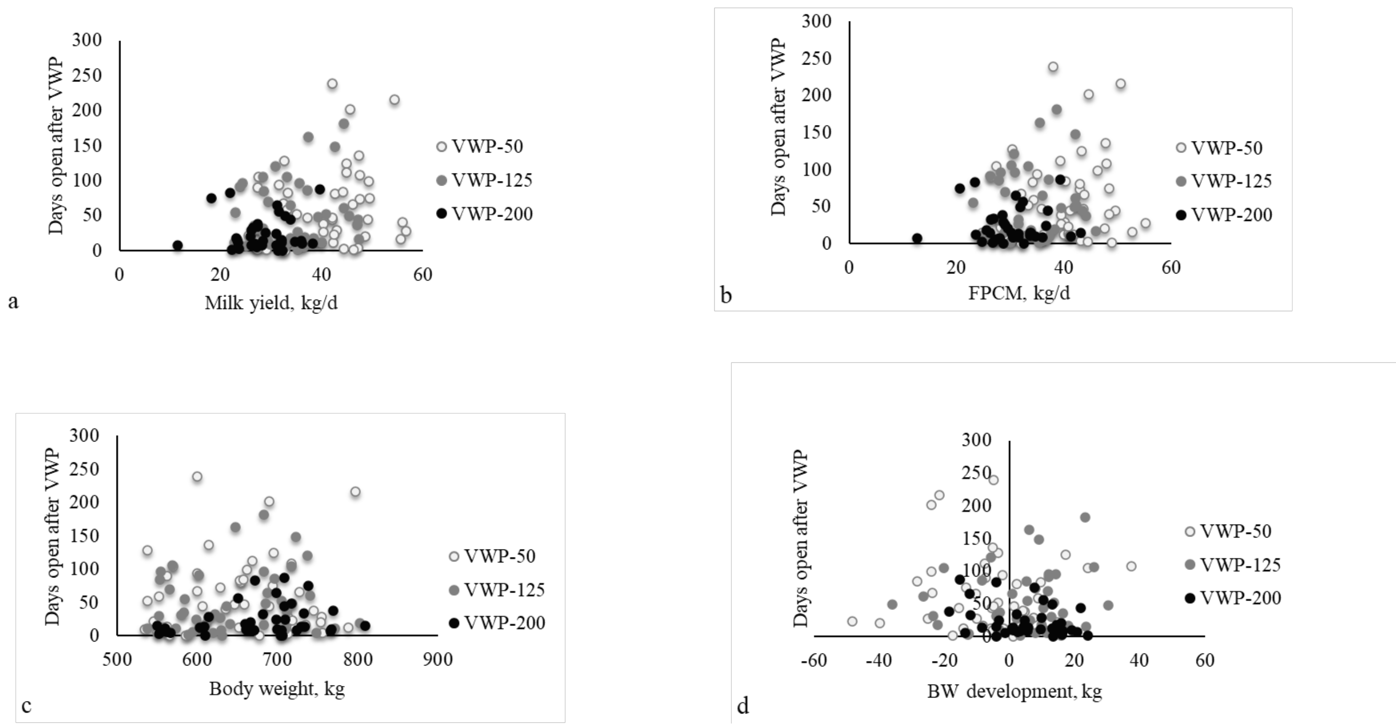

Figure 3. Relationship between days until pregnancy after end of VWP with Milk yield (a), FPCM (fat- and protein-corrected milk production) (b), Body weight(c) and Bodyweight (BW) development(d) within -2 to 2 weeks around the end of VWP for cows with a VWP of 50 d, 125 d, or 200.

Discussion

Extending the VWP from 50 to 200 days reduced the days until pregnancy after the end of the VWP. This is in accordance with Larsson and Berglund (Larsson and Berglund, 2000), who reported fewer days from insemination to pregnancy after extending the VWP from 50 to 140 days. Ratnayake et al. (1998) also reported fewer days from first insemination to pregnancy by extending the VWP from 150 days to 240 days. Other studies, however, found no difference in days until pregnancy after the end of the VWP between a conventional versus extended VWP (Schindler et al., 1991, Arbel et al., 2001).

Fewer days until pregnancy after the end of an extended VWP likely resulted from an increase in conception rate and fewer inseminations per pregnancy. In the current study, number of inseminations per pregnancy was 0.5 lower for cows in VWP-200, compared with cows in VWP-125. In addition, conception rate after first insemination was 63.3% for cows in VWP-200, compared with 43.8 and 42.0 % for cows in VWP-50 and VWP-125. In earlier studies, an increase in the VWP was also related with an increase in conception rate (Foote and Riek, 1999, Caraviello et al., 2006) and a decrease in the number of AIs per conception (Larsson and Berglund, 2000). This was explained by the delay of insemination until a cow is likely no longer in a state of NEB. The NEB in early lactation is typically accompanied by low glucose and increased free fatty acid and ketone body concentrations, which may impair oocyte quality and embryo development (Jorritsma et al., 2003, Leroy et al., 2006, Fouladi-Nashta et al., 2007).

In earlier studies, more cycles or ovulations prior to insemination were related with an increase in conception rate after first AI (Stevenson et al., 1983, Butler and Smith, 1989, Butler, 2003). Furthermore, ovarian cycles of normal length (18-24 days) were related with an increased pregnancy rate and fewer days open (Lamming and Darwash, 1998, Shrestha et al., 2004, Ma et al., 2020). In the current study, cows in VWP-125 and VWP-200 had a greater percentage of normal ovarian cycles around the end of the VWP, and cows in VWP-200 had a lower percentage of prolonged ovarian cycles than cows in VWP-50. Prolonged cycles are a common ovarian disturbance in dairy cows (Shrestha et al., 2004), and have been associated with a reduced pregnancy rate and extended days open (Ranasinghe et al., 2011). In our study, the increased percentage of normal cycles in VWP-125 and VWP-200 and reduced percentage of prolonged cycles for the cows in VWP-200 were not associated with improved conception rates, but may have contributed to the fewer days until pregnancy after the end of the VWP in VWP-200. The higher percentage of prolonged cycles for cows in VWP-50 could be related with the higher milk yield around end of the VWP.

We hypothesized that after an extended VWP, a reduction in milk yield during the breeding period might contribute to an improvement in reproductive performance. 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), possibly due to its association with a catabolic state (Santos et al., 2009) and inflammatory status in the uterus (Ribeiro and Carvalho, 2018, Pascottini et al., 2020). Other previous revious reports were ambivalent about the relation between milk yield and fertility and suggested that the interaction between milk yield and fertility is complex (Faust et al., 1988, López-Gatius et al., 2006, Bello et al., 2013). High milk production, as a consequence of genetic selection and improvements in nutrition and management, has coincided with a corresponding decline in fertility (Hansen et al., 1983, Roxström et al., 2001). In the current study, extending the VWP from 50 to 125 or 200 days decreased milk yield and FPCM around the end of the VWP. In our experiment, however, milk yield was only weakly correlated with days until pregnancy after the end of the VWP. This indicated that a lower milk yield at time of insemination can only explain part of the improved fertility after an extended VWP in our study. Other aspects of an extended VWP, such as a longer period for uterine recovery and insemination at a time of more neutral energy balance, may contribute to this improved fertility as well (Ma et al., 2020).

Next to milk yield, also an improvement in body condition or body weight might be related to more regular ovarian cyclicity, as indicated by a greater percentage of normal cycles around the end of the VWP, and improved reproductive performance for cows with an extended VWP. Several studies have shown a negative relationship between reproductive performance and body condition in early lactation. Veerkamp et al. (2001) reported that the correlation between BCS and calving interval and days to first service was between -0.44 and -0.59. In earlier work, greater BW loss during the early postpartum period did not affect fertilization of oocytes, but was hypothesised to be associated with an increase in the percentage of degenerated embryos by 7 days after insemination, thus providing important evidence for a carryover effect of NEB on embryo development 6 to 7 weeks later (Britt et al., 1986). Cows that lost more body condition in the first 65 days postpartum were more likely to be anovular, had decreased pregnancy per AI, and increased risk of pregnancy loss (Santos et al., 2009). In our study, we found no difference of BCS around end of the VWP in different groups, but cows in VWP-50 were still losing BW around end of the VWP, while cows in VWP-125 and VWP-200 were gaining BW around the end of the VWP. Additionally, cows in VWP-125 gained most weight (5.3 kg) in the 4 weeks around the end of VWP. This is probably because the peak dry matter intake occurs between 70 to 140 days post calving (Tesfaye and Hailu, 2019), and cows are restoring body condition after the period of NEB, whereas cows in VWP-200 have already recovered from the NEB. That the number of days until pregnancy after the end of the VWP were not reduced for cows in VWP-125 might be related with a too positive energy balance (Ma et al., 2020).

In our experiment, ovarian cyclicity and reproductive performance were intensively monitored for the first 100 days after the end of the VWP. This makes sense when comparing physiology of cows during the peri-insemination period, but for practical implications a more extended period, beyond 100 days after end of the VWP, might be relevant. In the current study, cows of all treatments were inseminated until 300 DIM. This implies that cows with a shorter VWP had more days to conceive after the end of the VWP. Within 300 DIM in our experiment, cows in VWP-200 had fewer days until pregnancy and less inseminations per pregnancy compared with cows in 50-d VWP. Although cows in VWP-200 had a better fertility, there was less time (100 d after end of the VWP) to inseminate the cows, which resulted in fewer cows being pregnant at 300 DIM. In this study, 92%, 86% and 81% of cows in VWP-50, VWP-125, and VWP-200 were pregnant by 300 DIM. To avoid increased culling for fertility reasons in practice, farmers should either apply a short VWP, or continue to inseminate the cows that do not conceive within 100 days after the VWP until later in lactation. Additionally, cows with calving intervals of ≥15 months (Lehmann, 2016) or days open after calving ≥130 days (Middleton and Pursley, 2019) had higher BCS at next calving, which might compromise health and metabolism during the subsequent transition period and start of lactation (Walsh et al., 2011; Schuh et al., 2019). At commercial farms applying an extended VWP for part of the herd or the total herd, farmers were accepting towards an extended calving interval with extended VWP, and were more inclined to inseminate a cow with difficulties to conceive multiple times, rather than replacing that cow (Burgers et al., 2020).

Conclusions

Extending the VWP from 50 d to 200 d improved ovarian cyclicity, as indicated by a greater percentage of normal ovarian cycles, a lower percentage of prolonged ovarian cycles in the 100 days around the end of the VWP and fewer days open after the end of the VWP. Extending the VWP from 50 to 125 d also improved the percentage of normal cycles in the 100 days around the end of the VWP but did not affect the days open after the end of VWP. In addition, extending the VWP from 50 to 125 or 200 days decreased the milk yield and FPCM around the end of the

VWP, which could only to a limited extent explain the improvement of the ovarian cyclicity and the reduction in days until pregnancy after the end of the VWP.

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References

Ancker, S., Norberg, E., Callesen, H., 2006. A synthesis of reproduction literature (Original Danish title: Vidensyntese Reproduktion. Rapport nr 113). Knowledge Centre for Agriculture, Skejby, Denmark.

Arbel, R., Bigun, Y., Ezra, E., Sturman, H., Hojman, D., 2001. The effect of extended calving intervals in high lactating cows on milk production and profitability. J. Dairy Sci. 84, 600-608.

Bello, N., Steibel, J., Erskine, R., Tempelman, R., 2013. Cows and herds constitute distinct hierarchical levels of heterogeneity in the variability of and association between milk yield and pregnancy outcome in dairy cows. J. Dairy Sci. 96, 2314-2326.

Britt, J., Scott, R., Armstrong, J., Whitacre, M., 1986. Determinants of estrous behavior in lactating Holstein cows. J. Dairy Sci. 69, 2195-2202.

Butler, W. R., 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83, 211-218.

Burgers E, Kok, A, Goselink, R, Hogeveen, H, Kemp, B, van Knegsel, A. 2021. Effects of extended voluntary waiting period from calving until first insemination on body condition, milk yield, and lactation persistency. J. Dairy Sci. 104, 8009-8022.

Butler, W. R., and Smith, R. D., 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72, 767-783.

Caraviello, D., Weigel, K., Craven, M., Gianola, D., Cook, N., Nordlund, K., Fricke, P., Wiltbank, M., 2006. Analysis of reproductive performance of lactating cows on large dairy farms using machine learning algorithms. J. Dairy Sci. 89, 4703-4722.

Chebel, R. C., Mendonça, L. G., Baruselli, P. S., 2018. Association between body condition score change during the dry period and postpartum health and performance. J. Dairy Sci. 101, 4595-4614.

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

Cheong, S. H., Filho, O. G. S., Absalón-Medina, V. A., Pelton, S. H., Butler, W. R., Gilbert, R. O., 2016. Metabolic and endocrine differences between dairy cows that do or do not ovulate first postpartum dominant follicles. Biol. Reprod. 94, 18, 11-11.

CVB. 2012. CVB tabellenboek veevoeding, Centraal Veevoeder Bureau Lelystad, the Netherlands.

Faust, M., McDaniel, B., Robison, O., Britt, J., 1988. Environmental and yield effects on reproduction in primiparous Holsteins. J. Dairy Sci. 71, 3092-3099.

Ferguson, J. D., Galligan, D. T., Thomsen, N., 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77, 2695-2703.

Foote, R. and Riek, P., 1999. Gonadotropin-releasing hormone improves reproductive performance of dairy cows with slow involution of the reproductive tract. J. Anim. Sci. 77, 12-16.

Fouladi-Nashta, A. A., Gutierrez, C. G., Gong, J. G., Garnsworthy, P. C., Webb R., 2007. Impact of dietary fatty acids on oocyte quality and development in lactating dairy cows. Biol. Reprod. 77, 9-17.

Hansen, L., Freeman, A., Berger, P., 1983. Yield and fertility relationships in dairy cattle. J. Dairy Sci 66, 293-305.

Inskeep, E. K., and Dailey R. A., 2005. Embryonic death in cattle. Veterinary Clinics: Food Animal Practice. 21, 437-461.

Jorritsma, R., Wensing, T., Kruip, T. A., Vos, P. L., Noordhuizen, J. P., 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Vet. Res. 34, 11-26.

Kafi, M., Mirzaei, A., Tamadon, A., Saeb, M., 2012. Factors affecting the occurrence of postpartum prolonged luteal activity in clinically healthy high-producing dairy cows. Theriogenology. 77, 421-429.

Kawashima, C., Matsui, M., Shimizu, T., Kida, K., Miyamoto, A., 2012. Nutritional factors that regulate ovulation of the dominant follicle during the first follicular wave postpartum in high-producing dairy cows. J. Reprod. Dev. 58, 10-16.

Lamming, G., Darwash, A., 1998. The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. Anim. Reprod. Sci. 52, 175-190.

Larsson, B., Berglund, B., 2000. Reproductive performance in cows with extended calving interval. Reproduction in Domestic Animals. 35, 277-279.

Leroy, J., Vanholder, T., Opsomer, G., Van Soom, A., de Kruif, A., 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. Domestic. Anim. 41, 119-123.

López-Gatius, F., García-Ispierto, I., Santolaria, P., Yániz, J., Nogareda, C., López-Béjar, M., 2006. Screening for high fertility in high-producing dairy cows. Theriogenology. 65, 1678-1689.

Lucy, M. 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84, 1277-1293.

Ma, J., van Hoeij, R., Bruckmaier, R., Kok, A., Lam, T., Kemp, B., van Knegsel, A., 2020. Consequences of transition treatments on fertility and associated metabolic status for dairy cows in early lactation. Animals. 10, 1100.

Middleton, E. and Pursley, J., 2019. Blood samples before and after embryonic attachment accurately determine non-pregnant lactating dairy cows at 24 d post-artificial insemination using a commercially available assay for pregnancy-specific protein B. J. Dairy Sci. 102, 7570-7575.

Niozas, G., Tsousis, G., Steinhöfel, I., Brozos, C., Römer, A., Wiedemann, S., Bollwein, H., Kaske, M., 2019. Extended lactation in high-yielding dairy cows. I. Effects on reproductive measurements. J. Dairy Sci 102, 799-810.

Österman, S. and Bertilsson, J., 2003. Extended calving interval in combination with milking two or three times per day: effects on milk production and milk composition. Livest. Prod. Sci. 82, 139-149.

