More milking days with lower yields

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Sustainability impacts of short or no dry periods in dairy cows

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Sustainability impacts of short or no dry periods in dairy cows

Akke Kok

Thesis

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Abstract

Farmers traditionally stop milking a cow 6 to 8 weeks before next calving. This 'dry period' (DP) maximises milk production in the next lactation. The resulting high milk production in early lactation, however, results in a negative energy balance and is associated with reduced health and fertility. Shortening or omitting the DP improves the energy balance in early lactation at the cost of milk production. This project aimed to evaluate and integrate sustainability impacts of shortening or omitting the DP, with a focus on cow welfare, cash flows at farm level, and greenhouse gas (GHG) emissions per unit milk. Welfare was addressed by monitoring lying and feeding behaviour of 81 cows with no DP or a 30-day DP in weeks -4 and 4 relative to calving. On average, cows with no DP had a 1 hour per day shorter lying time in week -4 than cows with a DP, but the absolute daily lying time (12.6 h) and relatively constant feeding rate suggest that welfare of cows with no DP was not impaired by milking in late gestation. Moreover, cows with no DP had a 1 hour longer lying time and a greater feed intake in week 4 of lactation, suggesting a better adaptation to the start of the next lactation. The number of meals, feed intake, and lying time of dairy cows were associated with physiological indicators of high metabolic load during this period. To compare milk yield between cows with different DP lengths, accounting for extra milk before calving and possible changes in calving interval, the 'effective lactation yield' measure was developed. The impact of DP length on effective lactation yields of second and greater parity cows was assessed over multiple lactations. The reduction in effective lactation yield compared with a standard DP was larger for no DP than for a short DP, and did not differ between the first and a subsequent shortening or omission of the DP, although the timing of milk yield changed. The overall impact of DP length on milk production, cash flows and GHG emissions were modelled based on production data of dairy farms that voluntarily managed cows for a short or no DP. First, introduction of no DP resulted in a dip in milk production of the herd in the second year the strategy was applied. On average over 5 years, applying a short DP reduced milk yield of the herd by 3.1%, and applying no DP reduced milk yield of the herd by 3.5%. Moreover, short and no DP reduced partial cash flows by €12 and €16 per cow per year, and increased GHG emissions per unit milk by 0.8% and 0.5%, respectively. These relatively small negative impacts of short and no DP on cash flows and GHG emissions can be offset by improved cow health and lifespan, which could result from the improved energy balance in early lactation (more pronounced for no DP than for a short DP) when these strategies are adopted. In conclusion, both shortening and omitting the DP can improve cow welfare with a small negative impact on cash flows and GHG emissions, which may be offset by improved cow health.

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Chapter 1

General introduction

1 Background

In 1953, average milk yield in the Netherlands was the highest of all countries, with about 4,000 litres per lactation (of about 305 days) (Ashton, 1956). The average lactation production of Dutch dairy cows increased to 9,442 kg in 351 days in 2016, of which 8,658 kg were produced in the first 305 days of lactation (CRV, 2017) (Figure 1). This increase in milk production per cow was the result of breeding, feeding and management, which was largely driven by economic incentives to reduce the costs per kg of milk. High milk production, however, can have implications for the welfare of dairy cows (Webster, 2000; Butler, 2003; Ingvartsen, 2006; Zobel et al., 2015). Good welfare has been defined as functioning well (e.g. good health), feeling well and being able to express natural behaviour (Fraser et al., 1997). Both functioning and feeling of dairy cows could be affected by high milk production, especially in early and late lactation, as described in the next section.



Figure 1: Average 305-day yield (kg milk) and calving interval (days) of recorded Dutch dairy cows from 1985 to 2016. Data from (CRV, 2017).

1.1 Consequences of high milk production

Early lactation

After calving, milk production and energy requirements increase rapidly, while feed intake lags behind (Huzzey et al., 2007). A cow thus faces an energy deficit, or negative energy balance (NEB) in the first months of lactation and uses her own body reserves to provide energy for milk production. A higher genetic merit for milk production is associated with a greater difference between energy intake and energy requirements and thus a greater NEB (Veerkamp, 1998). As a result, high-producing dairy cows mobilise body fat reserves and lose weight in the first 3 months of lactation (Rastani et al., 2005; Van Knegsel et al., 2014b).

The mobilisation of body fat to meet energy requirements results in an elevated concentration of free fatty acids (FFA) in the blood, which increases the risk of the metabolic disorders ketosis and fatty liver (Ingvartsen, 2006). A severe NEB and ketosis also are associated with an increased risk of other diseases, such as metritis, mastitis and lameness (Heuer et al., 1999; Collard et al., 2000; Berge and Vertenten, 2014; Esposito et al., 2014). Incidence of disease in dairy cows, therefore, is highest in the first weeks after calving, when diseases related to the calving process occur (dystocia, retained placenta, metritis) and when the increase in milk production and the NEB are greatest (Ingvartsen, 2006).

Moreover, high milk production and NEB are associated with reduced fertility (Lucy, 2001). A more severe NEB is associated with a longer period from calving to first ovulation, less regular ovarian cycles (i.e. short or long cycles) and a reduced conception rate (Butler, 2003; Chen et al., 2015b). Reduced fertility increases the period from calving to conception and consequently increases the average calving interval. In the Netherlands, the average calving interval increased from 390 days in 1995 to 413 days in 2016, with a peak of 418 days in 2010 (CRV, 2017) (Figure 1). Reduced fertility results in economic losses, because milk yield is lower in late lactation and because cows that do not become pregnant are culled (Inchaisri et al., 2010b). Reduced fertility is a reported reason for culling dairy cows in 18% to 35% of all culling (Pinedo et al., 2010; Brickell and Wathes, 2011).

Although we cannot ask a cow how she feels, it has been hypothesised that a high-producing cow does not feel well during early lactation (Webster, 2000; Roche et al., 2009; Oltenacu and Broom, 2010). Prolonged NEB could cause a feeling of hunger, and the physiological stress of high production may result in metabolic or physical exhaustion (Webster, 2000). In addition, it has been hypothesised that cows with a severe NEB have to spend more time feeding and ruminating, which

could constrain time for other behaviours, such as resting (Roche et al., 2009; Oltenacu and Broom, 2010).

Late lactation

Dairy cows also face significant transitions in physiology and management at the end of lactation, in preparation for the next lactation. Before subsequent calving, cows are generally subjected to a non-lactating or 'dry period' (DP) of 6 to 8 weeks. This DP facilitates the renewal of udder cells (Capuco et al., 1997), and maximises milk production in the next lactation (Kuhn et al., 2005; Van Knegsel et al., 2013). Also, the DP is used to cure intramammary infections at dry-off with antibiotics (Bradley and Green, 2001).

The cessation of milking at the start of the DP results in udder pressure, because the milk is produced but not removed from the udder (Zobel et al., 2015). A higher milk yield at dry-off increases udder pressure, milk leakage and stress levels (blood cortisol and faecal glucocorticoid metabolites) (Odensten et al., 2007; Bertulat et al., 2013), and increases the risk of intramammary infections at calving (Rajala-Schultz et al., 2005). Moreover, high udder pressure at dry-off and intramammary infections can be painful for dairy cows (Webster, 2000; Leitner et al., 2007; Bertulat et al., 2013; Zobel et al., 2015). The impact of the cessation of milking can be reduced by gradual cessation of milking and a low-energy ration to reduce milk yield prior to dry-off (Valizaheh et al., 2008; Tucker et al., 2009; Zobel et al., 2013).

On top of the physiological challenge of drying off, the DP generally involves changes in routine, diet and social group. These stressors can negatively affect feed intake, resting behaviour, health and feelings (Ingvartsen, 2006; Schirmann et al., 2011; Zobel et al., 2013, 2015; Chapinal et al., 2014). Not being milked is a major change in daily routine for the cow. Moreover, cows are commonly switched to a low-energy 'far-off' ration at the start of the DP, then to a medium-energy 'close-up' ration one month later and then back to a lactation ration upon calving (Rastani et al., 2005; Pezeshki et al., 2007; Santschi et al., 2011a). The different rations are often provided in different pens; therefore dietary change often requires regrouping of cows. After calving, cows have to adapt to being milked, the lactation ration and the lactating group again, which could reduce feed intake and slow recovery from NEB during early lactation (Huzzey et al., 2005; Rastani et al., 2005).