Pascottini, O. B., Van Schyndel, S., Spricigo, J., Carvalho, M., Mion, B., Ribeiro, E., LeBlanc, S., 2020. Effect of anti-inflammatory treatment on systemic inflammation, immune function, and endometrial health in postpartum dairy cows. Sci. Rep. 10, 1-9.

Pryce, J., Royal, M., Garnsworthy, P., Mao, I. L., 2004. Fertility in the high-producing dairy cow. Livest. Prod. Sci. 86, 125-135.

Ranasinghe, R., Nakao, T., Yamada, K., Koike, K., Hayashi, A., Dematawewa, C., 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.

Ratnayake D., Berglund, B., Bertilsson, J., Forsberg, M., Gustafsson, H., 1998. Fertility in dairy cows managed for calving intervals of 12, 15 or 18 months. Acta. Vet. Scand. 39, 215-228.

Ribeiro, E. S., and Carvalho, M. R., 2018. Impact and mechanisms of inflammatory diseases on embryonic development and fertility in cattle. Anim Reprod (AR). 14, 589-600.

Roche, J. R., Friggens, N. C., Kay, J. K., Fisher, M. W., Stafford, K. J., Berry, D. P., 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92, 5769-5801.

Roelofs, J., Van Eerdenburg, F., Hazeleger, W., Soede, N., Kemp, B., 2006. Relationship between progesterone concentrations in milk and blood and time of ovulation in dairy cattle. Anim. Reprod. Sci. 91, 337-343.

Roxström, A., Strandberg, E., Berglund, B., Emanuelson, U., Philipsson, J., 2001. Genetic and environmental correlations among female fertility traits, and between the ability to show oestrus and milk production in dairy cattle. Acta Agriculturae Scandinavica, Section A-Animal Science. 51, 192-199.

Santos, J., Rutigliano, H. M., Sá Filho, M., 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110, 207-221.

Schindler, H., Eger, S., Davidson, M., Ochowski, D., Schermerhorn, E., Foote, R., 1991. Factors affecting response of groups of dairy cows managed for different calving-conception intervals. Theriogenology. 36, 495-503

Schuh, K., Sadri, H., Häussler, S., Webb, L., Urh, C., Wagner, M., Koch, C., Frahm, J., Dänicke, S., Dusel G., 2019. Comparison of performance and metabolism from late pregnancy to early lactation in dairy cows with elevated v. normal body condition at dry-off. Animal. 13, 1478-1488.

Shrestha, H. K., Nakao, T., Suzuki, T., Higaki, T., Akita M., 2004. Effects of abnormal ovarian cycles during pre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. Theriogenology. 61, 1559-1571.

Siddiqui, M., Das, Z., Bhattacharjee, J., Rahman, M., Islam, M., Haque, M., Parrish, J., Shamsuddin, M., 2013. Factors affecting the first service conception rate of cows in smallholder dairy farms in Bangladesh. Reprod Domest Anim. 48, 500-505.

Stevenson, J., Schmidt, M., Call, E., 1983. Factors affecting reproductive performance of dairy cows first inseminated after five weeks postpartum. J. Dairy Sci. 66, 1148-1154.

Tesfaye, A., and Hailu, Y., 2019. The Effects of Probiotics Supplementation on Milk Yield and Composition of Lactating Dairy Cows. J. Phytopharmacol. 8, 12-17.

Tillard, E., Humblot, P., Faye, B., Lecomte, P., Dohoo, I., Bocquier F., 2008. Postcalving factors affecting conception risk in Holstein dairy cows in tropical and sub-tropical conditions. Theriogenology. 69, 443-457.

Veerkamp, R., Koenen, E., De Jong G., 2001. Genetic correlations among body condition score, yield, and fertility in first-parity cows estimated by random regression models. J. Dairy Sci. 84, 2327-2335.

Walsh, S., Williams E., Evans A., 2011. A review of the causes of poor fertility in high milk producing dairy cows. Anim. Reprod. Sci. 123, 127-138.

Chapter 5

Udder health of dairy cows with an extended voluntary waiting period from calving until the first insemination

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Abstract

This study aimed to evaluate the effect of an extended voluntary waiting period (**VWP**) on SCC, SCC elevations, and clinical mastitis incidence during the complete lactation and the first 6 weeks of the next lactation. Holstein-Friesian dairy cows (N=154) were blocked for parity, expected milk yield, calving season, and breeding value for persistency and were randomly distributed across 3 VWP (50, 125, or 200 days: **VWP-50, VWP-125, VWP-200**). Cows were monitored from calving until 6 weeks in the next lactation, or until culling. An elevation of SCC in milk was defined as SCC in milk ≥200,000 cells/mL after two previous weeks with SCC <200,000 cells/mL. Over the complete lactation, extending the VWP did not affect SCC elevations and the occurrence of clinical mastitis per lactation or per cow per year. There was no clear effect of VWP length on SCC in the complete lactation, except that multiparous cows in VWP-125 had a higher SCC compared with multiparous cows in VWP-50. Dry-off antibiotic usage per cow per year was lower in VWP-200 compared with VWP-50 for multiparous cows. In the first 6 weeks of the next lactation, cows in VWP-200 had a higher SCC compared with cows in VWP-50, with no effect of VWP on the number of elevations of SCC or the occurrence of clinical mastitis. Extending the VWP may therefore be used to reduce the frequency of transition periods and the associated use of dry-cow antibiotics, with limited impact on udder health, and a similar occurrence of SCC elevations and clinical mastitis per year.

Introduction

Considering the greatest economic results, indicated by yearly peak milk, a 1-year calving interval (**CI)** is generally advised to dairy farmers as the optimum lactation cycle (Hanks and Kossaibati, 2012; Temesgen et al., 2022). This 1-year CI usually includes a 10-month lactation, and a 2-month dry period (Kolver et al., 2006, Auldist et al., 2007). As a consequence of this yearly lactation cycle, cows experience multiple transitions every year, including drying-off, calving, and the start of the next lactation. During these transitions, large changes in both physiology (e. g. calving, onset of lactation) and management (e. g. regrouping, start of milking) are associated with an increased risk of diseases and disorders, such as clinical mastitis, hypocalcemia, and ketosis (Butler, 2000, Friggens et al., 2004, Fetrow et al., 2006, Pinedo et al., 2014) and possibly culling (Olechnowicz et al., 2011). In total, approximately 75% of disease incidences within herds occur within the first month of lactation (Erb et al., 1985, Ingvartsen et al., 2003, LeBlanc et al., 2006).

One possible solution to reduce the frequency of transitions and associated health disorders in dairy cows is to extend the lactation length and CI. Extending the voluntary waiting period from calving until first insemination (**VWP**) is one of the strategies to extend the lactation length beyond 305 d (Österman and Bertilsson, 2003, Knight, 2005, Lehmann *et al*., 2016, Sehested et al., 2019), resulting in an extended lactation length and CI. With an extended CI, the risk of diseases per year can be expected to reduce as there will be fewer calving events per year (Sehested et al., 2019). Cows with an extended VWP (150 days) had a lower incidence of metabolic disorders, lower veterinary costs, and lower culling rates compared with cows with a short VWP (60 days) (van Amburgh et al., 1997). Moreover, a 1-year CI was also associated with a high proportion of cows that were dried off at high production levels (above 18 kg/d), which probably has a negative impact on udder health (Österman and Bertilsson, 2003). For every 5-kg increase in milk yield at dry-off above 12.5 kg, the odds of a cow having an intramammary infection (**IMI**) with an environmental pathogen at calving increased at least by 77% (Rajala-Schultz et al., 2005). Extending the VWP from 40 to 180 days resulted in a greater proportion (34.2 vs. 54.6 %) of cows being dried off at lower milk yields (< 15 kg) (Niozas *et al*., 2019), which could reduce udder health problems around calving related to high milk production at dry-off (Rehn *et al*., 2000, Rajala-Schultz et al., 2005, Odensten *et al*., 2007). The incidence rate of 12 different diseases, including mastitis, decreased by 9.9 or 19.7% when all cows were managed for a CI of 15 or 17 months, respectively, in comparison with a CI of 13 months (Lehmann *et al*., 2016).

Extending the VWP may also have negative consequences on udder health. Cows with an extended CI have more days in late lactation, and cows in late lactation have a greater risk for increased SCC (Singh and Ludri, 2001) related to the decline in milk yield (Hagnestam-Nielsen et al., 2009) and low tight junction integrity (Nguyen et al., 2001). A lower milk yield could have a lower dilution effect and result in a greater SCC (Steeneveld *et al*., 2013). The low tight junction integrity prior to parturition is related to an increased number of epithelial cells in milk derived from augmented cell shedding and apoptosis which might also contribute to the higher SCC (Kessler et al., 2019). Niozas *et al*. (2019) reported a gradual increase of SCC with increasing days in milk (**DIM**), but no difference among 3 VWP groups (40, 120, and 180 days) regarding the SCC and incidence of clinical mastitis up to 330 DIM. To our knowledge, the impact of extending the VWP on SCC or incidence of clinical mastitis during the complete lactation and in the first weeks of the subsequent lactation has not been reported yet.

The aim of this study was to evaluate the effect of 3 VWP lengths (50, 125, and 200 days) on SCC, SCC elevations, and clinical mastitis incidence during the complete lactation and the start of the subsequent lactation.

Materials & Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University & Research (Netherlands) and complies with the Dutch law on Animal Experimentation (protocol number 2016.D-0038.005).

Animals, Experimental design, and Housing

The experiment was conducted at the Dairy Campus research farm (Leeuwarden, Netherlands) between December 2017 and January 2020. The experimental design, cow management, and diet composition have been reported previously (Burgers et al., 2021b). In summary, Holstein Friesian cows (**N = 154**) were selected based on (1) no twin pregnancy, (2) no clinical mastitis or SCC > 250,000 at the final 2 milk test days before dry-off, and (3) expected to finish a complete lactation. In week 6 after calving, cows were blocked for parity, calving season, milk yield in the previous lactation (multiparous cows) or expected milk yield (primiparous cows), and the breeding value for persistency (CRV, Arnhem, Netherlands). The experiment started with 50 blocks, each block consisted of 3 cows. After the removal of 2 cows before the end of VWP due to culling as a result of diseases, 2 more blocks of 3 cows were added. The cows were randomly distributed within blocks over 3 treatment groups: a VWP of 50 days (VWP-50), 125 days (VWP-125), or 200 days (VWP-200). Cows were inseminated when estrous was detected after the end of VWP. Estrous detection was visually by the animal caretaker and carried out with Nedap Smarttag system. Cows were inseminated until 300 DIM, in other words, cows in VWP-50 had 250 d to conceive, cows in VWP-125 had 175 d to conceive, and cows in VWP-200 had 100 d to conceive. The partial mixed ration for lactating cows consisted of grass silage, corn silage, soybean meal, and wheat meal, supporting 22 kg of milk. Concentrate supply started at 1 kg per day on the day of calving and increased stepwise to 9 kg (primiparous) or 10 kg (multiparous) per day from day 21 onwards. After 100 DIM, individual concentrate supply was decreased to match reductions in milk production based on the last 5 days of milk production. In the milking parlor, 1 kg of additional concentrate was supplied daily. The ration for dry cows consisted of grass silage and corn silage, supplemented with wheat straw and concentrate. In the last 10 days before the expected calving date, cows received 1 kg concentrate daily. Cows were dried off between 42 and 49 days before the expected calving date. From 7 days before dry-off, cows were given the dry-cow ration. From 4 days before dry-off, cows were milked once daily. When cows had an average SCC > 150,000 cells/mL or at least one case of clinical mastitis in the complete lactation, cows were treated with antibiotics at dry-off (Orbenin Dry Cow Extra, Zoetis, Netherlands). All cows were treated with teat sealant at dry-off (Orbeseal, Zoetis, Netherlands).

Cows were milked twice daily around 6 am and 6 pm in a 40-cow rotary milking parlor (GEA, Dusseldorf, Germany). The experimental period started at calving and ended 6 weeks after the next calving, or at 530 DIM if cows were not pregnant. Animals that were culled were followed until the moment they left the farm.

Cows in the 3 treatment groups were inseminated after their VWP when estrous was detected. Estrous detection was carried out by using the Nedap Smart tag system (Nedap, Groenlo the Netherlands) as well as visually by the animal caretaker. Cows were inseminated until 300 DIM. Cows that did not conceive within 300 DIM stayed in the experiment until 530 DIM as long as they produced at least 10 L of milk/d.

Clinical mastitis was diagnosed and recorded by the staff at the Dairy Campus research herd, during the morning or evening milking. A case of clinical mastitis was defined as a case of visibly abnormal milk, visible changes in the udder due to inflammation, or both. All cows with clinical mastitis were treated with antibiotics according to the herd-specific treatment plan based on the severity of the disease.

Milk Collection and Analysis

Milk yield was recorded from the day of calving until dry-off and the first 6 weeks of the next lactation at every milking. Milk samples for SCC analysis [(**ISO 9622**, 2013), Qlip, Zutphen, Netherlands] were collected 4 times per week (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning), and were analyzed as a pooled sample of 2-morning milkings and 2-afternoon milkings per cow per week.

Statistical Analyses

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The natural logarithm of SCC (cells × 10^3 /mL) was used for statistical analyses to approximate normality. Significance of effects was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

*SCC, Elevations of SCC, and Clinical Mastitis of Cows with Different VWP Lengths***.**

Analyses were done for 3 lactation periods: the complete lactation; the 9 weeks before dry-off; and the first 6 weeks of the subsequent lactation. To obtain SCC corrected for milk yield (to account for possible dilution effects), average weekly milk yield (kg/d) was included in the model for SCC (Steeneveld et al., 2013). Per lactation period, weekly milk yield and SCC were analyzed using a mixed linear model (PROC MIXED) with the cow as the repeated subject (Model 1). Fixed effects of treatment (VWP-50, VWP-125, or VWP-200), parity class (1 or ≥2), week (only for lactation periods of fixed length, i.e. the 9 weeks before dry-off and the first 6 weeks in subsequent lactation) and their 2-way interactions were included in the model. A first-order autoregressive covariance matrix was the best fit according to the Akaike information criterion and was used to account for within-cow variation.

An elevation of SCC was defined as SCC≥200,000 cells/mL after 2 previous weeks with SCC <200,000 cells/mL (Schukken *et al*., 2003). The binary variables “at least 1 elevation of SCC” and “at least 1 case of clinical mastitis” in the 3 lactation periods (the complete lactation, 9 weeks before dry-off, and the first 6 weeks of the subsequent lactation) were analyzed using a generalized linear regression model with logit link function (PROC GLIMMIX; Model 2). Fixed effects of treatment (VWP-50, VWP-125, or VWP-200), parity class (1 or ≥2), and their 2-way interactions were included in the model. The number of elevations of SCC and number of cases of clinical mastitis per cow in the complete lactation and the subsequent lactation after different VWP lengths were analyzed using a Poisson distribution for the dependent variable and the default log link function, with the same fixed factors as model 2 (Model 3). The number of SCC elevations per cow per year was calculated by dividing total elevations per lactation by CI length and subsequently multiplying by 365. For the non-pregnant and culled cows, the number of elevations was divided by lactation length. Effects of VWP treatment and parity class on the occurrences of clinical mastitis per cow per year and dry-off antibiotic use per cow per year were assessed through non-parametric tests. Effects of VWP, parity class, and of the 6 parity×treatment groups were tested separately using Kruskal-Wallis tests (PROC nparway; Model 4). Post-hoc comparisons were made between treatment groups of the same parity using Wilcoxon multiple comparisons.

To evaluate the time to the first elevation of SCC or time to the first case of clinical mastitis after different VWP, a survival analysis was used to obtain Kaplan-Meier curves (PROC LIFETEST; Model 5**)**. To evaluate statistical differences in Kaplan-Meier curves among the different VWP treatments, a Cox proportional hazards model was used (PROC PHREG).

*Occurrence of high SCC across the dry period**.***

To evaluate whether the SCC status of cows at the onset of a new lactation was related to SCC status before the end of the previous lactation, the SCC recorded at week 10 or 9 prepartum was compared with the first SCC recorded between 10 and 24 DIM (Van Hoeij et al. 2018). Somatic cell count was considered high when SCC was ≥200,000 cells/ mL (Schukken et al., 2003). Cows were classified as having a chronically high SCC (SCC ≥200,000 cells/mL before and after calving), cured high SCC (SCC ≥200,000 cells/mL before and SCC <200,000 cells/mL after calving), new high SCC (SCC <200,000 cells/mL before and SCC ≥200,000 cells/mL after calving), or consistently low SCC (SCC <200,000 cells/mL before and after calving).

The difference in the incidence of postpartum high SCC (binary; 0 or 1) between different VWP lengths was analyzed within cows with low or high SCC before the DP (binary; 0 or 1) using a logistic regression model (Model 6; PROC LOGISTIC in SAS 9.4; SAS Institute Inc., Cary, NC).

Values are presented as least squares means ± standard error of the mean. All *P*-values of pair-wise comparisons of least squares means were corrected with a Bonferroni adjustment.

Results

This experiment started with 154 cows in total. Within the experiment, 14 cows did not become pregnant during the first lactation within the experiment (2 from VWP-50, 3 from VWP-125, 9 from VWP-200), and 13 cows were culled due to health issues (5 from VWP-50, 4 from VWP-125, 4 from VWP-200). As a result, 127 cows were followed for a complete lactation and 6 weeks into the second lactation within this experiment. The mean CI of these 127 cows was 384, 452, and 501 days for cows in VWP-50, VWP-125, and VWP-200 (Table 1), and dry period length did not differ. The lactation length of all 154 cows was 363, 445, or 481 days for cows in VWP-50, VWP-125, or VWP-200.

Udder health in the complete lactation

There was no clear directional effect of VWP length on SCC in the complete lactation, yet there was an interaction effect of VWP class and parity (Table 2). Multiparous cows in VWP-125 had a greater SCC than multiparous cows in VWP-50 (5.02 vs 4.74 log-transformed SCC $\times 10^3$ cells/mL, $P = 0.01$; Figure S1 in Appendix). Extending VWP did not have an effect on the occurrence of at least 1 elevation of SCC, the number of elevations of SCC per affected cow, or the number of SCC elevations per cow per CI or per year. Extending VWP did not have an effect on the occurrence of at least 1 case of clinical mastitis, the total occurrence of clinical mastitis per treated cow, or the occurrence of clinical mastitis per lactation, or per year (Table 2).

Udder health before dry-off

The VWP did not affect the percentage of cows that were dried off with antibiotics (Table 3). However, due to the longer lactation length and CI, the dry-cow antibiotic use per cow per year for multiparous cows was lower in VWP-200 than in VWP-50 (0.37 vs 0.65, $P<0.01$; Appendix Table S2). During the 9 weeks before dry-off, the occurrence of at least 1 elevation of SCC and the number of elevations of SCC did not differ among VWP treatments. There was no clinical mastitis diagnosed in the 9 weeks before dry-off (Table 3).

The milk yield of cows in VWP-50 was higher compared with cows in VWP-125 and VWP-200, whereas there was no effect of VWP on average SCC. Over the 9 weeks before dry-off, SCC increased and milk yield decreased (Figure 1a,b).

Occurrence of high SCC across the dry period after different VWP lengths.

Based on weekly milk samples, the occurrence of SCC≥200,000 cells/mL on the last test day before dry-off and the first test day after 10 DIM did not differ among VWP treatments (Table 4). Similarly, the proportion of cows with a chronically or cured high SCC or new or no high SCC did not differ among VWP treatments.

Udder health in the subsequent lactation

In the first 6 weeks of the subsequent lactation in the experiment, milk yield tended to be higher for cows in VWP-50 than for cows in VWP-200 (P=0.08; Table 5). The average SCC was greater for cows in VWP-200 than cows in VWP-50 (Figure S2 in Appendix). The VWP did not have an effect on the occurrence of at least 1 elevation of SCC or at least 1 case of clinical mastitis, the number of elevations of SCC per affected cow, or the number of clinical mastitis cases per treated cow in this period.

Table 1. Calving interval and dry period length of the 127 cows that had a second calf, and lactation length of all 154 cows within the experiment after a voluntary waiting period from calving until the first insemination of 50, 125, or 200 days (VWP-50, VWP-125, or VWP-200).

	VWP			SEM	*P*-Value
	VWP50	VWP125	VWP200		
Cows, n	47	42	38		
Calving interval, d	384[a]	452[b]	501[c]	7.56	<0.01
	(324-565)	(400-586)	(469-575)		
Dry period length, d	41	42	43	1.7	0.69
	(18-63)	(8-72)	(8-75)		
Cows, n	54	49	51		
Lactation length, d	363[a]	445[b]	481[b]	12.9	0.02
	(283-528)	(361-543)	(422-526)		

Table 2. Variables regarding udder health for cows that were pregnant with a voluntary waiting period from calving until the first insemination of 50, 125, or 200 days (VWP-50, VWP-125, or VWP-200) in the complete lactation.

	VWP				Parity			*P*-value[1]		
	VWP50	VWP125	VWP200	SEM	1	≥2	SEM	VWP	Par	VWPxPar
Cows, n	47	42	38		36	91				
Average SCC[2] within full lactation	4.32	4.45	4.41	0.07	3.90	4.88	0.07	0.37	<0.01	0.02
Average SCC[2] corrected for milk yield	4.28	4.35	4.28	0.07	3.73	4.88	0.06	0.64	<0.01	0.07
Elevations of SCC (% of cows)	77.8	69.6	81.6	7.13	66.5	86.2	6.34	0.41	<0.01	0.08
Elevations of SCC (n cases/ affected cow)	2.0	2.4	2.2	0.32	1.5	2.8	0.30	0.64	<0.01	0.36
Elevations of SCC (n, per lactation)	1.6	1.6	1.9	0.30	1.0	2.4	0.26	0.73	<0.01	0.15
Elevations of SCC (n, per cow per year)	1.4	1.3	1.2	0.26	0.85	0.93	0.16	0.74	<0.01	0.21
Clinical mastitis (% of cows)	17.1	26.8	24.5	8.43	11.0	34.6	7.51	0.62	<0.01	0.93
Clinical mastitis (n cases/ affected cow)	1.2	1.7	1.5	0.7	1.3	2.0	1.2	0.70	<0.01	0.75
Clinical mastitis (n, per lactation)	0.2	0.5	0.4	0.2	0.1	0.7	0.2	0.44	<0.01	0.78

[1]VWP = voluntary waiting period; Par = parity class (Parity = 1 and parity ≥2); VWP×Par = interaction of VWP with parity.

[2]Somatic cell count ($\times 10^3$ cells/mL) is shown and analyzed as the natural logarithm of SCC.

Table 3. Variables regarding udder health and milk yield for cows with a voluntary waiting period after calving until the first insemination of 50, 125, or 200 days (VWP-50, VWP-125, or VWP-200) in the 9 weeks before dry-off.

	VWP				Parity			*P*-value[1]		
	VWP50	VWP125	VWP200	SEM	1	≥2	SEM	VWP	Par	VWP×Par
Cows with dry period[2], n	47	42	38		36	91				
Dry-off antibiotic use (% of cows)	48.1	28.1	30.4	0.40	20.6	33.4	1.5	0.17	<0.01	0.24
Week -9 until -1 relative to dry-off										
Elevations of SCC[3] (% of cows)	17.8	7.2	16.7	9.0	14.0	27.0	7.0	0.33	<0.01	0.33
Elevations of SCC (n cases/affected cows)	1.2	1.1	1.2	0.07	1.1	1.3	0.1	0.33	<0.01	0.33
Clinical mastitis (% of cows)	0	0	0		0	0				
Milk yield, kg/d[4]	19.9^{a}	17.2^{b}	16.4^{b}	0.98	19.3	16.4	0.87	0.01	<0.01	0.19
SCC[5]	4.65	4.56	4.52	0.12	4.03	5.13	0.10	0.63	<0.01	0.32

[a,b]Values within VWP within a row with different superscript letters differ ($P < 0.05$)

[1]VWP = voluntary waiting period; Par = parity class (Parity = 1 and parity ≥2); VWP×Par = interaction of VWP with parity.

[2]Of the 127 cows, 2 cows had a second calving without a dry period

[3]Somatic cell count

[4]P values for Week, VWP×Week, and Par×Week were all <0.01 for milk yield

[5]Somatic cell count ($\times 10^3$ cells/mL) is shown and analyzed as the natural logarithm of SCC. P values for week, VWP×Week, and Par×Week were <0.01, 0.40, and 0.85, respectively.

5

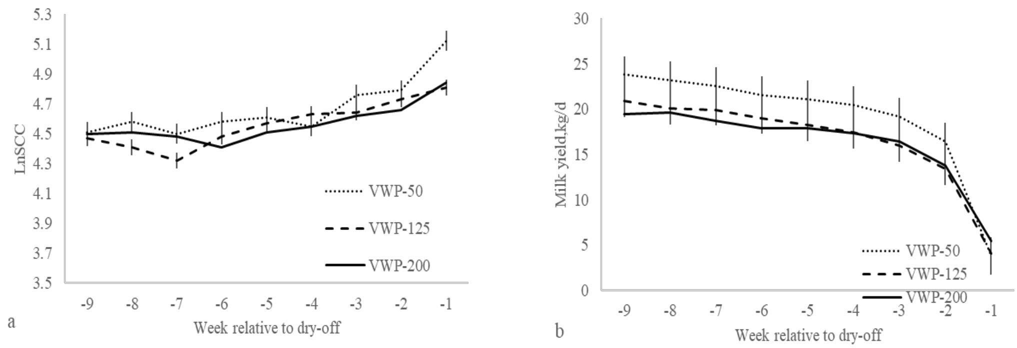

Figure 1. a-b: Development of somatic cell count (SCC; expressed as the natural logarithm of SCC) (a) and milk yield (b) in cows with a 50 d, 125 d, and 200 d voluntary waiting period (VWP-50, VWP-125, and VWP-200, respectively) during the 9 weeks relative to dry-off at the end of the first lactation. Values represent LSMEANS±SEM.

Table 4. Mean incidence (%) and number of cows with a low somatic cell count (SCC) (<200,000 cells/ml) or high SCC (≥ 200,000 cells/ml) around the dry period before the second lactation (at the last test-day before dry-off and first test-day after calving) for cows with a voluntary waiting period after calving until the first insemination of 50, 125, or 200 days (VWP-50, VWP-125, or VWP-200).

	VWP			*P*-value
	VWP50	VWP125	VWP200	
Total cows, n	47	42	38	
	%(n)	%(n)	%(n)	
SCC<200,000 prepartum	77 (36)	74 (31)	68 (26)	
SCC≥200,000 prepartum	23 (11)	26(11)	32 (12)	0.57
SCC<200,000 postpartum	60 (28)	60 (25)	61 (23)	
SCC≥200,000 postpartum	40 (19)	40 (17)	39 (15)	0.86
High SCC prepartum				
Chronic	55 (6)	27 (3)	50 (6)	
Recovered	45 (5)	73 (8)	50 (6)	0.33
Low SCC prepartum				
Elevation	39 (14)	45 (14)	35 (9)	
Healthy	61 (22)	55 (17)	65 (17)	0.64

Table 5. Variables regarding udder health for cows with a voluntary waiting period after calving until the first insemination of 50, 125, or 200 days (VWP-50, VWP-125, or VWP-200) in the first six weeks in the second lactation in the experiment.

	VWP				Parity			*P*-value[1]		
	VWP50	VWP125	VWP200	SEM	2	≥3	SEM	VWP	Par	VWP×Par
Cows, n	47	42	38		36	91				
Milk yield[2]	38.3	35.8	34.8	1.20	35.2	37.5	1.06	0.06	0.07	0.31
Average SCC[3]	4.25[a]	4.61[ab]	4.95[b]	0.21	4.54	4.67	0.18	0.04	0.54	0.71
Average SCC corrected for milk yield[3]	4.31	4.53	4.78	0.16	4.39	4.69	0.17	0.09	0.15	0.40
Elevations of SCC (% of cows)	12.14	23.63	17.31	8.57	19.21	16.18	6.93	0.39	0.66	0.91
Elevations of SCC (n, cases /affected cow)	1.1	1.2	1.2	0.1	1.2	1.2	0.1	0.39	0.66	0.91
Clinical mastitis (% cows)	7.50	4.41	13.46	4.61	5.56	11.36	5.44	0.40	0.29	0.27
Clinical mastitis (n cases/ affected cow)	1.1	1.1	1.2	0.0	1.1	1.1	0.1	0.48	0.46	0.32

[a,b]Values within VWP within a row with different superscript letters differ ($P < 0.05$)

[1]VWP = voluntary waiting period; Par = parity class (Parity = 1 and parity ≥2); VWP×Par = interaction of VWP with parity.

[2]P values for Week, VWP×Week and Par×Week were <0.01, <0.01, and 0.18, respectively for milk yield

[3]Somatic cell count ($\times 10^3$ cells/mL) is shown and analyzed as the natural logarithm of SCC. P values for week, VWP×Week, and Par×Week were <0.01, 0.02, and 0.43, respectively for average SCC; and 0.83, 0.06, and 0.26m respectively for average SCC corrected for milk yield.

Discussion

Extending VWP from calving until the first insemination increased lactation length and CI in our study. Aside from an extended VWP, cows with a short VWP that repeatedly failed to conceive following AI or did not show oestrus could also end up with an extended lactation or CI. In this experiment, cows in VWP-50 had a maximum lactation length of 528 d and CI of 565 d. In contrast, cows in VWP-200 had only 100 days to conceive, resulting in a smaller range in CI and fewer cows that became pregnant compared with cows in VWP-50 or VWP-125.

In the current study, extending the VWP did not affect SCC in complete lactation for primiparous cows. For multiparous cows, the SCC for cows with VWP-125 was higher than for cows with VWP-50 (back-transformed median: 367 vs 229 $\times 10^3$ cells/mL), but not for cows with VWP-200. Niozas *et al*. (2019) reported that extending the VWP from 40 days to 120 days or 180 days did not affect SCC throughout the lactation. Österman *et al* (2005) also found no effects of lactation length on SCC during the complete lactation, when comparing a production system with an 18-month extended CI with a traditional 12-month CI. In addition, in the study of Österman *et al* (2005), part of the cows was milked 3 times per day to increase their peak milk yield and persistency. Cows with better persistency are more suitable for an extended lactation length (Burgers et al., 2021b). Increasing the frequency of milking had no adverse effect on udder health (Waterman et al., 1983; Wright et al., 2013) or reduced udder diseases (Smith et al., 2002; Dahl et al.,2004; Sitkowska et al., 2018) in different studies. In the current study, no attempt was made to manage the cows for increased persistency.

In the current study, extending the VWP did not affect the incidence of SCC elevations and clinical mastitis per lactation or per year. Niozas *et al*. (2019) compared cows with VWP of 40, 120, or 180 days and reported no difference in the number of mastitis cases in 305 days in milk or the whole lactation. However, a lower annual rate of clinical mastitis may be expected when the lactation of cows is extended, due to fewer critical transitions related to dry-off, calving, and the start of a new lactation (Allore and Erb, 2000). Such an impact of lactation length on annual disease incidence is only expected if diseases occur more often around calving or in early lactation. Extending the VWP did not affect the annual rate of mastitis in the current study, which may be explained by the low incidence of clinical mastitis around calving and in early lactation (Appendix Figure S4). In the study of Heather *et al*. (2000), the annual rate of clinical mastitis was 0.95 and 0.68 cases per cow per year for cows with a standard (50 days) versus an extended VWP (150 days), which is much higher than 0.20, 0.23 and 0.19 cases per year for cows in VWP-50, VWP-125, and VWP-200 in the current study.

In the 9 weeks before the dry period, average SCC, SCC elevations, and incidence of clinical mastitis were not affected by an extended VWP. This is in line with Osterman *et al*. (2005), who reported that cows with a 15-month CI did not have higher SCC in the last 10 weeks prior to dry-off compared with cows with a 12-month CI. In earlier studies, an increased SCC in late lactation was mainly explained by a lower dilution of the somatic cells in milk due to the lower milk yield in late lactation (Miller *et al*., 1983), and by the low tight junction integrity before calving, increasing the epithelial cells in milk (Kessler et al., 2019). In the current study, milk yield in the last 9 weeks before dry-off was lower for cows with a 125-d VWP or 200-d VWP, compared with cows with 50-d VWP, while SCC in late lactation was not affected by VWP. Similarly, Pollott *et al.* (2011) studied cows with different lactation lengths (305 d, 370 d, or 440 d) and reported that throughout lactation, differences in SCC were observed between the 3 lactation-length curves were small even in late lactation, with a rise in SCC during pregnancy.