1.2 Consequences of shortening or omitting the dry period

Several strategies have been suggested to improve energy balance and cow health during early lactation. Energy balance can be improved through greater energy intake (Reist et al., 2003) or through reduced energy requirement for milk production. Reducing milk production during early lactation can be realised through breeding or through management, such as once-daily milking during early lactation (Patton et al., 2006; Schlamberger et al., 2010) or shortening or omitting the DP (Rastani et al., 2005; Van Knegsel et al., 2014b). Alternatively, frequency of calving and the associated NEB can be reduced by extending lactation length (Knight, 2001; Dobson et al., 2007; Lehmann et al., 2014). This thesis focuses mainly on the consequences of shortening or omitting the DP. The following 2 sections, therefore, give an overview of the consequences of shortening or omitting the DP during early and late lactation.

Early lactation

Shortening the DP in practice often implies a DP of 3 to 5 weeks instead of 6 to 8 weeks, whereas omission of the DP implies continuous milking (Santschi et al., 2011a; Steeneveld et al., 2013). By shortening or omitting the DP, the number of milking days before calving increases, whereas milk production directly after calving decreases (Rastani et al., 2005; Van Knegsel et al., 2014b). As a result, energy requirements partly shift from the months after calving to the months before calving. A recent meta-analysis estimated the average decrease in milk production after calving at 1.4 kg per day (-4.5%) in the case of a short DP of 4 to 5 weeks, and at 5.9 kg per day (-19%) in the case of no DP, compared with a conventional DP (Van Knegsel et al., 2013). With an improved or similar feed intake during early lactation, a short and no DP reduced the duration of the NEB from 3 months to about 2 months for a short DP and to less than 1 month for no DP (Rastani et al., 2005; Van Knegsel et al., 2014b). The severity of the NEB, which was greatest in week 2 after calving for all DP lengths, was reduced by 20-30% for a short DP and by 70-90% for no DP (Rastani et al., 2005; Van Knegsel et al., 2014b).

The improved energy balance in cows managed for a short or no DP results in an improved metabolic status and fertility, indicated by lower concentrations of FFA in plasma, increased incidence of normal resumption of ovarian cyclicity and fewer days from calving to first ovulation and conception (Andersen et al., 2005; Gümen et al., 2005; Watters et al., 2008, 2009; De Feu et al., 2009; Chen et al., 2015a; b). Improvements in fertility due to a short or no DP were not found in all studies (Pezeshki et al., 2007). Moreover, potential improvements in disease incidence during early lactation after a short or no DP are difficult to assess in experiments due to the small number

of animals, and remained unclear in a meta-analysis (Van Knegsel et al., 2013). In three experiments, however, ketosis did not occur in cows with no DP, whereas it did occur in 4.8% to 19% of the cows with a standard DP (Rastani et al., 2005; Schlamberger et al., 2010; Köpf et al., 2014).

Late lactation

Shortening or omitting the DP not only affects energy balance in early lactation, but also reduces the impact and number of changes in management in late gestation. A short DP, with the same calving interval, is achieved by more milking days and thus can be expected to result in a lower milk production at dry-off. This reduces udder pressure and the risk of new intramammary infections at dry-off (Rajala-Schultz et al., 2005; Bertulat et al., 2013). In addition, a short DP can be executed with only two, or no ration changes, whereas a standard DP often involves three ration changes from late gestation to calving (Rastani et al., 2005; Santschi et al., 2011a). No DP requires neither dry-off nor ration changes. The cow simply continues the standard milking routine and lactation ration and can remain in the milking herd during late gestation until she is moved to a calving pen.

Milk production in the 4 to 8 extra weeks of lactation averaged 426 kg (15 kg per day) in the case of a short DP and 762 kg (14 kg per day) in the case of no DP, and cows remained in a positive energy balance before calving (Rastani et al., 2005; Van Knegsel et al., 2014b). The positive energy balance indicates that cows with a short or no DP have an adequate feed intake before calving. Time for feeding and being milked two or three times a day, however, could constrain resting time during late gestation for cows with no DP.

1.3 Impact of shortening or omitting the dry period on sustainability

Shortening or omitting the DP could affect the sustainability of dairy farming. Key sustainability issues in Dutch dairy farming include animal welfare, economic viability and environmental impacts (Van Calker et al., 2005; LTO Nederland, 2011). Good animal welfare is important not only for cows but also for the image and social acceptability of dairy farming (Keeling, 2005; Van Calker et al., 2005). Economic viability of a dairy farm is necessary to sustain the livelihood of farmers. Environmental impacts should be minimised to ensure the long-term viability of the biophysical system (Fischer et al., 2007). Minimising the environmental impacts of milk production implies minimising emissions into the air, water and soil, and using scarce natural resources in an efficient manner (De Boer, 2003). A major environmental challenge is climate change (Steffen et al., 2015),

induced by the emission of greenhouse gases (**GHG**). In Europe, the dairy sector is responsible for about 30-40% of the GHG emissions associated with the livestock sector (Lesschen et al., 2011; Weiss and Leip, 2012). The Sustainable Dairy Chain (*'Duurzame Zuivelketen'*), a joint initiative between the Dutch Dairy Association (NZO) and the Dutch agricultural sector (LTO Nederland), aims for a 20% reduction of GHG by 2020 relative to 1990 (Reijs et al., 2016).

As described above, shortening or omitting the DP improves metabolic status and may improve the welfare of dairy cows, but at the cost of milk production during early lactation. This milk loss will be partly compensated by the extra milk produced before calving. Improved health and fertility, furthermore, could reduce the incidence of culling. These changes affect the production of milk and meat (from culled cows and calves), feed use and youngstock rearing. Consequently, shortening and omitting the DP can affect farm profitability and GHG emissions from milk production.

It is unknown how shortening or omitting the DP affects these sustainability issues, i.e. whether multiple issues are improved (synergies), or one issue is improved at the cost of another (trade-off). For informed decision-making by farmers, it is relevant to have a holistic assessment of the impact of a change in DP length. This thesis focuses on the impact of shortening or omitting the DP on animal welfare, farm income and GHG emissions in the Dutch dairy sector. The next section describes what is known about the effects of shortening or omitting the DP with regard to these sustainability issues.

2 Knowledge gaps

2.1 Effect of dry period length on animal welfare

Shortening or omitting the DP can affect multiple aspects of animal welfare (i.e. good health, feeling well, natural behaviour). The main motive to shorten or omit the DP is to improve energy balance and metabolic status during early lactation. As a result, assessments of the effects of DP length on welfare aspects have thus far mainly focused on good health, understood via energy balance and metabolic status (Gümen et al., 2005; Rastani et al., 2005; Van Knegsel et al., 2014b; Chen et al., 2015a). The impact of DP length on disease incidence is not clear from the literature (Van Knegsel et al., 2013). Multiple studies focused on the aspect of feeling well in relation to drying off and assessed the short-term impacts of drying off on udder pressure and behaviour (e.g. lying, feeding, steps, vocalisations) (Leitner et al., 2007; Valizaheh et al., 2008; Tucker et al., 2009; Zobel et al., 2013; Chapinal et al., 2014). The idea of shortening or omitting the DP raised at least three

additional welfare concerns. First, the feeling aspect of welfare may be negatively affected when the cow has no DP, and consequently no non-productive period to rest. Second, udder health may be negatively affected by the absence of the non-productive period to recover. Third, the health implications of short and no DP for the unborn and new-born calf were unknown. To address the first concern, this thesis focuses on longer-term impacts of having or not having a DP on cow behaviour during late gestation and early lactation, and its association with energy balance and metabolic status. Behavioural and physiological parameters are used as indirect measures of how an animal feels (Broom, 1996). Consequences for udder and calf health are not studied in this thesis but are discussed in Chapter 9.

On the one hand, effects of shortening or omitting the DP on cow welfare are expected to be positive, based on improved biological functioning during early lactation (better energy and metabolic status, improved fertility), drying off at a lower milk production or not at all, and fewer changes in routine that can act as stressors (Rastani et al., 2005; Van Knegsel et al., 2013; Zobel et al., 2015). Lower energy requirements could also reduce feeding time and thereby ease time constraints during early lactation.