Different VWP had no effect on udder health before dry-off or dry-off antibiotic usage at lactation level. However, due to the longer average CI resulting from extended VWP, annual usage of dry-off antibiotics was lower in VWP-200 compared with VWP-50 for multiparous cows. In the current study, selective use of dry cows' antibiotics was based on average SCC and the occurrence of clinical mastitis during lactation. Results indicate that, for multiparous cows, extended VWP may be used to reduce the annual antibiotic use at dry-off. Reduction of annual antibiotics use is of importance not only as a reduction in veterinary costs but also in relation to the development of bacterial strains which are resistant to antibiotics (Kuipers *et al.*, 2016; Vanhoudt *et al.*, 2018).

In the current experiment, cows with VWP-200 had a higher SCC in the first 6 weeks in the subsequent lactation after the extended VWP and tended to have lower milk yield compared with cows with VWP-50 (Burgers et al., 2021b). Partly, the greater postpartum SCC of cows with VWP-200 was explained by the lower milk yield for cows with VWP-200 compared with cows with VWP-50. A lower milk yield could result in a greater SCC because of the lower dilution effect (Steeneveld *et al*., 2013). However, also with a correction for milk yield, SCC in the first 6 weeks of the subsequent lactation was greater for cows with VWP-200, compared with cows with VWP-50. This indicates that the contrast in milk yield did not explain the difference in SCC in the subsequent lactation completely in the current study. In addition, fewer cows in VWP-200 were treated with antibiotics at dry-off compared with cows in VWP-50

(30.4% vs 48.1% respectively). Not using antibiotics in cows that had a low SCC before dry-off significantly increased the SCC and also incidence rate of clinical mastitis in subsequent lactation (Scherpenzeel *et al*., 2014). This could also partly explain the higher SCC for cows with VWP-200 in the current study, compared with cows with VWP-50. In this perspective, the incidence of clinical mastitis during the 6 weeks after the second calving was 13.5% and 7.5% for cows in VWP-200 and VWP-50, respectively, which might also be related to the higher SCC of cows in VWP-200. In the current study, multiparous cows with VWP-125 tended to have higher SCC compared with multiparous cows with VWP-50 in the complete lactation. High SCC in VWP-125 was not related to a greater incidence of clinical mastitis, which was not different between VWP treatments with 35.3% and 32.9% for cows with VWP-125 and VWP-50, respectively.

In this study, extending the VWP had limited effects on udder health in the current lactation. In practice, farmers are interested in extending VWP also for other reasons. First, managing cows for extended lactations would lead to fewer transition periods per cow per year and associated management labor (i.e., drying-off, calving, and the start of lactation). Second, fewer calves were born and the associated reduction in excess calves and calf care was also a reason for farmers to extend the VWP (Burgers et al., 2021a). Third, a reduction in annual disease occurrence and associated veterinary costs can be expected for diseases specifically associated with the transition period when cows are managed for extended lactations (Lehmann et al., 2016; van Amburgh *et al*., 1997).

Conclusion

Extending the VWP did not have an effect on the occurrence of SCC elevations and clinical mastitis per lactation or per year. In multiparous cows, cows with VWP-125 had a higher SCC than cows with VWP-50 in complete lactation. Extending the VWP reduced milk yield in the 9 weeks relative to dry-off, but did not affect SCC, or the occurrence of SCC elevations or mastitis in the same period. Dry-off antibiotic usage per year was reduced in VWP-200 compared with VWP-50 for multiparous cows. In the first 6 weeks of the next lactation, SCC was increased after an extended lactation with VWP-200 compared with VWP-50, with no effect on the number of SCC elevations or the occurrence of mastitis. These results indicate that extending the VWP may be used to reduce the frequency of transition periods and the associated use of dry-cow antibiotics with limited impact on udder health.

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Appendix:

Table S1. Variables regarding udder health for all cows with a voluntary waiting period from calving until the first insemination of 50, 125, or 200 days (VWP-50, VWP-125, or VWP-200) in the complete lactation.

	VWP				Parity			P-value[1]		
	VWP50	VWP125	VWP200	SEM	1	≥2	SEM	VWP	Par	VWPxPar
Cows, n	54	49	51		41	113				
Average SCC[2] within full lactation	4.34	4.44	4.31	0.07	3.88	4.84	0.06	0.26	<0.01	0.04
Average SCC[2] corrected for milk yield	4.31	4.35	4.16	0.06	3.69	4.85	0.06	0.05	<0.01	0.07
Elevations of SCC (% of cows)	77.1	67.2	75.6	9.4	65.9	80.8	7.6	0.50	0.05	0.38
Elevations of SCC (n cases/ affected cow)	2.0	2.4	2.1	0.3	1.5	2.8	0.3	0.48	<0.01	0.35
Elevations of SCC (n, per lactation)	1.6	1.7	1.6	0.2	1.1	2.7	0.2	0.96	<0.01	0.21
Elevations of SCC (n, per cow per year)	1.7	1.3	1.1	0.2	0.9	2.0	0.2	0.90	<0.01	0.13
Clinical mastitis (% of cows)	19.8	24.3	22.1	10.0	9.6	34.6	8.1	0.89	<0.01	0.96
Clinical mastitis (n cases/ affected cow)	1.3	1.5	1.4	0.2	1.1	1.7	0.2	0.66	<0.01	0.95
Clinical mastitis (n, per lactation)	0.2	0.3	0.3	0.6	0.1	0.8	0.6	0.44	<0.01	0.71

[1]VWP = voluntary waiting period; Par = parity class (Parity = 1 and parity ≥2); VWP×Par = interaction of VWP with parity.

[2]Somatic cell count (×10^3 cells/mL) is shown and analyzed as the natural logarithm of SCC.

Table S2. Dry-off antibiotic use and mastitis occurrence per cow per year of cows with a voluntary waiting period after calving until the first insemination of 50, 125, or 200 days (VWP-50, VWP-125, or VWP-200).

	Primiparous			Multiparous			*P*-value		
	VWP50	VWP125	VWP200	VWP50	VWP125	VWP200	VWP	Par	VWP×Par
Cows, n	13	14	8	34	27	29			
Dry-off antibiotic use (%)[1]	15 (2/13)	0 (0/14)	13 (1/8)	68 (23/34)	56 (15/27)	52 (15/29)	0.17	<0.01	0.24
Dry-off antibiotic use per cow per year[2]	0.15	0	0.09	0.65[a]	0.45[ab]	0.37[b]	0.01	<0.01	<0.01
Cows, n	14	15	12	40	34	39			
Mastitis (%)[3]	7 (1/14)	7 (1/15)	0 (0/12)	31 (14/40)	29 (10/26)	31 (12/39)	0.89	<0.01	0.96
Mastitis per cow per year[2]	0.08	0.06	0	0.52	0.60	0.44	0.75	<0.01	0.12

[1]P-values for % of cases are derived from the GLIMMIX model and repeated from table 3

[2]P-values for incidence of dry-off antibiotics and mastitis per year are based on three independent non-parametric comparisons, of parity class, treatment group, and the 6 parity*treatment groups. Letters indicate the difference between treatment groups of the same parity based on Wilcoxon multiple comparisons after the Kruskal-Wallis test.

[3]P-values for % of cases are derived from the GLIMMIX model and repeated from table 2.

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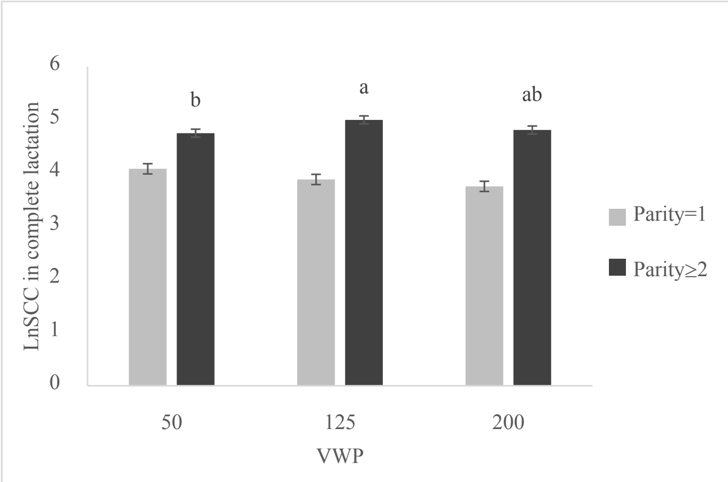

Figure S1. Somatic cell count (SCC; expressed as the natural logarithm of SCC) in cows with a 50 d, 125 d, and 200 d voluntary waiting period (VWP) and different parity class (parity=1; parity ≥2) in first complete lactation. Values represent LSMEANS±SEM.

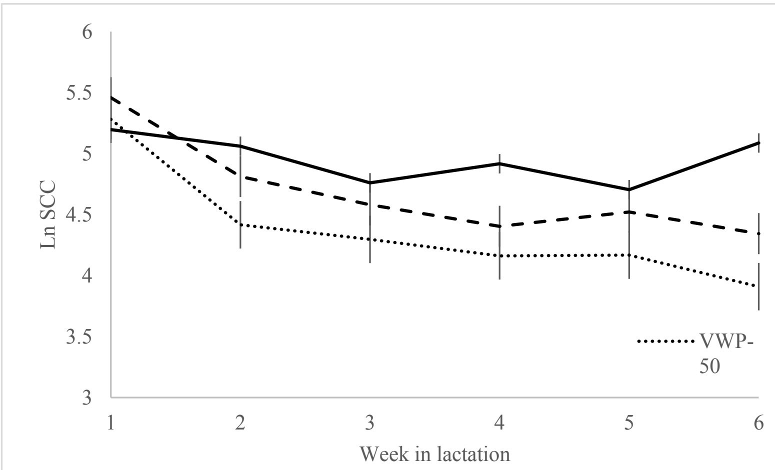

Figure S2. Development of somatic cell count (SCC; expressed as the natural logarithm of SCC) in cows with a 50 d, 125 d, and 200 d voluntary waiting period (VWP-50, VWP-125, and VWP-200, respectively) in the first 6 weeks in subsequent lactation (b). Values represent LSMEANS±SEM.

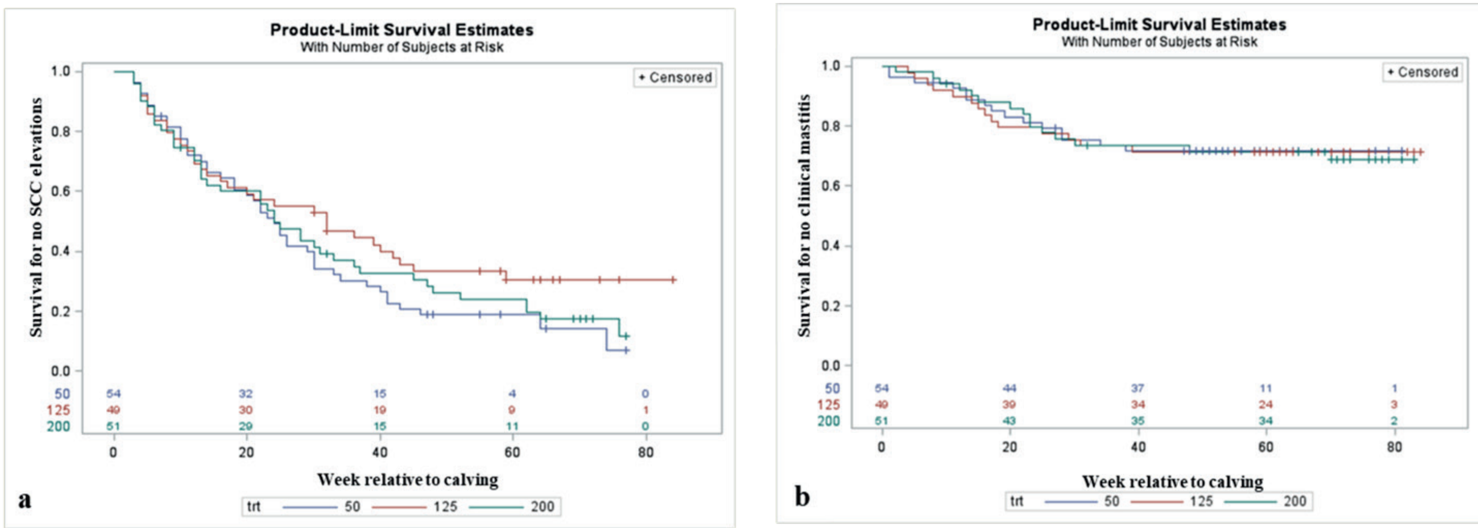

Figure S3. a-b: Kaplan-Meier survival curves showing (a) the time of survival for no elevation (i.e. SCC ≥ 200,000 cells/mL after two previous weeks SCC<200,000 cells/mL), and (b) the time of survival for no case of clinical mastitis in the first full lactation for cows with a 50 d, 125 d, and 200 d voluntary waiting period (VWP-50, VWP-125, and VWP-200, respectively).

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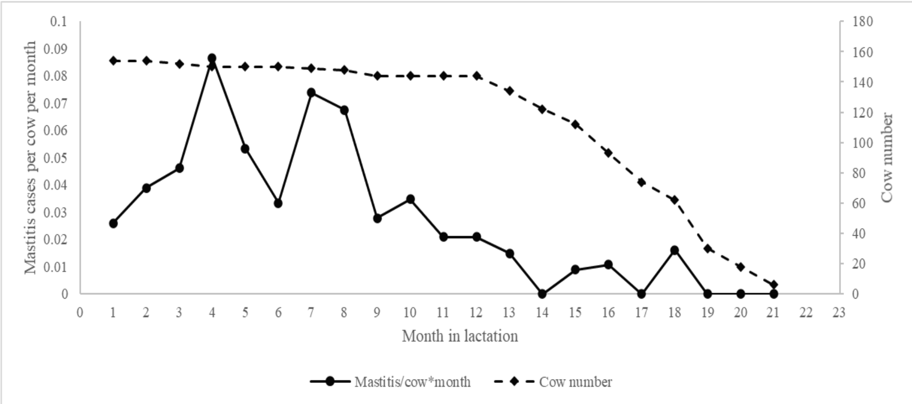


Figure S4: Mastitis incidence per cow per month and number of cows in lactation.

References

Allore, H.G., Erb, H.N., 2000. Simulated effects on dairy cattle health of extending the voluntary waiting period with recombinant bovine somatotropin. Prev. Vet. Med. 46, 29-50.

Auldist, M., O'brien, G., Cole, D., Macmillan, K., Grainger, C., 2007. Effects of varying lactation length on milk production capacity of cows in pasture-based dairying systems. J. Dairy Sci. 90, 3234-3241.

Burgers, E., Kok, A., Goselink, R., Hogeveen, H., Kemp, B., Van Knegsel, A., 2021a. Fertility and milk production on commercial dairy farms with customized lactation lengths. J. Dairy Sci. 104, 443-458.

Burgers E, Kok, A., Goselink, R., Hogeveen, H., Kemp, B., Van Knegsel, A., 2021b. Effects of extended voluntary waiting period from calving until first insemination on body condition, milk yield, and lactation persistency. J. Dairy Sci. 104, 8009-8022.

Butler, W., 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci. 60, 449-457.

Dahl, G.E., Wallace, R.L., Shanks, R.D., Lueking, D., 2004. Hot topic: effects of frequent milking in early lactation on milk yield and udder health. J. Dairy Sci. 87, 882–885.

Erb, H., Smith, R., Oltenacu, P., Guard, C., Hillman, R., Powers, P., Smith, M., White, M., 1985. Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in holstein cows. J. Dairy Sci. 68, 3337-3349.

Fetrow, J., Nordlund, K., Norman, H., 2006. Invited review: Culling: Nomenclature, definitions, and recommendations. J. Dairy Sci. 89, 1896-1905.

Friggens, N.C., Andersen, J.B., Larsen, T., Aaes, O., Dewhurst, R.J., 2004. Priming the dairy cow for lactation: A review of dry cow feeding strategies. Anim Res, 53. 453-473.

Hagnestam-Nielsen, C., Emanuelson, U., Berglund, B., Strandberg, E., 2009. Relationship between somatic cell count and milk yield in different stages of lactation. J. Dairy Sci. 92, 3124-3133.

Hanks, J., Kossaibati, M., 2012. Key Performance Indicators for the UK national dairy herd. A study of herd performance in 500 Holstein/Friesian herds for the year ending 31st August 2020.

Ingvartsen, K.L., Dewhurst, R.J., Friggens, N., 2003. On the relationship between lactational performance and health: Is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livest Prod Sci 83, 277-308.

Kessler, E.C., Wall, S., Hernandez, L., Gross, J., Bruckmaier, R., 2019. Mammary gland tight junction permeability after parturition is greater in dairy cows with elevated circulating serotonin concentrations. J. Dairy Sci. 102, 1768-1774.

Knight, C.H., 2005. Extended lactation: Turning theory into reality. Advances in Dairy Technology. 17, 113-123.

Kolver, E., Roche, J., Burke, C., Aspin, C., 2006. Effects of genotype and diet on milk solids production, body condition, and reproduction of cows milked continuously for 600 days. Conference of the New Zealand Society of Animal Production 2006.

Kuipers, A., Koops, W., Wemmenhove, H., 2016. Antibiotic use in dairy herds in the Netherlands from 2005 to 2012. J. Dairy Sci. 99, 1632-1648.

LeBlanc, S., Lissemore, K., Kelton, D., Duffield, T., Leslie, K., 2006. Major advances in disease prevention in dairy cattle. J. Dairy Sci. 89, 1267-1279.

Lehmann, J.O., 2016. Extended lactation in danish dairy production. PhD dissertation, Aarhus University.

Miller, R., Emanuelsson, U., Persson, E., Brolund, L., Philipsson, J., Funke, H., 1983. Relationships of milk somatic cell counts to daily milk yield and composition. Acta Agric Scand. 33, 209-223.

Niozas, G., Tsousis, G., Malesios, C., Steinhöfel, I., Boscos, C., Bollwein, H., Kaske, M., 2019. Extended lactation in high-yielding dairy cows. Ii. Effects on milk production, udder health, and body measurements. J. Dairy Sci. 102, 811-823.

Nguyen, D., Parlow, A., Neville, M., 2001. Hormonal regulation of tight junction closure in the mouse mammary epithelium during the transition from pregnancy to lactation. J. Endocrinol. 170, 347-356.

Nor, N.M., Steeneveld, W., Mourits, M., Hogeveen, H., 2012. Estimating the costs of rearing young dairy cattle in the Netherlands using a simulation model that accounts for uncertainty related to diseases. Prev Vet Med. 106, 214-224.

Odensten, M., Berglund, B., Waller, K.P., Holtenius, K., 2007. Metabolism and udder health at dry-off in cows of different breeds and production levels. J. Dairy Sci. 90, 1417-1428.

Olechnowicz, J., Jaskowski, J.M., 2011. Reasons for culling, culling due to lameness, and economic losses in dairy cows. Med. Weter. 67, 618-621.

Österman, S., Bertilsson, J., 2003. Extended calving interval in combination with milking two or three times per day: Effects on milk production and milk composition. Livest Prod Sci. 82, 139-149.

Pinedo, P., Daniels, A., Shumaker, J., De Vries, A., 2014. Dynamics of culling for jersey, holstein, and jersey× holstein crossbred cows in large multibreed dairy herds. J. Dairy Sci. 97, 2886-2895.

Pollott, G., 2011. Do holstein lactations of varied lengths have different characteristics? J. Dairy Sci. 94, 6173-6180.

Rajala-Schultz, P.J., Hogan, J.S., Smith, K., 2005. Association between milk yield at dry-off and probability of intramammary infections at calving. J. Dairy Sci. 88, 577-579.

Rehn, H., Berglund, B., Emanuelson, U., Tengroth, G., Philipsson, J., 2000. Milk production in swedish dairy cows managed for calving intervals of 12 and 15 months. Acta Agric. Scan. A Anim. Sci. 50, 263-271.

Scherpenzeel, C., Den Uijl, I., van Schaik, G., Riekerink, R.O., Keurentjes, J., Lam, T., 2014. Evaluation of the use of dry cow antibiotics in low somatic cell count cows. J. Dairy Sci. 97, 3606-3614.

Schukken, Y.H., Wilson, D.J., Welcome, F., Garrison-Tikofsky, L., Gonzalez, R.N., 2003. Monitoring udder health and milk quality using somatic cell counts. Vet Res. 34, 579-596.

Sehested, J., Gaillard, C., Lehmann, J., Maciel, G., Vestergaard, M., Weisbjerg, M., Mogensen, L., Larsen, L., Poulsen, N., Kristensen, T., 2019. Extended lactation in dairy cattle. Animal. 13, 65-74.

Sharma, N., Kang, T.Y., Lee, S-J., Kim, J.N., Hur, C.H., Ha, J.C., Vohra, V., Jeong, D.K., 2013. Status of bovine mastitis and associated risk factors in subtropical jeju island, south korea. Trop Anim Health Prod. 45, 1829-1832.

Singh, M., Ludri, R., 2001. Somatic cell counts in marrah buffaloes (bubalus bubalis) during different stages of lactation, parity and season. Asian-Australasian J. Anim Sci. 14, 189-192.

Sitkowska, B., Piwczyński, D., Wójcik, P., 2018. Milking traits affected by milking frequency during first month of lactation. Italian J. Anim Sci. 17, 777-784.

Smith, J.W., Ely, L.O., Graves, W.M., Gilson, W.D., 2002. Effect of milking frequency on DHI performance measures. J. Dairy Sci. 85, 3526–3533.

Steeneveld, W., Hogeveen, H., Barkema, H.W., van den Broek, J., Huirne, R.B., 2008. The influence of cow factors on the incidence of clinical mastitis in dairy cows. J. Dairy Sci. 91, 1391-1402.

Steeneveld, W., Schukken, Y.H., Van Knegsel, A., Hogeveen, H., 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.

Temesgen, M., Assen, A.A., Gizaw, T.T., Minalu, B.A., 2022. Factors affecting calving to conception interval (days open) in dairy cows located at Dessie and Kombolcha towns, Ethiopia. PloS One. 17, e264029.

Van Amburgh, M.E., Galton, D.M., Bauman, D.E., Everett, R.W., 1997. Management and economics of extended calving intervals with use of bovine somatotropin. Livest Prod Sci. 50, 15-28.

Van Hoeij R.J., Lam, T., Bruckmaier R.M., Dijkstra, J., Remmelink, G.J., Kemp, B., Van Knegsel, A., 2018. Udder health of dairy cows fed different dietary energy levels after a short or no dry period without use of dry cow antibiotics. J. Dairy Sci. 101, 4570-4585.

Vanhoudt, A., Van Hees-Huijps, K., van Knegsel, A., Sampimon, O.C., Vernooij, J., Nielen, M., Van Werven, T., 2018. Effects of reduced intramammary antimicrobial use during the dry period on udder health in Dutch dairy herds. J. Dairy Sci. 101, 3248-3260.

Waterman, D. F., Harmon, R., Hemken, R., Langlois, B., 1983. Milking frequency as related to udder health and milk production. J. Dairy Sci. 66, 253-258.

Wright, J., Wall, E., McFadden T., 2013. Effects of increased milking frequency during early lactation on milk yield and udder health of primiparous Holstein heifers. J. Anim Sci. 91, 195-202.

Chapter 6

General Discussion

Introduction

The rapid increase in milk production in early lactation is related to a negative energy balance (**NEB**) in dairy cows, as feed intake is insufficient to cover the energy requirements for milk production (Bell, 1995, Rastani et al., 2005, Van Knegsel et al., 2014). The NEB increases the risk for impaired fertility and health, which are indicated by abnormal ovarian cycles, reduced conception rates (Chen et al., 2015), a high incidence and increased severity of metabolic disorders, infectious diseases, inflammation, and oxidative stress in dairy cows in early lactation (Mallard et al., 1998, Sordillo et al., 2009, Trevisi et al., 2012). Traditionally, with a 1-year calving interval (**CI**), including a 60 d dry period (**DP**) and a 40-60 d voluntary waiting period (**VWP**), cows have yearly peak milk, yet also have a yearly challenging transition period and NEB. A major amount of the health issues in early lactation can be traced back to the physiology and management of dairy cows during the transition period (Reviewed by Drackley et al., 2001, Sundrum et al., 2015, Pascottini et al., 2020). Shortening and omitting the DP are of interest as they shift milk yield partially from the post-calving to the pre-calving period and, herewith, improve the EB in early lactation (Rastani et al., 2005, Van Knegsel et al., 2014). Extending the VWP is a possible solution to reduce the frequency of the challenging transition period (Lehman et al., 2016, Burgers et al., 2021). Both shortening or omitting the DP and extending the VWP are expected to improve the health and fertility of dairy cows.

Relationships between inflammatory biomarkers and health in early lactation

In **chapter 2**, We explored the relationships between inflammatory biomarkers and health status in early lactation. We reported that the incidence of clinical health problem (CHP) in early lactation is related to lower concentrations of albumin, paraoxonase, a lower LAI, and a higher concentration of haptoglobin in plasma. This is in line with Mayasari et al. (2017), who also showed clinical health problem in early lactation is related with a lower concentration of albumin and higher concentration of haptoglobin. The general decrease in albumin concentrations in sick animals may be attributed to the role of albumin as a negative APP (Gruys et al., 1994). Serum haptoglobin has been stated as a valid biomarker that can distinguish diseased animals from healthy animals (Eckersall and Bell, 2010) and is especially effective in the diagnosis and prognosis of mastitis, enteritis, and endometritis (Murata et al., 2004, Petersen et al., 2004). Paraoxonase is commonly used as a biomarker for liver status around calving (Bionaz et al., 2007, Bertoni et al., 2008). Low paraoxonase level has been associated with liver damage (Bionaz et al., 2007, Bertoni et al., 2008).

In **chapter 2**, we defined CHP when they can cause an inflammatory response, including clinical mastitis, metritis, retained placenta and fever. We also noticed that around 50% CHP in current study happened in the first week after calving. The occurrence of clinical health problems is related to prolonged or severe inflammation around calving (Trevisi et al., 2011). During NEB and inflammation, pro-inflammatory cytokines stimulated hepatic synthesis of positive APP, such as haptoglobin and ceruloplasmin, and impaired hepatic synthesis of negative APP such as albumin and cholesterol (Bionaz et al., 2007, LeBlanc, 2012).In this perspective, we recommend that the changes in biomarkers, especially around calving, maybe a good indicator for the health status of the dairy cow.

Relationships between metabolic status and health in early lactation

Chapter 2described cows with CHP tended to have a lower DMI compared other cows. Cows are still udder NEB early after calving, lower DMI is related to a stronger NEB, resulting in lower levels of glucose, insulin, and IGF-1, and an increased risk of health problems (Mair et al., 2016). However, we did not find the direct relationship between health status and NEB in early lactation. The CHP cows had lower insulin and IGF-1 concentrations in plasma compared with healthy cows in the week 1 after calving, which may explain the high incidence of CHP in week 1. In addition, health disorders showed high initial effects on feed intake decrease, and the largest decrease in total feed intake was observed during the first occurrence of mastitis (Bareille et al 2003). Therefore, early diagnosis and treatment of health problems in early lactation are essential for the success of the adaptation to the new lactation.

Consequences of different DPs and VWPs on fertility

Shortening or omitting the dry period

Shortening or omitting the DP was reported to improve fertility, indicated by an earlier onset of first ovulation postpartum (Watters et al., 2009) and overall improved resumption of ovarian cyclicity (Chen et al.,2015b, Santschi et al., 2011, Watters et al., 2009), improved conception rate (Chen et al.,2015b) and reduced days open (Van Hoeij et al., 2017).

Unfavorable consequences of omitting the DP, however, can be a reduction in milk yield and fattening of cows in the subsequent lactation (Chen et al., 2016). Adjusting dietary energy levels in early lactation could be a strategy to limit these negative consequences of a short or 0-d DP (Van Hoeij et al., 2017). Earlier we reported that reducing postpartum dietary energy level to match expected energy requirements for cows with 0-d DP did not affect milk yield or milk composition, but resulted in a less positive energy balance (**EB**) and less body weight gain in the subsequent lactation, compared with feeding cows with 0-d DP a standard energy level (based on the energy requirement for the expected milk yield of cows with a short DP) (Van Hoeij et al., 2017). Cows fed a lower energy level after 0-d DP had fewer days open compared with cows fed a standard energy level (Van Hoeij et al., 2017). In **chapter 3**, we further explored that fewer days open was related to fewer services per conception, fewer days to onset of luteal activity (OLA), and a higher percentage of ovarian cycles of normal length (18-24 d). In addition, reducing postpartum dietary energy levels for cows after 0-d DP decreased the incidence of delayed resumption of ovarian cyclicity, which was defined as OLA occurring at more than 45 days post-calving (**Table 6.1**). Gümen et al. (2005) reported that the days to first ovulation were negatively correlated with EB in early lactation. The severe NEB in early lactation inhibits follicular development by suppressing plasma insulin-like growth factor-1 (IGF-1) concentration and pituitary luteinizing hormone pulsatility, which decreases the number 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). In this thesis, cows with an earlier (< 21 d) OLA had better EB compared with cows with later OLA (≥21 d) (**Chapter 3**). Contrary to this result, other studies reported that the interval to first ovulation was not related to EB (Reist et al., 2002, Spicer et al., 1990). It was suggested that other factors also contribute to delayed first ovulation, such as uterine infection (Opsomer et al., 2000), mastitis (Huszenicza et al., 2005), and lameness (Petersson et al., 2006).

Several fertility variables seem to relate to each other. Days open is influenced by both the interval between calving to insemination and the success of insemination (Britt et al., 1974). Also, the resumption of ovarian cyclicity, which is a combined trait consisting of the onset of ovarian activity and regularity of the ovarian cycles, is related to the timing of the first insemination and the success of insemination (Pushpakumara et al., 2003). Fewer days to the resumption of ovarian cyclicity were related to a lower number of AI per conception and fewer postpartum days open (Lucy et al., 1992, Thatcher and Wilcox, 1973). This is in line with our results in **chapter 3**, showing that cows with short (<80 d) and medium (80-130 d) days open had fewer days from calving till first AI compared with cows with long days open (>130 d). Cows with short (<80 d) and medium (80-130 d) days open had fewer services per conception compared with cows with long days open (>130 d). However, some studies reported that early resumption of ovarian cyclicity was related to more days to conception and a lower conception rate in multiparous cows (Smith and Wallace, 1998). Differences in genetic population could be a reason to explain this discrepancy. Although early resumption of ovarian cyclicity seems beneficial for pregnancy, an exact relationship between days to the resumption of ovarian cyclicity and pregnancy remains unknown.

Table 6.1 Classification of resumption of ovarian cyclicity within 100 days in milk (DIM) of dairy cows with different transition treatments[1].

	Transition treatment[1]			SEM	P-value	
	0-d DP (LOW)	0-d DP (STD)	30-d DP (STD)		DP	P
Cows, n	42	43	43			
Normal resumption (%)[2]	23.81(10/42)	18.60(8/43)	30.23(13/43)	4.32	0.21	0.09
Abnormal ovarian cyclicity[2]:						
Delayed resumption (%)	2.38(1/42)[a]	6.98(3/43)[a]	16.28(7/43)[b]	1.09	0.01	0.65
Prolonged luteal phase (%)	64.29(27/42)	67.44(29/43)	46.51(20/43)	3.22	0.73	0.27
Cessation (%)	9.52(4/42)	6.98(3/43)	6.98(3/43)	2.06	0.69	0.87

[1]Low: Energy level based on the requirement for the expected milk yield of cows with a 0-d DP; STD: Energy level based on the requirement for the expected milk yield of cows with a 30-d DP; The 3 transition treatments were: cows with a 30-d DP and an STD dietary energy level (30-d DP(STD)), cows with a 0-d DP with the same STD dietary energy level as cows with a 30-d DP (0-d DP(STD)), and cows with a 0-d DP with a LOW energy level (0-d DP(LOW)).

[2]Normal resumption of ovarian cyclicity: OLA occurring at 45 DIM or less, followed by regular ovarian cycles of 18 to 24 d in length; Abnormal resumption, type I: OLA occurring at more than 45 DIM (delayed resumption); 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); Abnormal resumption, type III: absence of luteal activity for more than 14 d between two ovarian cycles (cessation of cyclicity).