On the other hand, lactating during late gestation will increase metabolic activity in the last months of pregnancy, and milking itself can put pressure on the time budget of dairy cows during late gestation (Gomez and Cook, 2010). Despite the fact that cows with no DP maintain a positive energy balance before calving (Rastani et al., 2005; Van Knegsel et al., 2014b), feeding and milking time could constrain lying time and might negatively affect the feeling aspect of welfare (Munksgaard et al., 2005). Effects of DP length on feeling, beyond the short-term impact of drying off, have not yet been studied.

2.2 Effect of dry period length on income

Shortening or omitting the DP will have economic consequences. From the outset of this project the assumed trade-off was that shortening or omitting the DP of dairy cows improves energy balance at the cost of milk production. Lower milk production due to a short or no DP could lower milk revenues and feed costs. Improved metabolic status, moreover, could reduce treatment costs, youngstock rearing costs and revenues from culled cows. Some studies evaluated economic impacts of shortening or omitting the DP (Sørensen et al., 1993; Santschi et al., 2011b; Heeren et al., 2014; Köpf et al., 2014). A short DP, compared with a standard DP, had a positive (Santschi et al., 2011b) or negative economic impact (Sørensen et al., 1993) depending on its impact on milk revenues. Omitting the DP always had a negative impact on milk revenues, which was outweighed by a relatively large reduction in culling rate (37% to 24%) (Heeren et al., 2014) or veterinary costs (-€91 per lactation) (Köpf et al. 2014).

Economic evaluations of DP length appeared very sensitive to the effect of DP length on milk yield, culling and disease incidence, which emphasises the importance of an accurate estimate of these effects. The accuracy of current comparisons of milk yield between cows or herds with different DP lengths, however, could be improved. For example, the effect of DP length on milk yield in the presented economic evaluations did not always account for changes in calving interval. An improved fertility in case of a short or no DP (Gümen et al., 2005; Watters et al., 2009; Chen et al., 2015b) could shorten the calving interval, and thereby reduce the days of lower production during late lactation. Also, the effect of DP on milk yield was always based on the lactation after the first time the DP was shortened or omitted, whereas it is so far unknown if the effect remains the same when the DP is shortened or omitted over multiple lactations.

An accurate estimate of consequences of shortening or omitting the DP on milk yield should ideally be derived from a large dataset. At the lactation level, this estimate should account for the additional milk in the extra lactation days before calving, and for the duration of the calving interval (Figure 2). Over multiple lactations, milk yield of heifers is not affected by DP length, and the impact of DP length on milk yield could be different for second parity than for older cows (Pezeshki et al., 2007; Santschi et al., 2011a) or when the DP is shortened or omitted over multiple lactations. At the herd level, the overall milk yield depends on herd composition (i.e. the parity distribution) and herd dynamics. Herd composition could be affected by DP length through improved health and lifespan. Herd dynamics, such as culling, may have a different impact on milk yield when the timing of milk yield changes due to a short or no DP.



Figure 2: Schematic lactations of a cow with a conventional DP (solid line) and a cow with no DP (dashed line) for 4 calendar years; from 1st calving until some months into the 4th lactation. No DP, compared with a standard DP, results in a lower milk yield (with identical absolute decline) and a shorter calving interval from the second lactation onwards.

2.3 Effect of dry period length on greenhouse gas emissions

The impact of shortening or omitting the DP on GHG emissions per unit of milk will depend, among other variables, on overall milk yield, changes in feed use and changes in health and lifespan of the dairy herd. A reduction in overall milk yield, with the same maintenance requirements per cow, could increase feed requirements and GHG emissions per unit of milk produced (Garnsworthy, 2004; Van Middelaar et al., 2014). Production and utilisation of feed are responsible for a major portion of GHG emissions along the milk production chain (Lesschen et al., 2011; Gerber et al., 2013). In addition to feed quantity, feed composition can have an important influence on the amount of GHGs produced (Van Middelaar et al., 2013). Shortening or omitting the DP could be accompanied by a change in overall feed composition, mainly because a lower daily milk yield could be matched by a reduction in energy density of the lactation ration (Garnsworthy, 2004; Van Hoeij et al., 2017). Shortening or omitting the DP could also increase the lifespan of dairy cows, as a consequence of reduced NEB and improved metabolic status (Rastani et al., 2005; Chen et al., 2015a). An increased lifespan would dilute the amount of GHG emissions related to the rearing phase per unit milk (Van Middelaar et al., 2014). To our knowledge, no evaluation of the impact of DP length on GHG emissions of milk production has been made.

3 Aim

The aim of this thesis is to evaluate and integrate sustainability impacts of short or no DP in dairy cows, with a focus on cow welfare, cash flows and GHG emissions. To this end, the objectives are to:

- assess effects of short or no DP on cow behaviour;
- develop a measure to compare milk yield of cows with different DP lengths;
- compare milk yield of cows with different DP lengths over multiple lactations;
- estimate effects of short or no DP on milk yield and cash flows at herd level and GHG emissions per unit milk;
- compare short and no DP with extended lactations, an alternative solution to the NEB.

4 Outline of the thesis

The outline of the thesis is visualised in Figure 3. Chapters 2, 3 and 4 focus on the dairy cow. In Chapter 2, a sensor is validated that is used in Chapter 3 to measure lying behaviour. In Chapter 3, lying and feeding behaviour of dairy cows with short or no DP is described, and in Chapter 4, associations between behaviour and metabolic status in these cows are assessed. The behavioural and physiological measures together are used as indicators for welfare. Chapter 5, 6 and 7 focus on the impacts of DP length on milk production, cash flows and GHG emissions. A new method to compare milk production of cows with different DP lengths and calving intervals is demonstrated in Chapter 5 and applied in Chapter 6 to assess milk production of cows with different DP lengths over multiple lactations. Results of Chapter 6 are incorporated in Chapter 7 in a simulated dairy herd, to evaluate cash flows and GHG emissions of dairy herds with conventional, short and no DP. The model of Chapter 7 is adapted in Chapter 8 to assess the cash flows and GHG emissions of dairy herds with extended calving intervals, in order to compare short or no DP with an alternative solution to the NEB in early lactation. In Chapter 9, the assessed sustainability impacts of short or no DP in dairy cows are integrated, and trade-offs and synergies are discussed.



Figure 3: Outline of the thesis.

Chapter 2

Validation of sensor-recorded lying bouts in lactating dairy cows using a 2-sensor approach

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Abstract

Lying behaviour is a relevant indicator for the evaluation of cow welfare. Lying can be recorded automatically by data loggers attached to one of the hind legs of a cow. A threshold for the duration of a lying bout (LB) record is required, however, to discard false records caused by horizontal leg movements, like scratching. Previously determined thresholds for similar sensors ranged from 25 s to 4 min. We aimed to validate LBs recorded by the IceQube sensor (with IceManager software) and to determine a threshold to distinguish true from false LB records in lactating dairy cows. A novel method of validation, that does not require time-consuming behavioural observations, was used to generate a larger dataset with potentially more incidental short LB records. Both hind legs of 28 lactating dairy cows were equipped with an IceQube sensor for a period of 6 days and used as each other's validation. Classification of LB records as true (actual LB) or false (recorded while standing) was based on three assumptions. First, all standing records (absence of LB records) were assumed to occur whilst standing. Second, false LB records due to short leg movements could not occur in both hind legs simultaneously. Third, true LBs only occurred if the LB records of the paired sensors coincided. False LB records constituted 4% of the records. Based on a maximum accuracy of 0.99, a minimum duration of LB records of 33 s was determined, implying a sensitivity of 0.99 and a specificity of 0.98. Applying this threshold of 33 s hardly affected estimates of daily lying time, but improved estimates of frequency and mean duration of LBs for individual cows. The importance of distinguishing short LBs was demonstrated specifically for detection of calving. The two-sensor approach, using sensor outputs on both hind legs as each other's validation, is a timeefficient method to validate LB records that can be applied to different sensors and husbandry conditions.