Extending voluntary waiting period

With a traditional VWP of 40 to 60 d, artificial insemination (AI) is planned to start during a period of high milk yield (Ancker et al., 2006) when most of the cows are still in NEB and mobilizing body reserves, which is associated with high reproductive failure (Santos et al., 2009), indicated by low conception rates after the first insemination, more inseminations per pregnancy, and more days open (Lucy, 2001, Pryce et al., 2004, Inskeep and Dailey, 2005). With extended VWP to 125 d or 200 d, cows had fewer days open after the end of the VWP compared with cows with a 50 d VWP (**Chapter 4**). The improvement was partially related with an increase in conception rate and fewer inseminations per pregnancy. However, we did not find an effect of VWP on conception rate and the number of services in our study (Chapter 4). Extending VWP from 50 to 125 or 200 days decreased milk yield and FPCM around the last AI (**Table 6.2**). This indicates that a lower milk yield at the time of insemination possibly contributed to the improved fertility after an extended VWP in our study. Other aspects of an extended VWP, such as a longer period for uterine recovery and insemination at a time of more neutral EB, may contribute to this improved fertility as well (**Chapter 3**).

Energy balance, metabolism, and fertility

The negative relationship between OLA and NEB (**Chapter 3**) is possibly associated with a compromised ovarian follicular development by suppressing plasma IGF-1 concentration and pituitary luteinizing hormone (LH) pulsatility (Lucy, 2000).

An NEB could decrease GnRH secretion from the hypothalamus, resulting in less LH secretion from the pituitary gland (Rasby et al., 1991). During an NEB, cows lose body weight and their body condition score (BCS) decreases. Cows that decreased BCS had lower serum LH concentrations compared with cows that maintained BCS after calving (Rutter and Randel, 1984). Plasma IGF-1 increased gene expression of follicle-stimulating hormone (FSH) receptor in granulosa cells and stimulated the proliferation of granulosa cells (Zulu et al., 2002). Lower IGF-1 is unable to synergize with the gonadotrophins on ovarian cells preventing the dominant follicle from ovulating (Beam and Butler, 1999) and delaying the resumption of cyclicity (Gutierrez et al., 1999), at last leading to a prolonged interval from calving to first ovulation (Shrestha et al., 2004). In contrast, high plasma IGF-1 concentration within 2 weeks postpartum increased the possibility of earlier resumption of ovarian cyclicity (Patton et al., 2007).

Table 6.2 Milk production, FPCM[1], body condition, and body weight around the last AI within 100 days after the end of VWP for cows with a VWP of 50, 125, or 200 days. Values represent LSMEANS and maximal SEM.

	Voluntary Waiting			SEM	*P*-value		
	50 d	125 d	200		VWP	Parity (P)	VWP
Cows, n	52	49	47				
Milk production (-2 to 2 weeks around the last AI),kg/d	33.4[a]	29.7[b]	25.	1.00	<0.0	<0.01	<0.0
FPCM[1] (-2 to 2 weeks[2] around the last AI),kg/d	32.6[a]	30.2[b]	27.	1.00	<0.0	<0.01	<0.0
Body weight (-2 to 2 weeks[2] around the last AI),kg	630	642	667	13	0.10	<0.01	0.69
Body weight change (-2 to 2 weeks[2] around the last AI),kg	-2.9[b]	4.7[a]	2.3[a]	3.01	0.03	0.07	0.46
Body condition score (-2 to 2 weeks[2] around the last AI)	2.5	2.5	2.5	0.2	0.76	0.07	0.49

[a b]Values within VWP within a row with different superscript letters differ ($P < 0.05$)

[1]FPCM: Fat and protein corrected milk production.

[2]Weeks around the last AI (week -2, -1, 1, 2 relative to the last AI).

More days to OLA was also related to a lower concentration of glucose, and insulin and a higher concentration of NEFA and BHB in weeks 1-7 after calving (chapter 3). Insulin improved follicular development by acting directly on the ovary and by stimulating IGF-1 secretion (Butler et al., 2003, Simpson et al., 1994). When insulin was infused into cows with NEB, the hypothalamic-pituitary axis recoupled which enabled the liver to respond to GH again and increased IGF-1 production (Butler et al., 2003). A high concentration of insulin in plasma was associated with an increased follicular diameter and follicular oestradiol concentration (Simpson et al., 1994), which is related to preovulatory follicles rather than anovulatory follicles (Ireland and Roche, 1983). Likewise, cows that ovulated the first dominant follicle tended to have a higher plasma insulin concentration postpartum compared with cows with an anovulatory first dominant follicle (Beam and Butler, 1997). Therefore, insulin seems to stimulate follicular development from early-stage until ovulation. Plasma NEFA is negatively related to blastocyst yield (Fouladi-Nashta et al., 2007) and oxygen consumption of blastocysts, indicating less viable embryos (Van Hoeck et al., 2011). High BHB combined with low glucose had less yield of blastocysts compared with high BHB combined with high glucose, probably due to the different metabolism of BHB in cumulus cells compared with glucose (Leroy et al., 2006).

Consequences of different DPs and VWPs on health status

Dry period length and disease

Cows with 0-d DP had a greater EB in early lactation than cows with a short or conventional DP (Rastani et al., 2005, De Feu et al., 2009, Van Knegsel et al., 2014), which potentially reduces the risk of metabolic diseases in early lactation (Butler et al., 1981, Collard et al., 2000, Ingvartsen et al., 2003). In this thesis, however, omitting the DP, compared with a short DP, did not reduce the incidence of metabolic disorders and diseases in early lactation (**Table 6.3**).

In previous studies, omitting the DP resulted in a numerically lower incidence of cows with mastitis (Rastani et al., 2005, Schlamberger et al., 2010) or ketosis (Rastani et al., 2005, Schlamberger et al., 2010, Vanholder et al., 2015), a numerically greater (Rastani et al., 2005) or lower (Schlamberger et al., 2010) number of cows with retained placenta, and a numerically greater number of cows with displaced abomasum (Rastani et al., 2005), compared with a short or conventional DP. Additionally, a meta-analysis reported that shortening and omitting the DP tended to reduce the risk of ketosis in the subsequent lactation, but that shortening the DP did not alter the risk for mastitis, metritis, or reproductive failure in the subsequent lactation compared with a conventional DP (Van Knegsel et al., 2013). Based on the greater EB in cows with an omitted DP, positive effects on (metabolic) disease incidence can be expected, compared with cows with a short or conventional DP. However, the combination of low numbers of animals and low incidences of diseases made it difficult to estimate potential health effects in experimental datasets.

Dry period length and udder health

Different DP lengths did not affect udder health, but cows with a 0-d DP fed a standard energy level tended to have a greater hazard for a case of clinical mastitis than cows with a 30-d DP during the complete lactation (Van Hoeij et al., 2017). Cows with a 0-d DP had a greater SCC in week 1-44 postpartum than cows with a 30-d DP (Van Hoeij et al., 2017). Differences in postpartum SCC among DP lengths could be related to the use of dry cows' antibiotics or lower milk yields postpartum (Steeneveld et al., 2013), or an increase in IMI across the DP or pre-calving period. Until now, effects of DP length on udder health are not fully clear, studies reported contrasting results of the effect of DP length on SCC, new intramammary infections (IMI), and clinical mastitis postpartum in cows (Gulay et al., 2003, Rastani et al., 2005, Pinedo et al., 2011, Steeneveld et al., 2013, Shoshani et al., 2014).

Voluntary waiting period effects on disease incidence

Extending the lactation length of high-producing dairy cows was hypothesized to have beneficial effects on cow health by the reduction in the frequency of the transition periods (Knight et al., 2005) and therefore less risk for metabolic disorders and disease. In our study, however, the VWP did not have an effect on the number of cows treated for disease during the complete lactation (**Table 6.4**). In total, 90% of the cows were treated at least once for disease, with claw and leg problems being the main issue. In addition, there was no effect of VWP on the number of disease treatments during the period from 6 weeks after calving until 6 weeks after the next calving, including the transition period after an extended lactation length (Burgers et al., 2022). Numerically, cows with a VWP of 125 or 200 d had more diagnoses per lactation than cows with a VWP of 50 d. However, on a yearly basis veterinary treatments were numerically less for cows with extended VWP (Burgers et al., 2022). Considering the limited number of transitions and low incidence of diseases in the transition period, beneficial results could be expected in further study.

Table 6.3 Disease treatments in the first 14 weeks after calving for cows with different transition treatments[1]

	Transition treatment			Total
	0-d DP(LOW)	0-d DP(STD)	30-d DP(STD)	
Milk fever	4	4	2	10
Fever	2	7	7	16
Mastitis	13	17	10	40
Claw problems	17	17	15	49
Placenta	3	6	6	15
White flu	2	8	7	17
Endometritis	3	2	8	13
Cystic	8	7	14	29
Other	0	2	6	8
Total disease treatments	52	70	75	197
Cows, n	42	43	43	128

[1]Low: Energy level based on the requirement for the expected milk yield of cows with a 0-d DP; STD: Energy level based on the requirement for the expected milk yield of cows with a 30-d DP; The 3 treatments: cows with a 30-d DP and an STD dietary energy level (30-d DP(STD)), cows with a 0-d DP with the same STD dietary energy level as cows with a 30-d DP (0-d DP(STD)), and cows with a 0-d DP with a LOW energy level (0-d DP(LOW)).

Table 6.4 Cows treated for the disease in a complete lactation of cows with a voluntary waiting period (VWP) of 50, 125, or 200 d.

	VWP50	VWP125	VWP200	Total
Number of cows	54	49	51	154
Milk fever	8(14.8%)	11(22.4%)	9(17.6%)	28(18.2%)
Ketosis	1(1.85%)	1(2.04%)	3(5.88%)	5(3.25%)
Clinical mastitis	17(31.5%)	12(24.5%)	16(31.4%)	45(29.2%)
Retained placenta	5(9.36%)	3(6.12%)	8(15.7%)	16(10.4%)
Endometritis	15(37.8%)	15(30.6%)	19(37.3%)	49(31.8%)
Cystic ovaries	8(14.8%)	7(14.3%)	12(23.5%)	27(17.5%)
Claw and leg problems	22(40.7%)	29(59.2%)	27(52.9%)	78(50.6%)
Stomach and intestine problems[1]	13(24.1%)	17(34.7%)	17(33.3%)	47(30.5%)
Other[2]	11(20.4%)	16(32.7%)	12(23.5%)	39(25.3%)
Treated for any disease				
Yes	48(88.9%)	44(89.8%)	47(92.2%)	139(90.3%)
No	6(11.1%)	5(10.2%)	4(7.8%)	15(9.7%)

[1]Main stomach and intestine problems: rotavirus, diarrhea, peritonitis.

[2]Main diagnoses in 'other': fever, cobalt deficiency, 3 teats.

Voluntary waiting period effects on udder health

In the current study, extending the VWP did not affect the occurrence of SCC elevations and clinical mastitis per lactation or year (**Chapter 5**). Studies showed contrasting results of different VWPs on SCC and clinical mastitis postpartum of cows (Allore and Erb, 2000, Osterman et al. 2005, Pollott et al. 2011, Steeneveld et al., 2013, Niozas et al. 2019). Chapter 5 of this thesis focused on udder health, more specifically SCC, elevations of SCC, and cases of clinical mastitis, of cows subjected to a 50 d, 125 d, or 200 d VWP, in different lactation periods. In **Chapter 5**, we also showed the dry-off antibiotic usage for cows on lactation and yearly basis. There were no VWP effects on dry-off antibiotic usage on a lactation basis, but yearly usage of antibiotics at dry-off was lower in multiparous cows with a VWP of 200 d compared with multiparous cows with a VWP of 50 d. Reduction of antibiotics use is of importance not only as a reduction in veterinary costs but also to prevent the development of bacterial strains which are resistant to antibiotics (Kuipers et al., 2016, Vanhoudt et al., 2018). Moreover, recently, the preventive use of antimicrobials in the EU has been restricted (EC, 2/2015 299/04).

Figure 6.1 Somatic cell count (SCC; expressed as the natural logarithm) of cows with a voluntary waiting period of 50 d (VWP-50), 125d (VWP-125), or 200d (VWP-200) across lactation.

Primiparous cows had a lower SCC and a lower incidence of at least 1 elevation of SCC or at least 1 case of clinical mastitis than cows with greater parity (**Chapter 5**). Previous studies also showed that younger cows had better udder health than older cows (Schepers et al., 1997), which may be related to a better EB (**Chapter 2**), with greater plasma IGF-1 (**Chapter 2 and 4**) and lower plasma NEFA concentration (**Chapter 2**), the lower immunosuppressive effect of plasma NFFA on neutrophils (Ingvartsen and Moyes, 2013) and better epithelial integrity in young cows than in greater parity cows (Chen et al., 2015). Lower daily milk yield in young cows may also reduce the risk of damage to the teat sphincter and pathogens entering the teat canal, leaving more of the bacteriostatic keratin lining of the teat canal in place which has antibacterial activity and traps invading pathogens (Suriyasathaporn et al., 2000, Ingvartsen et al., 2003). Furthermore, the presence of high SCC (≥ 200,000 cells/mL) in the previous late lactation increases the risk for clinical mastitis in the following lactation (Pantoja et al., 2009). Cows with greater parity have experienced a period of late lactation more often, have a greater daily milk yield throughout lactation that may damage the teat sphincter or keratin lining, and, therefore, may have a greater risk for IMI in late lactation and thus greater risk for IMI in the subsequent lactation than cows with lower parity. Due to the greater risk for IMI in cows with greater parity, these cows may more often need treatment with dry cow antibiotics than cows with lower parity.

Different DPs and VWPs strategies applied to practice

Commercial dairy farmers have applied a short or 0-d DP on their farms (Santschi et al. 2011; Steeneveld et al., 2013). Dutch farmers that applied 0-d DP reported healthier cows, improved fertility, and a decreased workload because of the fewer dietary and group transitions (Steeneveld et al., 2013).

However, the main concern for farmers to apply a short or 0-d DP is milk yield losses in the subsequent lactation. In a meta-analysis, milk losses in the lactation following a short or 0-d DP were 4.5%, and 19%, respectively (Van Knegsel et al. 2013). Partly, milk losses in the subsequent lactation will be compensated by additional milk produced in the weeks before calving, and possibly by improved fertility and a shorter calving interval (Kok et al., 2016). Moreover, a reduction in disease occurrence and replacement rate may to some extent financially compensate for a reduction in milk yield (Kok et al., 2017).

Farmers that apply extended VWP reported multiple reasons for doing so (Burgers et al., 2020). First, managing cows for extended lactations would lead to fewer transition periods per cow per year and associated management labor (i.e., drying-off, calving, the start of lactation) and veterinary services associated with calving (Kok et al., 2019). Second, fewer calves born was a reason for farmers to extend the VWP. Fewer calves result in less income from calves sold, but it can be compensated or might be a benefit by a reduction in calf care costs (Nor et al., 2012) and labor related to calf care. Third, farmers expected a reduction in annual disease occurrence and associated veterinary costs for diseases specifically associated with the transition period (Lehmann et al., 2016). No effect of different VWP on clinical mastitis incidence was found in the current thesis, but the annual dry-off antibiotic use was lower for multiparous cows with a VWP-200 (**Chapter 5**). Similar to the DP strategy, milk yield losses are a possible negative issue for farmers to apply extended VWP. Extending VWP until 200 d for primiparous cows did not affect their daily milk yield, but multiparous cows with a 200-d VWP had a reduced milk yield per day of calving interval compared with cows with a 50-d VWP (Burgers et al., 2021a). Milk yield across studies was similar or reduced when VWP was extended (Reviewed by Van Knesgel et al., 2022).

Some cows may be more suitable for an extended VWP, or a short or 0-d DP, to limit milk losses and maximize the benefits of these strategies. Cows with a high risk of transition diseases may be suitable for short or 0-d DP, or an extended VWP, as these strategies would reduce the severity or frequency of the transition period. Cows with a high SCC in late lactation may benefit from a conventional dry period, and cows with a low milk yield in late lactation may not be able to have a short or 0-d DP (Kok et al., 2021). The different parity effects of different VWPs on milk yield may indicate the potential in selecting certain cows for extended lactation rather than all cows, especially for multiparous cows (Burgers et al., 2021b). Lehmann et al. (2016) reported that random selection of multiparous cows for extended lactation resulted in an average loss of milk, whereas selecting the most suitable cows allows milk production to be maintained. Persistent cows are suitable for extended lactation length to minimize milk loss (Burgers et al., 2019). Very persistent lactations could reduce milk losses, or possibly increase production, with an extended lactation (Arbel et al., 2001, Inchaisri et al., 2011, Kok et al., 2019). Other characteristics, including maximum milk yield, BCS, and BW, can also be used to determine for which cows to extend the lactation (Burgers et al., 2019). Together with these cow characteristics, a VWP based on milk level might contribute to an individual approach for extended VWP management to maximize the benefits of an extended VWP and minimize milk losses.