1 Introduction

Diseases, housing conditions, stocking density, temperature and several other factors can cause changes in lying behaviour (EFSA, 2009). Assessing lying behaviour, therefore, can yield insight into the welfare of dairy cows. Lameness, for example, has been associated with an increase in total lying time (Ito et al., 2010). Furthermore, cows that had a difficult calf delivery alternated between lying and standing more often, resulting in a higher number of lying periods or 'lying bouts' (LB) per day (Proudfoot et al., 2009).

Currently, lying behaviour can be assessed using continuous observations from video recordings or data from sensors. Sensors have the potential to record lying behaviour automatically, thus time-efficiently. In addition, increasing use of activity sensors for estrus detection leads to an increasing on-farm presence of sensors that could also record lying behaviour (Steeneveld and Hogeveen, 2015). Validation of the sensor output is necessary, however, to ensure that recorded data accurately reflect true behaviour. LB records have been validated against the golden standard of time-consuming behavioural observations to determine a threshold that retains true and discards false records (Trénel et al., 2009; Ledgerwood et al., 2010; Tolkamp et al., 2010; Mattachini et al., 2013). In other studies, however, thresholds are not used or not underpinned by scientific validations (Endres and Barberg, 2007; Ito et al., 2010; Blackie et al., 2011; Kokin et al., 2014).

IceTag sensors (IceRobotics, South Queensferry, UK), attached to one of the hind legs of a cow, have been used to record activity and lying behaviour by several research groups (Endres and Barberg, 2007; Tolkamp et al., 2010; Blackie et al., 2011; Mattachini et al., 2013). Tolkamp et al. (2010) validated IceTag LB records against continuous observations of late-pregnant beef cows. They transformed data about the percentage of lying and standing per min into lying episodes per s and defined a threshold of 4 min to discard false episodes. This threshold reduced the number of lying episodes with 62% to 88%. Later, IceTag-data was produced per s and a threshold of 25 s was validated for dairy cows by Mattachini et al. (2013). The new IceManager software (2010) for the IceTag and similar IceQube sensor, that replaced IceTagAnalyser software, automatically creates a separate file with recorded LBs. No LB record threshold has been formulated or validation has been performed for these LB records.

Thresholds for sensor output of lying behaviour have been validated, so far, against behavioural observations (Ledgerwood et al., 2010; Tolkamp et al., 2010; Mattachini et al., 2013). Because behavioural observations take time, however, datasets to validate sensor output are often small (Trénel et al., 2009; Mattachini et al., 2013). Incidental short LBs may not be observed frequently

enough in such a dataset to influence the threshold, while their detection will depend on it. Moreover, short LBs could be highly relevant to detect as indicator for acute discomfort or restlessness. Therefore, a larger dataset would be more suitable to establish an optimal threshold to ensure that sensor data accurately reflect lying behaviour.

We aimed to validate LBs recorded by the IceQube sensor and to determine an optimal threshold to distinguish true from false LB records in lactating dairy cows. Moreover, to generate a larger dataset that potentially includes more incidental short LBs, we used a time-efficient novel method of validation that does not require behavioural observations. In addition, we specifically analysed LB records of periparturient cows to illustrate the importance of detection of short LBs.

2 Experimental setup and data collection

In October and November 2014, data from 28 cows were obtained on the research farm 'Dairy Campus' in Lelystad (the Netherlands). Cows were housed in free stalls with mattress and saw-dust bedding and concrete slatted floors. They were milked twice daily and supplied with fixed amounts of concentrates and ad libitum roughage. Stage of lactation ranged from 20 to 133 days in milk (90 \pm 29, mean \pm SD). Parity ranged from 2 to 6 (3.3 \pm 1.1). Cows were equipped with two IceQube sensors (IceRobotics, UK) simultaneously. Sensors were attached to the left and the right hind leg of each cow, for a period of 7 days, yielding paired records per cow. On 7 November 2014, the research farm was declared to have a Salmonella outbreak. Two weeks before that, fever and diarrhea were already observed in the herd. It is unclear how many animals were infected. Diseases could affect total lying time, but are not expected to interfere with the recording and validation of LB data. Therefore, data from all cows were included. One cow died, resulting in only 1 complete day of recording.

To illustrate the importance of detecting short LBs, we analysed IceQube LB records around calving (2 days before until 2 days after calving) from another 6 cows, that calved on the research farm between August 2014 and July 2015.

IceReader (hardware; IceRobotics, UK) was used to download IceQube data and IceManager (software; IceRobotics, UK) processed these data into LB records, with a start date, start time (hh:mm:ss) and duration (s). Per sensor, a file with LB records and a file with the number of LBs recorded and lying time per day were produced. This output differs from earlier versions of IceTagAnalyser software, which only yielded the percentage of lying and standing recorded on a

per-min or per-s basis. The yet unvalidated LB records are referred to as raw LB records. Data were processed and analysed in SAS (version 9.3; SAS Institute Inc., Cary, NC). The analysis at LB record level included raw LB records collected from the 12 h following attachment of IceQube sensors, and from the subsequent 6 complete days. For analyses on a per-day level, only the 163 (27 cows 6 days and 1 cow 1 day) completely recorded days were included.

Classification of raw LB records as true (actual lying) or false (recorded while standing) LBs was based on three assumptions. First, all standing records (i.e. absence of a raw LB record between two consecutive raw LB records) were assumed to occur whilst standing (Tolkamp et al., 2010). Second, false LB records due to short leg movements from a vertical to a horizontal position could not occur in both hind legs simultaneously. Third, following from the first two assumptions, true LBs only occurred if lying was recorded on both hind legs, thus when LB records of the paired sensors coincided. To classify the raw LB records, all were combined in one file and sorted by cow, start date and start time. If the start date, start time and duration of a raw LB record by the sensor on the right hind leg (R) were identical to a raw LB record by the sensor on the left hind leg (L) of the same cow, they were classified as a true LB record, and assumed to correspond to a LB. However, start time and duration of coinciding raw LB records by R and L could differ slightly. This difference could be due to differences in leg movements when lying down and getting up, or could result from minor differences in the internal clock of both sensors. Therefore, the allowed difference in start time and duration was relaxed stepwise with 2 s, until 14 s, at which point all raw LB records that overlapped in time with raw LB records by the opposite sensor were classified as true. True LB records shorter than 30 s were manually checked to verify overlap of the records in time.

3 Data Analyses

After raw LB records were classified as true or false LBs, the duration in s was log_e-transformed. We subsequently determined the accuracy, sensitivity, specificity, and the positive and negative predictive value of thresholds for lying bout duration, that were increased stepwise from o by 0.5 log_e. Accuracy was defined as the sum of correctly discarded false LB records and correctly retained true LB records divided by the total amount of LB records (Weiss and Koepsell, 2014); sensitivity as the number of true LBs retained divided by the total number of true LBs; specificity as the number of false LBs discarded divided by the total number of false LBs; positive predictive value as the number of true LBs retained divided by the total number of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total number of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total number of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total number of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total number of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total number of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total number of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total amount of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total number of the total amount of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total amount of LB records retained; and negative predictive value as the amount of false LBs discarded divided by the total amount of LB records retained divided by the total number of the total amount of LB recor

discarded. Maximum accuracy was used to determine the most accurate threshold to distinguish true from false LB records.

To assess the effect of the determined threshold on estimates of lying time, number of LBs per day and LB duration (lying time per day / number of LBs per day), these variables were expressed per day. This was done by summing LB records per cow per leg per start date of the LB record. The lying variables were computed using the following criteria: all raw LB records, only records longer than 33 s, only records longer than 4 min and only records classified as true LBs. Lying variables were averaged per cow per sensor (n=56). We subsequently assessed the differences between variable estimates based on no threshold, thresholds of 33 s and 4 min and variable values computed from true LBs, using descriptive statistics.

To determine the difference in number of LBs per day around calving, repeated measures ANOVA and Bonferroni post-hoc tests were performed on LB records that exceeded the thresholds of 33 s and 4 min (PROC MIXED), in which measures of the subject cows (n=6) were compared between 5 subsequent days (-2 to 2 days relative to calving).

4 Results and discussion

On average, 12.1 ± 3.3 raw LB records were recorded per cow per day, which corresponded to a lying time of about 13 h per cow per day ($13:02 \pm 2:18$, hh:mm). The difference in number of raw LB records per day between R and L on the same cow ranged from 0 to 7. The dataset comprised 4,279 raw LB records. Only 307 of these raw LB records (7.2%) had a duration shorter than 4 min, of which more than 60% (196) did not exceed 30 s (figure 1).