From the economic point of view, depending on different variables included, extending lactations had a positive or negative impact. Kok et al (2019) reported a negative effect of extended lactation lengths on the net partial cash flow of cows with a stochastic simulation model, based on revenues for milk, surplus calves, and culled cows, and costs for feed, artificial insemination, calving management and rearing of young stock, and the negative effect was larger for multiparous than for heifers. Arbel et al. (2001) found positive economic effects of extended lactation but did not include calves and changed labor. Arbel et al. (2001) selected only high-yielding cows to be extended in a confinement system. The economic impact may be differed by type of study, milk production level, calculation method, extended lactation strategy and time lasts of the application. Therefore, more studies are needed to evaluate the economic effect of implementing extended lactation thoroughly.

Knowledge gap

- The main reason to extend the VWP of dairy cows is the lower frequency of critical transition periods and associated diseases per unit time. However, we did not find an effect of VWP on the incidence of diseases and udder health in the current thesis. We followed only one lactation after extended VWP and selected the good health status of cows before they went into the experiment. Considering the study size and low incidence of transition diseases, a large and long-term controlled study with accurate monitoring of disease incidence could give more information on the relationships between VWP, disease occurrence, and productive lifespan.
- Extending the VWP results in different periconception and prenatal conditions, which were reported to have effects on the development and health in the early and later life of calves (Fleming et al., 2018). The effect of extending the VWP on the growth of calves is still unclear. Moreover, as discussed in the current thesis, dairy farmers get fewer calves born after applying an extended VWP, which may reduce the chances for genetic improvement and selection. This possible long-term effect remains to be studied.
- In this thesis, relationships between the inflammatory biomarkers and health status in early lactation were found. Some studies suggested that inflammation plays an important role in the etiology and results in health disorders in early lactation (Dubuc et al., 2010, Qu et al., 2014), but it is also proposed that subclinical disorders before disease diagnosis could cause an increase in inflammatory mediators, in which case the inflammatory state would be an effect of the disease rather than a cause (Mayasari et al., 2017). Further research can study the cause-effect relation between inflammatory biomarkers and disease, and models can be made to predict health status by inflammatory biomarkers in early lactation.

Conclusion

This thesis evaluated the consequences of shortening or omitting the DP and extending the VWP for the fertility and health status of dairy cows. Relationships between inflammatory biomarkers, metabolism, and health status in early lactation were studied (chapter 2), followed by consequences of DP treatments on fertility and associated metabolic status in early lactation (Chapter 3). Last, the consequences of extending the VWP on fertility (Chapter 4) and udder health status (Chapter 5) of dairy cows were explored.

In the first 6 weeks of lactation, a more severe NEB was related to a lower DMI, lower levels of IGF-1 and insulin, and higher levels of NEFA and BHB in plasma. The occurrence of clinical health problems in the first 6 weeks was related to lower levels of albumin and paraoxonase, a lower LAI, and a higher level of haptoglobin in plasma in weeks 1, 2, and 4 of the lactation.

For cows in parity ≥ 3, omitting the DP together with a reduced dietary energy level improved fertility, which is indicated by fewer days open compared with cows fed standard energy level, together with a 30-d or 0-d DP. Fewer days open was related to fewer days to onset of luteal activity and fewer insemination services. Fewer days to onset of luteal activity were associated with a better energy balance and better metabolic status in early lactation.

Extending the VWP from 50 d to 200 d reduced days open after the end of the VWP, which may be related to the improved ovarian cyclicity in the 100 days around the end of the VWP. The improved ovarian cyclicity of cows in VWP-200 was indicated by a higher percentage of normal ovarian cycles and a lower percentage of prolonged ovarian cycles compared with cows in VWP-50. In addition, extending the VWP from 50 to 125 or 200 days decreased the milk yield and FPCM with 4 weeks around the end of the VWP, but the lower milk yield could only explain the reduced days open after the end of VWP for cows with a 200-d VWP to a limited extent.

Extending the VWP did not have a risk on udder health, as VWP did not affect SCC in the complete lactation, nor the occurrence of SCC elevations and clinical mastitis per lactation or year. Extending the VWP reduced milk yield in the 9 weeks prior to dry-off, but did not affect SCC, or the occurrence of SCC elevations or mastitis in this period. Dry-off antibiotic usage per year was reduced in VWP-200 compared with VWP-50 for multiparous cows. Therefore, extending the VWP may be used to reduce the frequency of transition periods and the associated use of dry-cow antibiotics with limited impact on udder health.

References

Ancker, S., Nordberg, E., Callesen, H., 2006. Vidensyntese Reproduktion. Rapport nr 113. Dansk Kvæg, Skejby.

Allore, H.G., Erb, H.N., 2000. Simulated effects on dairy cattle health of extending the voluntary waiting period with recombinant bovine somatotropin. Prev Vet Med. 46, 29-50.

Arbel, R., Bigun, Y., Ezra, E., Sturman, H., Hojman, D., 2001. The Effect of Extended Calving Intervals in High Lactating Cows on Milk Production and Profitability. J. Dairy Sci, 84, 600-608.

Beam, S.W., Butler, W., 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. Suppl. 54, 411–424.

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.

Bell, A., Slepetis, R., Ehrhardt, U., 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci. 78, 1954-1961.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88, 2017-2026.

Bertoni, G., Trevisi, E., Han, X., Bionaz, M., 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91, 3300-3310.

Bionaz, M., Trevisi, E., Calamari, L., Librandi, F., Ferrari, A., Bertoni, G., 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90, 1740-1750.

Booth C. J., L. D. Warnick and Y. T. Gröhn. (2004). Effect of Lameness on Culling in Dairy Cows. J. Dairy Sci, 87(12), 4115-4122.

Britt, J. H., Kittok, R. J., Harrison, D. S., 1974. Ovulation, estrus and endocrine response after GnRH in early postpartum cows. J. Anim Sci. 39, 915-919.

Burgers, E., Goselink, R., Kemp, B., Van Knegsel, A., 2020. Reducing the number of critical transitions for dairy cows: effects on milk yield and health. Pages 226-226 in Proc. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science.

Burgers, E., Kok, A., Goselink, R., Hogeveen, H., Kemp, B., Van Knegsel, A., 2021. Fertility and milk production on commercial dairy farms with customized lactation lengths. J. Dairy Sci. 104, 443-458.

Burgers, E., Kok, A., Goselink, R., Van Knegsel, A., 2019. Customising lactation length: impact of calving interval, parity, and lactation persistency on milk production of dairy cows. Pages 148-148 in Proc. Proceedings of the 17th International Conference on Production Diseases in Farm Animals.

Butler, W., Everett, R., Coppock, C., 1981. The relationships between energy balance, milk production and ovulation in postpartum Holstein cows. J. Anim Sci. 53, 742-748.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83:211- 218.

Carlen, E., Strandberg, E., Roth, A., 2004. Genetic parameters for clinical mastitis, somatic cell score, and production in the first three lactations of Swedish Holstein cows. J. Dairy Sci. 87, 3062-3070.

Chen, J., Remmelink, G., Gross, J. J., Bruckmaier, R., Kemp, B., van Knegsel, A., 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.

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.

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., 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.

Eckersall, P. D., Bell, R., 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The Vet. J. 185:23-27.

Fouladi-Nashta, A. A., Gutierrez, C. G., Gong, J. G., Garnsworthy, P. C., Webb, R., 2007. Impact of dietary fatty acids on oocyte quality and development in lactating dairy cows. Biol Reprod. 77, 9-17.

Gruys, E., Obwolo, M.J., Toussaint, M.J., 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. Veterinary Bulletin, 64, 1009-1018.

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.

Gutiérrez-Yurrita, P. J., Montes, C., 1999. Bioenergetics and phenology of reproduction of the introduced red swamp crayfish, Procambarus clarkii, in Do· ana National Park, Spain, and implications for species management. Freshwater Biology. 42, 561-574.

Huszenicza, G., Jánosi, S., Kulcsár, M., Kóródi, P., Reiczigel, J., Kátai, L., Peters, A., De Rensis, F., 2005. Effects of Clinical Mastitis on Ovarian Function in Post-partum Dairy Cows. Reprod. Domest. Anim. 40, 199–204.

Inchaisri, C., Jorritsma, R., Vos, P. L. A. M., van der Weijden, G. C., Hogeveen, H., 2011. Analysis of the economically optimal voluntary waiting period for first insemination. J Dairy Sci. 94, 3811-3823.

Ingvartsen, K.L., Dewhurst, R.J., Friggens, N., 2003. On the relationship between lactational performance and health: Is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livest Prod Sci 83, 277-308.

Ingvartsen, K. L., Moyes, K., 2013. Nutrition, immune function and health of dairy cattle. Animal, 7,112-122.

Inskeep, E. K., Dailey, R. A., 2005. Embryonic death in cattle. Veterinary Clinics: Food Animal Practice. 21, 437-461.

Ireland, J., Roche, J., 1983. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles. J. Anim Sci. 57, 157-167.

Jacometo, C. B., Osorio, J. S., Socha, M., Corrêa, M. N., Piccioli-Cappelli, F., Trevisi, E., Loor, J. J., 2015. Maternal consumption of organic trace minerals alters calf systemic and neutrophil mRNA and microRNA indicators of inflammation and oxidative stress. J. Dairy Sci. 98, 7717-7729.

Kok, A., Chen, J., Kemp, B., Van Knegsel, A., 2019. Dry period length in dairy cows and consequences for metabolism and welfare and customised management strategies. Animal. 13, s42-s51.

Kok, A., van Hoeij, R., Kemp, B., van Knegsel, A., 2021. Evaluation of customized dry-period strategies in dairy cows. J. Dairy Sci. 104, 1887-1899.

Kok, A., van Hoeij, R. J., Tolkamp, B. J., Haskell, M. J., van Knegsel, A., de Boer, I. J., Bokkers, E. A., 2017. Behavioural adaptation to a short or no dry period with associated management in dairy cows. Appl Anim Behav Sci. 186, 7-15.

Kok, A., van Middelaar, C., Engel, B., van Knegsel, A., Hogeveen, H., Kemp, B., de Boer, I., 2016. Effective lactation yield: A measure to compare milk yield between cows with different dry period lengths. J. Dairy Sci. 99, 2956-2966.

Konvičná, J., Vargová, M., Paulíková, I., Kováč, G., Kostecká, Z., 2015. Oxidative stress and antioxidant status in dairy cows during prepartal and postpartal periods. Acta Veter Brno. 84, 133-140.

Kuipers, A., Koops, W., Wemmenhove, H., 2016. Antibiotic use in dairy herds in the Netherlands from 2005 to 2012. J. Dairy Sci. 99, 1632-1648.

LeBlanc, S., Lissemore, K., Kelton, D., Duffield, T., Leslie, K., 2006. Major advances in disease prevention in dairy cattle. J. Dairy Sci. 89, 1267-1279.

Lehmann, J.O., 2016. Extended lactation in danish dairy production. PhD dissertation, Aarhus University.

Leroy, J., Vanholder, T., Opsomer, G., Van Soom, A., de Kruif, A., 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 Ddomest Anim. 41, 119-123.

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, 277-1293.

Lucy, M., Staples, C., Thatcher, W., Erickson, P. S., Cleale, R., Firkins, J., Clark, J., Murphy, M. R., Brodie, B., 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 Sci. 54, 23-331.

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.

Niozas, G., Tsousis, G., Malesios, C., Steinhöfel, I., Boscos, C., Bollwein, H., Kaske, M., 2019. Extended lactation in high-yielding dairy cows. Ii. Effects on milk production, udder health, and body measurements. J. Dairy Sci. 102, 811-823.

Nor, N. M., Steeneveld, W., Mourits, M., Hogeveen, H., 2012. Estimating the costs of rearing young dairy cattle in the Netherlands using a simulation model that accounts for uncertainty related to diseases. Prev Vet Med 106, 214-224.

Österman, S., Östensson, K., Svennersten-Sjaunja, K., Bertilsson, J., 2005. How does extended lactation in combination with different milking frequencies affect somatic cell counts in dairy cows? Livest Prod Sci. 96, 225-232.

Pantoja, J., Hulland, C., Ruegg, P., 2009. Dynamics of somatic cell counts and intramammary infections across the dry period. Prev Vet Med. 90, 43-54.

Pascottini, O. B., Leroy, J. L., Opsomer, G., 2020. Metabolic stress in the transition period of dairy cows: Focusing on the prepartum period. Animals 10, 1419.

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.

Petersen, H. H., Nielsen, J. P., Heegaard, P., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35, 163-187.

Petersson, K.-J., Strandberg, E., Gustafsson, H., Berglund, B., 2006. Environmental effects on progesterone profile measures of dairy cow fertility. Anim. Reprod. Sci. 91, 201–214.

Pollott, G., 2011. Do holstein lactations of varied lengths have different characteristics? J. Dairy Sci. 94, 6173-6180.

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.

Pushpakumara, P., Gardner, N., Reynolds, C., Beever, D., Wathes, D., 2003. Relationships between transition period diet, metabolic parameters and fertility in lactating dairy cows. Theriogenology. 60, 1165–1185.

Rasby, R., Wettemann, R., Geisert, R., Wagner, J., Lusby, K., 1991. Influence of nutrition and body condition on pituitary, ovarian, and thyroid function of nonlactating beef cows. J. Anim. Sci. 69, 2073-2080.

Rastani, R. R., Grummer, R. R., Bertics, S. J., Gumen, A., Wiltbank, M. C., Mashek, D. G., Schwab, M. C., 2005. Reducing dry period length to simplify feeding transition cows: Milk production, energy balance, and metabolic profiles. J Dairy Sci, 88,1004-1014.

Santos, J., Rutigliano, H. M., Sá Filho, M., 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci.110, 207-221.

Santschi, D.E., Lefebvre, D., Cue, R., Girard, C., Pellerin, D., 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.

Schepers, A., Lam, T., Schukken, Y., Wilmink, J., Hanekamp, W., 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J. Dairy Sci. 80, 1833-1840.

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.

Shrestha, H.K., Nakao, T., Suzuki, T., Higaki, T., Akita, M., 2004. Effects of abnormal ovarian cycles during pre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. Theriogenology. 61, 1559–1571.

Simpson, R., Chase, C., Spicer, L., Vernon, R., Hammond, A., Rae, D., 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. Reprod.102, 483-492.

Smith, M.C.A., Wallace, J.M., 1998. Influence of early post partum ovulation on the re-establishment of pregnancy in multiparous and primiparous dairy cattle. Reprod. Fertil. Dev. 10, 207–216.

Sordillo, L. M., Contreras, G., Aitken, S. L., 2009. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Animal health research reviews. 10, 53-63.

Staples, C., Thatcher, W., Clark, J., 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73, 938-947.

Steeneveld, W., Schukken, Y.H., Van Knegsel, A., Hogeveen, H., 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.

Sundrum, A., 2015. Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. Animals. 5, 978-1020.

Suriyasathaporn, W., Heuer, C., Noordhuizen-Stassen, E. N., Schukken, Y. H., 2000. Hyperketonemia and the impairment of udder defense: a review. Vet. Res. 31, 397-412.

Thatcher, W., Wilcox, C., 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 56, 608-610.

Trevisi, E., Amadori, M., Cogrossi, S., Razzuoli, E., Bertoni, G., 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res. Vet. Sci. 93, 695-704.

Van Altena, S., 2016. Biomarkers and mechanisms of natural disease resistance in dairy cows. Wageningen University and Research.

Van Hoeck, V., Sturmey, R. G., Bermejo-Alvarez, P., Rizos, D., Gutierrez-Adan, A., Leese, H. J., Bols, P. E., Leroy, J. L., 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. PloS one. 6, e23183.

Van Hoeij, R., Dijkstra, J., Bruckmaier, R., Gross, J. J., T. Lam, Remmelink, G., Kemp, B., Van Knegsel. A., 2017. The effect of dry period length and postpartum level of concentrate on milk production, energy balance, and plasma metabolites of dairy cows across the dry period and in early lactation. J. Dairy Sci. 100, 5863-5879.