Across cows, the number of true LBs varied from 3.5 to 27 per day (8.5 to16.5 when the two most extreme cows were excluded) and their duration ranged from 4 s to 4 h and 20 min. The number of false LB records averaged 0.5 and ranged from 0 to 3.3 per day. Within cows, the difference in false LB records between hind legs ranged from 0 to 1.6 records per day. False LB records (4.7%) included 177 short false LB records (from 1 to 50 s in duration), and 24 longer false LB records (> 20 min). The longer false LBs occurred in case one record from the sensor on one hind leg matched two subsequent records from the sensor on the opposite leg, separated by a non-lying period (of 5 to 13 s). These 24 LB records were not regarded as false, but were assumed to be 8 long true LBs that were falsely interrupted by leg movements while lying.



Figure 1. Duration and frequency of lying bout records. White bars represent true records, black bars represent false records.

As illustrated in figure 1, the duration distributions of false and true LBs overlapped. Therefore, no threshold could be defined that discarded all false and retained all true LBs. A LB record threshold of 33 s (table 1), however, yielded a maximum accuracy of 0.992, a sensitivity of 0.993 and a specificity of 0.977. This threshold retained 2.5% more LB records than the threshold of 4 min indicated by Tolkamp et al. (2010). The retained dataset still included 2.3% of the false LB records, which were outnumbered by true LB records of similar duration. In addition, the threshold of 33 s is close to the threshold of 25 s that was validated by Mattachini et al. (2013) and the sampling interval of 30 s in combination with a single-event data filter recommended by Ledgerwood et al. (2010).

Threshold		False	True	Acc	Sen	Sp	PPV	NPV	
Ln(LB(s))	LB (s)	records (n)	records (n)						
0	1	177	4102	0.959	1.000	0.000	0.959	NA ¹	
0.5	2	176	4102	0.959	1.000	0.006	0.959	1.000	
1	3	171	4102	0.960	1.000	0.034	0.960	1.000	
1.5	4	152	4101	0.964	1.000	0.141	0.964	0.962	
2	7	93	4098	0.977	0.999	0.475	0.978	0.955	
2.5	12	52	4092	0.986	0.998	0.706	0.987	0.926	
3	20	18	4082	0.991	0.995	0.898	0.996	0.888	
3.5	33	4	4073	0.992	0.993	0.977	0.999	0.856	
4	55	0	4056	0.989	0.989	1.000	1.000	0.794	
4.5	90	0	4036	0.985	0.984	1.000	1.000	0.728	
5	148	0	4000	0.976	0.975	1.000	1.000	0.634	
5.5	245	0	3967	0.968	0.967	1.000	1.000	0.567	
6	403	0	3906	0.954	0.952	1.000	1.000	0.475	

Table 1. False and true lying bout (LB) records retained (n) with increasing LB record thresholds and the associated accuracy (acc), sensitivity (sen), specificity (sp), positive predictive value (PPV) and negative predictive value (NPV).

 $^{1}NA = not applicable.$

Table 2 shows the effect of using different thresholds for LB record duration, i.e. no threshold, the determined threshold of 33 s, and a threshold of 4 min as determined by Tolkamp et al. (2010), on estimates of daily lying time, frequency of LBs and mean duration of LBs per cow. The choice of threshold (i.e. no, 33 s or 4 min) hardly affected estimates of daily lying time, i.e the average difference between estimates using no threshold and using a threshold of 4 min was 1 min only. However, the choice of threshold did affect estimates of frequency and mean duration of LBs.

Table 2. Difference between lying variable estimates per sensor per cow using no threshold, thresholds of 33 s and 4 min and variable values based on true lying bouts (LB).

	Δ total lying time (min/d)			Δ LB d	min/bout)	ΔΙ	Δ LB frequency (n/d)		
	all	33s	4min	all	33s	4min	all	33s	4min
mean	0	0	-1	-4	1	2	0.5	5 -0.1	-0.7
SD	0	0	4	10	2	3	0.7	0.1	1.8
min	0	0	-22	-59	-2	0	0.0) -0.5	-10.0
max	1	0	0	0	13	13	3.3	0.3	0.0

The mean duration of LBs was underestimated using no threshold, whereas it was overestimated by thresholds of 33 s and 4 min. This was caused by the estimates of LB frequency, which were overestimated up to 3.3 LBs per day using no threshold, whereas they were underestimated up to 0.5 LBs per day with a threshold of 33 s and 10 LBs per day with a threshold of 4 min. The proportion of false LBs (4.7%, mean), however, varied from zero to 49% between cows. As a result, the mean duration of LBs for individual cows was underestimated up to 59 min when no LB record threshold was applied (see table 3 for extreme cases). Both thresholds for LB record duration generally equalled or improved this estimate, with a maximum difference of 13 min between variable estimates and the true mean duration of LBs for both thresholds. Overall, the LB record threshold of 33 s obtained the most accurate and least biased estimates for all lying variables.

Table 3. Estimates of mean lying bout (LB) duration and frequency for right (R) and left (L) hind legs of individual cows, computed from true LB records (true), all LB records (all), and LB records that exceed thresholds of 33 s and 4 min. Only the data of 3 extreme individuals are presented for illustrative purposes.

		LB duration (h/bout)				LB freq	LB frequency (n/d)		
Cow	Leg	true	all	33s	4min	true	all	33s	4min
5177	L	2.02	1.29	2.13	2.24	3.5	5.5	3.3	3.2
5177	R	2.02	1.04	2.24	2.24	3.5	6.8	3.2	3.2
6356	L	1.43	1.18	1.43	1.46	9.3	11.3	9.3	9.2
6356	R	1.43	1.32	1.43	1.46	9.3	10.2	9.3	9.2
6459	L	1.28	1.18	1.28	1.28	10.0	10.8	10.0	10.0
6459	R	1.28	1.04	1.24	1.28	10.0	12.3	10.3	10.0

The distribution of LB records presented here is different from the results of Tolkamp et al. (2010), in which more than 80% of the lying episodes was shorter than 4 min. All these episodes were false in their experimental group of late-pregnant beef cows and resulted from leg movements such as scratching, from being displaced at the feeding rack and from teething problems such as loose wiring (B.J. Tolkamp and M.J. Haskell, SRUC, UK; personal communication). In our study, relatively few (7.2%) LB records had a duration shorter than 4 min, and about half of those were assumed to be true LBs. The great reduction in short LB records can probably be explained by the improved hardware, as reported by de Mol et al. (2013), and adjusted software that processes raw data into LBs.

Whether the shortest LB records classified as true indeed correspond to true LBs could be questioned, because our classification was not based on a golden standard but on the assumption that coinciding LB records only occur when the cow is lying. However, short LBs (< 4 min) have regularly been observed in the herd. That such short true LBs were not encountered in the study of Tolkamp et al. (2010) may be due to differences in lying behaviour of late-pregnant beef cattle and lactating dairy cows.

Short LBs could be informative for detection of restlessness, which could indicate issues such as (difficult) calving (Huzzey et al., 2005; Proudfoot et al., 2009) or other suboptimal conditions. In this study, the cow that died, for example, displayed restless lying behaviour on the day before she died, including 10 (out of 27) LBs between 33 s and 4 min in duration. For periparturient cows, the total number of LBs per day using a threshold of 33 s peaked at 27.3 ± 4.7 (mean \pm SD) on the day of calving, which was significantly higher than the number of LBs on the 2 days before and after calving (P < 0.003 for all contrasts of day -2, -1, 1 and 2 versus day of calving; figure 2). Using a threshold of 4 min, the number of LBs numerically peaked at 22.0 ± 3.3 on the day of calving, but this was not significantly different from days -2 and -1. The restlessness displayed by the cow that died and the periparturient cows would (at least partly) have been wrongly discarded by a threshold of 4 min, indicating the potential importance of having a threshold as small as possible to retain accurate information on true lying behaviour.