Van Knegsel, A., Burgers, E. E., Ma, J., Goselink, R., Kok, A., 2022. Extending Lactation Length: Consequences for Cow, Calf and Farmer. J. Anim. Sci.

Van Knegsel, A., Remmelink, G., Jorjong, S., Fievez, V., Kemp, B., Van Knegsel, A.T., 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.

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.

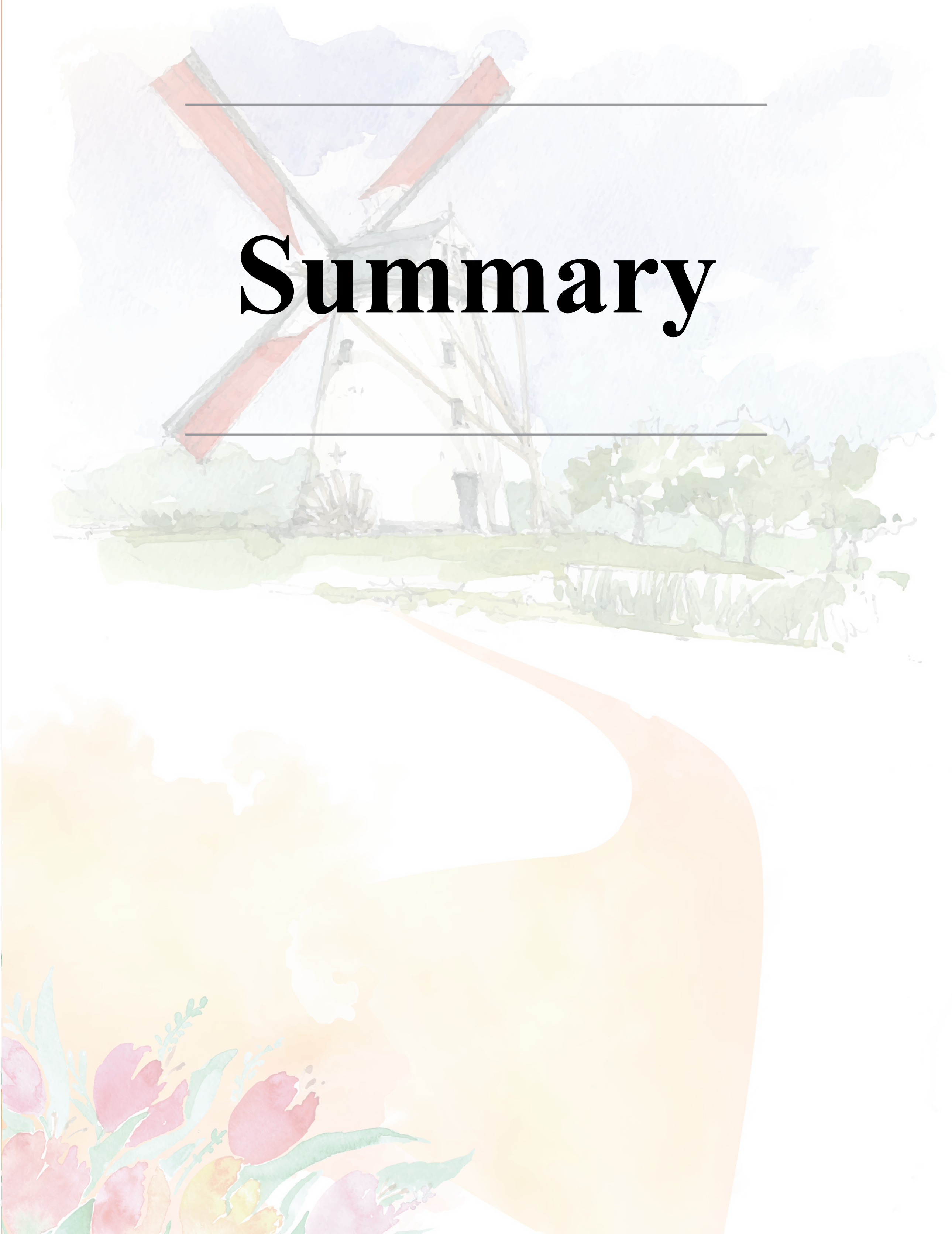
Vanholder, T., Papen, J., Bemers, R., Vertenten, G., Berge, A., 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J. Dairy Sci. 98, 880-888.

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.

Zulu, V. C., Sawamukai, Y., Nakada, K., Kida, K., Moriyoshi, M., 2002. Relationship among insulin-like growth factor-I, blood metabolites and postpartum ovarian function in dairy cows. J. Vet. Med. Sci. 64, 879-885.

6

Summary



Modern dairy cows are managed to have a typical one year lactation cycle, with a lactation length of 305 d and a dry period (DP) length of 60 d, which is considered to result in the greatest economic output. Within such a lactation period, cows have a 40-60 d voluntary waiting period (VWP) from calving until the first insemination to allow them to recover from calving and start of the lactation. However, it can be questioned whether one year is the ideal length of a lactation cycle for modern dairy cows. Nowadays, high milk yield at dry-off has become a challenge to udder health and cow welfare. Also, a yearly calving event is associated with a yearly peak in risk for disease related with both the calving process and the start of a new lactation. The start of a new lactation, with a high milk production and a limited dry matter intake is accompanied by a negative energy balance (NEB). Altered metabolic and inflammatory status play a role in the relationship between NEB and an increased risk for diseases in early lactation. Shortening and omitting the DP can reduce the NEB in early lactation through a reduced milk production with similar feed intake, but increase the risk of fattening in the subsequent lactation. Adjusting the dietary energy level to correct for lower milk production in cows without a DP was reported to result in less body weight gain and fewer days open compared with cows fed a standard energy level. However, the underlying relationships between metabolic changes and fertility remain to be elucidated. Extending the VWP is another strategy hypothesized to improve fertility, because at the delayed insemination moment milk production is lower and energy balance is better than earlier in lactation. It is unclear how extending the VWP would affect udder health of cows, as udder health might be negatively affected by longer lactations, but positively affected by a lower milk yield at dry-off. Both strategies, shortening the DP and extending the VWP, are expected to improve cow health, because they reduce the NEB in early lactation (after no or a short DP) or the frequency calving events (with extended VWP).

The aims of this thesis were 1) to evaluate relationships between inflammatory biomarkers, metabolic status and clinical health problems in early lactation, 2) to evaluate effects of omitting the DP in cows fed 2 different dietary energy levels in early lactation on fertility, 3) to evaluate consequences of extending the VWP on fertility of dairy cows, and 4) to evaluate effects of extending VWP period on udder health of dairy cows.

To study these aims, two large experiments were conducted. Chapter 3 describes experiment 1. In experiment 1, 128 cows were assigned randomly to 1 of 3 transition treatments with either a 0-d DP or a 30-d DP. Cows with a 0-d DP were fed either a low energy level, which was based on the energy requirements for their expected milk yield [0-d DP(LOW)] (n = 42) or a standard energy level [0-d DP(STD)] (n = 43) based on the requirement for the expected milk yield of cows with a 30-d DP. Cows with a 30-d DP were fed a standard energy level based on the requirement for the expected milk yield of cows with a 30-d DP [30-d DP(STD)] (n = 43). Chapter 2, 4 and 5 describe experiment 2. In experiment 2, 154 cows were assigned randomly to a VWP of 50-d (VWP-50) (n =54), 125-d (VWP-125) (n = 49), or 200-d (VWP-200) (n =51). Cows in the 3 treatment groups were inseminated at the first detected estrous after their VWP.

Relationships between inflammatory biomarkers, metabolism, energy balance and health status of cows in early lactation were described in **Chapter 2**. Cows were grouped into three groups based on their health status in the first 6 weeks postpartum. Cows in the first group were cows that were treated for clinical health problems associated with an inflammatory response (CHP) (endometritis, fever, clinical mastitis, or retained placenta). The second group consisted of cows that were only treated for health problems not associated with an inflammatory response, described as other health problems (OHP) such as milk fever, vaginal discharge, cystic ovaries, claw and leg problems, stomach and intestine problem, cobalt deficiency, or cows with 3 teats. The third group were cows were not treated for any health problems (NHP). Subsequently cows were clustered based on NEB status in first 6 weeks postpartum within 4 clusters (SP: stable positive cluster; MN: mild negative cluster; IN: intermediate negative cluster; SN: severe negative cluster). Then we assessed the relations of the defined health groups and EB clusters with metabolic variables and biomarkers for inflammation, liver function, and oxidative stress. In early lactation, cows with clinical health problems had a lower concentration of albumin, paraoxonase, a lower liver activity index (LAI), and a higher concentration of haptoglobin in plasma. Moreover, CHP cows had higher concentrations of glucose, insulin and IGF-1 in plasma in the first week of lactation. Cows in more severe NEB clusters had was significantly related to metabolic status, milk yield, and DMI, but NEB cluster only tended to be related to inflammatory biomarkers, liver index and oxidative stress.

In **chapter 3**, ovarian cyclicity and reproductive performance of cows without a dry period with a standard or low energy level was compared with cows with a short dry period and a standard dietary energy level. Cows with 0-d DP (LOW) were reported to have fewer days open compared with cows with 0-d DP (STD) or 30-d DP (STD). Ovarian cyclicity for cows in 0-d DP (LOW) was improved compared with cows in 0-d DP (STD) or 30-d DP (STD). Older cows (parity ≥ 3) with 0-d DP (LOW) had a better fertility through a reduced interval from calving to onset of luteal activity (OLA), a reduced number of services per conception, and, consequently, a reduced number of days open compared with cows with 0-d DP (STD) or 30-d DP (STD). Fewer days open was associated with fewer days to OLA, fewer services per conception, and better metabolic status in week 1 till 7 of lactation.

Consequences of extending VWP on fertility were presented in **chapter 4**. Cows with VWP-200 had an improved fertility compared with cows with VWP-50, as indicated by a greater percentage of normal ovarian cycles and a lower percentage of prolonged ovarian cycles in the 100 d around the end of the VWP, and fewer days open after the end of the VWP. In addition, cows with an extended VWP (VWP-125 and VWP-200) had a lower milk yield and FPCM around the end of VWP compared with cows with VWP-50. In conclusion, extending the VWP from 50 to 125 or 200 days resulted in a greater percentage of normal ovarian cycles and a lower milk yield around the end of VWP, but only VWP-200 reduced days open after the end of the VWP.

Chapter 5 describes udder health of cow after a 50, 125, or 200-d VWP. The SCC, SCC elevations and incidence of clinical mastitis were analyzed as health status variables. An elevation of SCC in milk was defined as SCC ≥200,000 cells/mL after two previous weeks with SCC <200,000 cells/mL. Extending the VWP did not affect the occurrence of SCC elevations or clinical mastitis per lactation or per year. Extending the VWP reduced milk yield in the 9 weeks prior to dry-off, but did not affect SCC, or the occurrence of SCC elevations or mastitis in the same period. Dry-off antibiotic usage per year was reduced in multiparous cows with VWP-200 compared with VWP-50 because of the lower frequency of dry periods. In the first 6 weeks of the next lactation, SCC was increased after an extended lactation with VWP-200 compared with VWP-50, while no effect on the number of SCC elevations or the occurrence of clinical mastitis was observed. This study showed that extending the VWP may be used to reduce the frequency of dry periods and calving events and the associated usage of dry-cow antibiotics with limited impact on udder health.

In the general discussion in **Chapter 6**, current finding are discussed in a broader perspective and the potential of application of the different DP and VWP strategies are highlighted. To conclude, occurrence of clinical health problems in early lactation was associated with changes in inflammatory biomarkers and metabolic status. A more severe NEB was related with greater NEFA and BHB concentrations, and lower glucose, IGF-1, and insulin, but was only to a limited extent related with inflammatory biomarkers. Omitting the dry period together with a reduced dietary level reduced days open for older cows, which was related with fewer days to OLA and fewer services per conception. Fewer days to OLA was associated with better metabolic status in early lactation. Extending the VWP from 50 d to 200 d improved ovarian cyclicity in the 100 days around the end of the VWP and reduced days open after the end of the VWP.

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About the author

Curriculum Vitae

Junnan Ma was born on 5 June 1990 in Zhoukou, Henan, China. In September 2010, she started BSc at College of Animal Science, Jiangxi Agriculture University, Nanchang. In 2014, she started MSc in Animal Feed Institute, Chinese Academy of Agriculture Science, Beijing, under the supervision of Prof. Dr. Yan Tu and Prof. Dr. Qiyu Diao. In May 2017, she obtained her MSc degree. In March 2018, she started her PhD entitled "Customised lactation length: Reproduction and health in dairy cows with different lactation lengths" at the Adaptation Physiology Group of Wageningen University, the Netherlands. The outcomes of her research are presented in this thesis.

Publications

Refereed Scientific Journals

Dong, L., **J Ma**, Y Tu, and Q. Diao. 2019. Weaning methods affect ruminal methanogenic archaea composition and diversity in Holstein calves. Journal of Integrative Agriculture 18(5):1080-1092.

Ma, J., R. J. van Hoeij, R. M. Bruckmaier, A. Kok, T. J. Lam, B. Kemp, and A. T. van Knegsel. 2020. Consequences of transition treatments on fertility and associated metabolic status for dairy cows in early lactation. Animals 10(6):1100.

Piao, M., **J. Ma,** Q. Diao, and Y. Tu. 2021. Effects of diets with different solid-to-liquid feed ratios with the same dry matter intake on the growth performance and gastrointestinal development of male Holstein calves. Animal Feed Science and Technology 274:114846.

Ma, J., E. E. Burgers, A. Kok, R. M. Goselink, T. J. Lam, B. Kemp, and A. T. van Knegsel. 2022. Consequences of extending the voluntary waiting period for insemination on reproductive performance in dairy cows. Animal Reproduction Science 244:107046.

Ma, J., E. E. Burgers, A. Kok, R. M. Goselink, T. J. Lam, B. Kemp, and A. T. van Knegsel. 2022. Effect of extended voluntary waiting period from calving until first insemination on udder health. Journal of Dairy Research. Accepted.

Expected Journal publications

Ma, J., E. E. Burgers, A. Kok, F. Vossebeld, E. Saccenti, R.M. Bruckmaier, R. M. Goselink, E. Trevisi, A. Minuti, T. J. Lam, B. Kemp, and A. T. van Knegsel. Relations between inflammation biomarkers and energy balance status in early lactation of dairy cows. To be submitted.

WIAS Training and Supervision Plan

The Basic Package (2 ECTS[1])	Year
WIAS Introduction Day	2018
Course on Philosophy of Science and/or Ethics	2018
Disciplinary Competences (6 ECTS)	
Writing own PhD Proposal	2018
Successful Dairy Heifer Rearing-feeding and Management	2018
Professional Competences (11 ECTS)	
The Essentials of Scientific Writing and Presenting	2018
Brain Training	2018
PhD Workshop Carousel	2018
Effective Behaviour in Your Professional Surroundings	2018
Bridging across Cultural Differences	2018
Project and Time Management	2018
Stress Identification & Management	2018
Supervising MSc and BSc student	2018
Critical thinking and Agrumentation	2019
Reviewing a Scientific Paper	2019
Research Data Management	2019

Career Perspective	2021
Effective and Efficient Communication in Academia and Beyond	2022
Mobilising Your Scientific Network	2022
Societal Relevance (1 ECTS)	
Organisation of "Lunch meetings in Adaptation Physiology group	2021-2022
Presentation Skills (4 ECTS)	
European Bovine Congress (EBC), oral presentation	2019
International Conference on Production Disease in Farm Animals (ICPD), oral presentation	2019
International Conference on Production Disease in Farm Animals (ICPD), poster	2020
American Dairy Science Association Annal meeting (ADSA), oral presentation	2021
Teaching competences (6 ECTS)	
Lecture in MSc course- Health, Welafre and Management (ADP-30306)	2019
Supervise Msc project of Adaptation Physiology group (ADP-30806)	2021
Msc thesis- Tamaki Uyama	2019
Msc thesis- Hanneke de Cock	2019
Bsc thesis- Noelle van Dijck	2020
Bsc thesis- Joas van der Linder	2021
Total ECTS	**30**

[1]One ECTS credit equals a study load of approximately 28 hours

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