It was assumed in this study that the absence of LB records could only occur while standing, after findings of Tolkamp et al. (2010) that all short standing bout records in their study were true. The 8 interruptions of LB records in one hind leg while the sensor on the other leg recorded one LB, however, could be a false interruption of lying. If true standing, the cow would have stood up briefly while one hind leg remained in a horizontal position. False standing bouts may have been recorded due to movement of the leg while a cow was lying on her side. Cows have been observed to lie on their side and rest their hind leg on top of the cow in the adjacent cubicle in a diagonal position. Although this would violate our assumption, it remains a safe assumption to apply because these short interruptions occurred only incidentally.

Figure 2. Mean (and SEM) number of lying bout records per cow (n=6) per day from day -2 to 2 relative to calving, grouped into classes of duration.



The 2-sensor approach, using sensor outputs from opposite legs as each other's validation, is an easy and efficient method that can be applied to determine a threshold to distinguish true from false LB records. This in contrast to visual observations, which in case of continuous observations would be more accurate, but far more time-consuming. It would be interesting to see if the currently proposed threshold of 33 s performs well for other sensors and under different husbandry conditions. Housing and management conditions, such as deep litter systems or grazing, influence lying behaviour and movement of cows. A much larger overestimation of LBs was reported for grazing than for cubicle-housed cows (27 versus 1 LB in 4 h), for example, but the duration of the false LB records was not reported (Rutter et al., 2014).

Next to validation, the usage of 2 sensors at a time provides a whole new scope of possibilities. It enables a classification of true and false LB records that is more accurate than a general LB threshold for all animals. Furthermore, it gives insight in the frequency and duration of false LB records. These false records could be indicative for frequency of grooming behaviour, such as scratching and licking while standing on three legs, which may in turn reflect slipperiness of the floor (Platz et al., 2008) and mobility of the cows. Kokin et al. (2014) reported that the amount of LB records on the diseased hind leg of severely lame cows was more than twice the number recorded

on the healthy leg. Although severe lameness may be detected visually, a change in false LB frequency could potentially be an early warning signal.

5 Conclusions

A minimum duration of LB records of 33 s was determined for lactating dairy cows to filter false records and accurately measure lying behaviour using sensors. Applying this threshold is relevant for estimation of mean duration and frequency of LBs of individual animals. Short LB records could be indicative of restlessness due to calving or illness. Moreover, using two identical sensors per cow is a time-efficient method for sensor validation and threshold determination, and can potentially yield information on cow health and welfare.

Chapter 3

Behavioural adaptation to a short or no dry period with associated management in dairy cows

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Abstract

From calving, dairy cows are typically milked for about a year, and subsequently managed to have a non-lactating or 'dry period' (DP) before next calving. However, the use of a DP may reduce cow welfare because typical DP management involves the cow changing groups and ration. Also, the DP results in a severe negative energy balance after calving. Shortening or omitting the DP may have beneficial effects on cow welfare through fewer changes in management before calving, and a lower milk yield after calving. Our objective was to assess the effects of no DP and a short DP (30 days) with associated management on feeding, lying, and number of steps of dairy cows in late gestation and early lactation. Feeding behaviour was recorded by computerised feeders for 122 periods (42 with a short DP and 80 with no DP) from week -6 to week 7 relative to calving. Steps and lying behaviour of 81 of these cows (28 with a short DP and 53 with no DP) were recorded with accelerometers in week -4 and in week 4 relative to calving only. Effects of DP treatment and parity on behaviour were analysed with mixed models. Before calving, cows with a short DP were fed a DP ration, and moved to a dry cow group. During this time, cows with a short DP spent more time lying (13.7 vs. 12.6 h per day; P = 0.01) and feeding (240 vs. 209 min per day; P < 0.01), and stepped less (663 vs. 1130 steps per day; P < 0.01) than cows with no DP. After calving, all cows were fed the same lactation ration and were housed in the same herd. Cows with a short DP, however, had a lower feed intake (35.7 vs 39.1 kg per day; P < 0.01), and spent less time lying (10.7 vs. 11.6 h per day; P = 0.03) after calving than cows with no DP. Milk yield was negatively correlated with daily lying time (r: -0.22; P < 0.05), but was not correlated with daily feeding time. Also, less time was spent on both lying and feeding after calving than before calving. These results indicate that lying time was not constrained by feeding time. Lying time was positively correlated with energy balance (r: 0.28; P < 0.01). Compared with a short DP with associated ration and group changes, no DP reduced lying time and increased the number of steps in late gestation, and resulted in a higher feed intake and longer lying time in early lactation.
1 Introduction

The lactation cycle of dairy cows starts with calving. From calving, cows are typically milked for about a year, and subsequently managed to have a non-lactating or 'dry period' (DP) of 6 to 8 weeks before next calving. The DP allows for treatment of intramammary infections (Robert et al., 2006), facilitates the renewal of udder cells (Capuco et al., 1997), and maximises milk yield in the next lactation (Kuhn et al., 2005; Van Knegsel et al., 2013). The DP is generally considered a rest period for the cow that allows for reduced metabolic and physical activity in the last two months of pregnancy.

Whether a DP is beneficial for dairy cow welfare has been questioned (Zobel et al., 2015). Good welfare has been defined as feeling well, functioning well, and living a natural life (Fraser et al., 1997). Planned cessation of lactation, as well as being unnatural, was shown to increase udder pressure and stress (as measured by faecal glucocorticoid metabolites) at the start of the DP (Tucker et al., 2009; Bertulat et al., 2013). In addition, cows need to adapt to a new social environment and to dietary changes at the start and end of the DP, because they are typically moved to a non-lactating group and fed a dry cow ration (von Keyserlingk et al., 2008; Martens et al., 2012; Santschi and Lefebvre, 2014). After the DP, a higher milk yield is associated with a more severe negative energy balance (Rastani et al., 2005; Van Knegsel et al., 2014b). Such a negative energy balance is associated with impaired fertility and reduced metabolic health (Butler, 2003; Chen et al., 2015a; b), and may last until 3 months into lactation after a conventional DP (Rastani et al., 2005; Van Knegsel et al., 2014b). The prolonged lipolysis to meet energy needs may also result in exhaustion, and may have consequences for a cow's affective state (Webster, 2000; Roche et al., 2009).

Behavioural adaptation may not interfere with welfare as long as it is within the limits of the adaptive capacity of the animal (Korte et al., 2007). Behaviour of cows is affected by external factors (such as housing) and internal factors (such as behavioural needs). The behaviour patterns that are expressed are the result of these internal and external factors. Behaviour patterns can be assessed by examining the time budget and the temporal distribution of behaviours (Winter and Hillerton, 1995). Much of the time budget of dairy cattle is made up of lying, feeding, ruminating, and – in lactating cows – being milked (Gomez and Cook, 2010; Norring et al., 2012). The daily duration of these activities depends on factors such as housing, access to pasture, milking facilities, lameness, and stage of lactation (Krohn et al., 1992; Huzzey et al., 2006; Fregonesi et al., 2007; Gomez and Cook, 2010). In addition to changes in feeding time, cows were found to increase feeding rate when given limited access to resources (Munksgaard et al., 2005) and when lame (González et al., 2008).

Feeding behaviour is often recorded as visits to the feeder or feed bunk. Multiple visits that occur shortly after one another can be clustered into distinct feeding bouts or meals (Yeates et al., 2001; Tolkamp et al., 2002). Meals are biologically more relevant than visits to understand short-term feeding behaviour (Tolkamp et al., 2002). Cow welfare may be compromised when cows cannot adapt their behaviour to the circumstances, or if short-term behaviour patterns result in a long-term reduction of welfare. Increased standing time, for example, is observed in early lactation (Fregonesi and Leaver, 2001; Munksgaard et al., 2005), but (on hard surfaces) is a risk factor for lameness (Cook and Nordlund, 2009).

Shortening or omitting the DP may have beneficial effects on cow welfare (Zobel et al., 2015). Both strategies improve the energy balance after calving, through a reduced milk yield and equal or better feed intake after calving (Rastani et al., 2005; Van Knegsel et al., 2014b). Moreover, milk yield before dry-off is lower for a short DP than for a standard DP (Pezeshki et al., 2007), because milk yield decreases towards the end of lactation. A lower milk yield before dry-off reduces udder pressure and stress in the DP (Bertulat et al., 2013), and reduces the risk of intramammary infections after calving (Rajala-Schultz et al., 2005). Cows with no DP can be kept in the herd, without regrouping and dietary changes.

It is unclear how dairy cows adapt behaviourally to a DP, and how the absence of a DP affects their time budget. Our objective, therefore, was to assess the effects of a short and no DP with associated management on feeding, lying, and number of steps of dairy cows in late gestation and early lactation. To assess possible reasons for changes in behaviour, we also studied associations between behaviour, milk yield, and energy balance in early lactation.

2 Materials and methods

2.1 Experimental design, animals, and housing

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol in compliance with Dutch law on Animal Experimentation (protocol number 2014125). The experiment was conducted at the Dairy Campus research farm (Lelystad, the Netherlands) using 125 Holstein-Friesian cows between January 2014 and July 2015. The study was initially designed to analyse the effect of DP length and dietary energy source on energy balance and metabolic health; sample size was based on a power analysis for these variables. Cows were included in the experiment at an average rate of 3 cows per week, based on the availability of cows

in late gestation. Inclusion criteria were an expected calving interval shorter than 490 days, a milk yield of >16 kg and no clinical or subclinical mastitis (a cell count > 250.000 cells/ml) at 90 days before expected calving. For practical reasons, six cows were used twice in the experiment, resulting in data for 131 periods around calving (60 periods of cows in parity 1 before calving and 71 periods of cows in parity > 1 before calving).

Treatment groups were balanced for parity (1 or > 1 before calving), expected calving date, and milk production in the previous lactation. This was done by grouping cows that were most similar in these aspects together in groups of 6, and randomly assigning the cows of each group to no DP (n=87), or a short DP of 30 days (n=44). Twice as many cows were assigned to the no DP treatment because of an additional contrast in concentrate allowance (further details will be given below).

Cows entered the experiment on Mondays, 44 ± 3 days before the expected calving date, and were kept in the study until 305 days in milk. All cows were housed in the same freestall barn with a concrete slatted floor in all alleys, and stalls (1.25 m × 2.20 m) fitted with rubber mattresses (4 cm thick) covered with sawdust. Lactating and dry cows were kept in separate groups. The stocking density in both groups was maintained at one cow per cubicle and a maximum of two cows per feeding bin throughout the experiment, with a space allowance of 7 m² per cow.

The drying-off protocol for cows with a short DP consisted of an abrupt transition to the DP ration at day 7 before dry-off and an abrupt transition to milking once daily at day 4 before dry-off. Cows were dried off (i.e. milked for the last time) on Mondays, 30 ± 3 days before the expected calving date. At dry-off no antibiotics were used. Dry cows were weighed in the milking parlour on Tuesdays. Lactating cows were milked and weighed in the milking parlour twice daily at about 06.00 h and 17.00 h.

2.2 Feed composition and provision

During the DP, cows received a DP ration (estimated net energy (NE): 5.4 MJ per kg DM) that consisted of grass silage, maize silage, wheat straw, and rapeseed meal in a ratio of 48:19:25:8 (DM basis), and vitamins and minerals. Cows with no DP received a lactation ration (estimated NE: 6.4 MJ per kg DM) that consisted of grass silage, maize silage, wheat straw, soybean meal, and sugar beet pulp in a ratio of 45:35:2:8:10 (DM basis), and vitamins and minerals. After calving, all cows received this lactation ration up to 49 days in milk (DIM).

Basal rations were provided in roughage intake control (RIC) feeders (Insentec, Marknesse, the Netherlands). One RIC feeder was available per two cows. Rations were mixed once daily before 10.00 h and fed twice daily around 10.00 h and 17.00 h. The RIC feeders could not be accessed by the cows when feeders were filled and from 23.45 h to 0.00 h when data records were saved. Cows had free access to water, that was provided in valve trough drinkers placed in between feeding bins and quick drainage troughs of 150L at opposite sides of the barn. Because cow density was kept constant, lactating cows had access to 3 or 4 troughs and dry cows had access to 1 or 2 troughs depending on group size.

Concentrate was provided separately from the basal ration, and the concentrate allowance differed between treatment groups. Cows with a short DP were fed a standard amount of concentrate for their expected milk yield (Short DP STD), which was based on previous data in this herd with this specific DP management (Van Knegsel et al., 2014b). In that study, the mean daily milk yield in the 14 weeks after calving was 40.4 kg fat-and-protein-corrected milk (FPCM) for cows with a short DP and 35.4 kg FPCM for cows with no DP, compared with 43.3 kg FPCM for cows with a standard DP (Van Knegsel et al., 2014b). Cows with no DP were assigned either to the same concentrate level as cows with a short DP (No DP STD), or to a lower concentrate level that matched their expected milk yield (No DP LOW).

All cows received standard concentrate (869 g DM per kg; estimated NE: 7.4 MJ per kg DM) at a level of 1 kg per day from -10 ± 3 days before the expected calving date. The concentrate allowance increased stepwise by 0.3 kg per day from 1.0 kg per day at 4 DIM up to 8.5 kg per day at 28 DIM for the short DP STD and no DP STD treatments, and stepwise by 0.3 kg per day from 1.0 kg per day at 4 DIM up to 6.7 kg per day at 22 DIM for the no DP LOW treatment. Concentrate was provided by two computerised feeders located in the freestall (Manus VC5, DeLaval, Steenwijk, the Netherlands). The individual daily allowance of concentrate was available in equal portions (minimum portion size: 0.4 kg) over six 4-h periods, and the actual quantity dispensed (kg per day) was recorded. Uncollected concentrate portions in one timeslot were added to the portion in the next timeslot. Additionally, lactating cows received 0.5 kg of a standard concentrate (887 g DM per kg; estimated NE: 7.7 MJ per kg DM) when they were milked (i.e. 1.0 kg per day).

2.3 Measurements and data analysis

Feeding behaviour

For each visit to a feeder, RIC feeders recorded cow identity, the start time and end time (hh:mm:ss) of the visit, and the start weight and end weight of the feed in the feeder to the nearest 0.1 kg. Visit duration (s), feed intake (kg), and feeding rate (kg per min feeding) were calculated from these records. Concentrate feeders only registered the amount of concentrate collected per cow per day.

RIC data were analysed from 6 weeks before calving until 7 weeks after calving. In total, 9 cows were excluded from the analysis for various reasons: 5 cows were removed from the experiment before 7 weeks in lactation for health reasons (severe clinical lameness (2x), broken hip, 2 acute deaths without diagnosis at 3 and 10 days after calving), and 4 cows did not have the assigned DP length due to early calving in case of the short DP group (n=1) and spontaneous drying off (i.e. the cow stopped lactating despite twice-daily milking; n=3) in case of the no DP group. The RIC dataset consisted, therefore, of 122 13-week periods, with a total of 332,524 recorded visits to the RIC feeders.

Criteria were used to clean the dataset prior to analysis. Visits with a feeding rate > 2 kg per min were discarded (0.4% of records), because inspection of sequentially recorded visits to the same feeder suggested that these records were erroneous. In addition, visit duration was discarded for visits that lasted longer than 3 h and visits with feeding rates below 0.02 kg per min (0.1% of records). Inspection of these records suggested that the recorded feed intakes were genuine for these visits, as evidenced by sequentially recorded feeding bin weights, whereas visit durations were likely long because of failed registration of the end time of the visit.

Visits can be clustered into meals based on the interval length between visits. For dairy cows, the distribution of short intervals within meals and longer intervals between meals can be described by a three-population model, which uses a combination of two Gaussian distributions for the short intervals and one Weibull distribution for the longer intervals (for further details see: Yeates et al., 2001). A meal criterion can be estimated from this distribution, to classify intervals as within meal and between meal intervals in the most accurate way. When the interval between visits is shorter than the meal criterion, the visits belong to the same meal.

Visit records were used to compute intervals between subsequent visits for each cow. A threepopulation model was fitted to the frequency distribution of the log_e-transformed intervals between visits, and a meal criterion was estimated from this distribution (Yeates et al., 2001; Tolkamp et al., 2002, 2011). To assess whether separate meal criteria for treatment groups or periods relative to calving would be more appropriate than one single meal criterion for all treatments, nested models were constructed. Three nested models were produced to estimate separate meal criteria for 1) the three treatment groups (Short DP STD, No DP STD, and No DP LOW), 2) the two periods (before and after calving), and 3) each treatment × period interaction. Comparisons of the log-likelihoods of nested model fit. However, the resulting meal criteria were very similar between treatment groups before calving (18.1, 17.4, and 17.7 min) and after calving (21.9, 20.2, and 21.2 min). Therefore, it was decided to use one meal criterion before calving (18.0 min) and one meal criterion after calving (20.9 min), calculated from the pooled data. These meal criteria were used to cluster visits into meals. Duration of meals (meal duration), duration of visits within meals (feeding duration), number of visits per meal, and feed intake per meal were calculated, and secondary variables (e.g. daily feed intake, feed duration and feeding rate) were derived from these variables. Weekly means of feeding behaviour characteristics per cow per day were used for the analysis.

Mixed models were used to analyse the effect of fixed factors treatment, parity (1 or > 1 before calving), and week, as well as interactions of parity and week with treatment, on feeding behaviour (PROC MIXED procedure in SAS version 9.1; SAS Institute Inc., Cary, NC). The combination of cow identity and parity before calving was specified as repeated subject. No DP STD and No DP LOW were grouped together (No DP), because preliminary analysis showed no difference for this (P > 0.05). The covariance structure with the best model fit, based on the lowest Akaike's information criterion, was selected from unstructured, compound symmetry, and autoregressive covariance structures. Statistical significance (P < 0.05) of fixed effects was evaluated with approximate F tests (Kenward and Roger, 1997); treatment contrasts were compared using Wald tests.

Lying behaviour and steps

Lying behaviour and steps were recorded with triaxial accelerometers (IceQube, IceRobotics, South Queensferry, UK) from June 2014 until July 2015. Lying behaviour is recorded when the hind leg is in a horizontal position; the step count measures the number of times the animal lifts its leg up and places it back down again. The step count was used as indicator for walking activity, although stepping may also be recorded while standing in one place (e.g. during milking; Gygax et al., 2008). Sensors were attached to the left or right hind leg and detached on Thursdays between 10.00 h and 12.00 h. Previous research found no effect of sensor attachment to the left versus right hind leg on lying behaviour (Gibbons et al., 2012). Each cow was moved into a cubicle for the attachment of the

sensor. Because of limited sensor availability, lying behaviour and steps of cows were recorded for 6 complete days (Friday until Wednesday) at 4 weeks (26 ± 3 to 21 ± 3 days) before the expected calving date, and then again at 4 weeks (22 ± 3 to 27 ± 3 days) after calving only. Cows were regrouped and switched to a DP ration 11 days before the precalving measurement period, and dried off 4 days before the precalving measurement period. We therefore expect to measure few short-term behavioural responses to the change in ration, change in social environment, or the process of drying off (von Keyserlingk et al., 2008; Tucker et al., 2009). Lying behaviour and steps of 81 unique cows were recorded in both periods (n=26 for no DP STD; n= 27 for no DP LOW; and n= 28 for short DP STD), including only cows that were also included in the analysis of feeding behaviour.

Data were downloaded from IceQube sensors using IceReader, and processed by IceManager (both from IceRobotics, South Queensferry, UK) to produce two data files per cow per time period. One file contained all recorded lying bouts, with a start date, start time (hh:mm:ss) and duration (s); the other file consisted of recorded lying time (s), standing time (s), and number of steps per 15-min interval. Recorded lying bouts with durations shorter than 33 s were discarded as false lying bouts (Kok et al., 2015). The filtered data of lying bouts were used to compute the number of lying bouts per cow per day. Daily lying time and number of steps per cow per day were used for the analysis.

To analyse the effect of treatment (no DP or short DP), parity (1 or >1 before calving), and week on lying time, number of lying bouts, and steps, the same mixed model approach was used as for the analysis of feeding behaviour characteristics. No DP STD and No DP LOW were grouped together (No DP), because preliminary analysis showed no difference for this (P > 0.05).

The daily number of steps in the period before calving was compared between days of the week, to assess the impact of going through the milking parlour for weighing on Tuesdays for cows with a short DP. Per treatment, the mixed model included a fixed effect for day of the week (Friday through Wednesday), and a random cow effect. All weekdays were compared using pairwise Wald tests with Tukey-adjusted P-values, and the estimate statement was used to compare the number of steps recorded on Tuesdays with all other days.

Associations between behaviour, milk yield, and energy balance

To assess possible reasons for differences in behaviour, we analysed associations between behaviour, milk yield and energy balance at 4 weeks after calving. Milk yield was recorded daily. Energy balance was calculated according to the Dutch net energy for lactation (VEM) system (Van Es, 1975) as the difference between intake of VEM with the requirement of VEM for maintenance, milk production, and pregnancy (1,000 VEM = 6.9 MJ of NE). Energy balance was expressed in kJ per kg^{0.75} per day (Van Es, 1975). Computation of the energy balance required milk yield, milk composition, body weight of the cow, feed intake, and energy content of the feed. Milk samples for fat and protein analysis (ISO 9622, Qlip, Zutphen, the Netherlands) were collected for four subsequent milkings and were analysed as a weighted pooled sample per cow. RIC feeders recorded feed intake of the basal ration (kg) and concentrate feeders recorded the quantity of concentrates dispensed (kg) per cow per d. Feed intake was converted to energy intake using the dry matter content and net energy (NE) of each diet component.

Means and standard deviations of milk yield and energy balance in week 4 after calving were computed per DP treatment (no DP or short DP) per parity (2 or >2). Associations between variables were assessed with Pearson correlations. Significant correlations (r; P < 0.05) were interpreted as slight (< 0.2), low (0.2 - 0.4), moderate (0.4 - 0.7), high (0.7 - 0.9), or very high (> 0.9) (Martin and Bateson, 1993).

3 Results

3.1 The effect of a short or no dry period on feeding behaviour

Over the 6 weeks before calving, cows with a short DP and cows with no DP had on average 7 meals per day with 5 visits per meal from the RIC feeders (i.e. excluding concentrate; Table 1). Average meal duration (i.e. the time from the start of the first visit within the meal until the end of the last visit within the meal), however, was longer for cows with a short DP than for cows with no DP, which resulted in total meal times of 293 min per day for cows with a short DP and 255 min per day for cows with no DP (P < 0.01). The feeding duration (i.e. the time spent with head in the feeder) was about 80% of the meal duration for both treatments. Meal size (i.e. the feed intake per meal) and feed intake per day were smaller for cows with a short DP than for cows with no DP, and cows with a short DP had a lower feeding rate (all P < 0.01). Young cows (parity 1) had longer total feeding times and lower feeding rates than older cows (parity >1; P < 0.01; Figure 1c, 1d).



Figure 1. Basal ration intake (kg fresh feed per day; panel a), number of meals per day (panel b), total feeding time (min per day; panel c), and feeding rate (g per min feeding; panel d) of cows with a short DP and cows with no DP from 6 weeks before to 7 weeks after calving (means and SE). Panels c and d depict behaviour for young cows (parity 1 before calving) and older cows (parity > 1 before calving) separately. Dashed lines indicate cows are fed the DP ration, solid lines indicate cows are fed the lactation ration.

Over the 7 weeks after calving, cows with a short DP and cows with no DP had on average 8 meals per day with 4 visits per meal from the RIC feeders (Table 2). The number of meals per day and feeding rate peaked in the first week after calving, whereas total feeding time and feed intake were lowest in this week (Figure 1). Average meal duration and total meal time were not different between DP treatments after calving (P > 0.05). Meal size was not different between cows with a short DP and cows with no DP (P > 0.05), but feed intake per day was 3.4 kg (1.3 kg DM) per day lower for cows with a short DP than for cows with no DP (P < 0.01). Young cows (parity 1 before calving) with a short DP had longer total feeding times (P = 0.04) and lower feeding rates (P < 0.01) than young cows with no DP (Figure 1c, 1d).

	Short DP		No DP		P-value ¹		
Item ²	mean	SE	mean	SE	DP	Par	DP×Par
Cows (No.)	42		80				
Meals per day	6.7	0.2	7.3	0.1	< 0.01	0.95	0.21
Visits per meal	5.0	0.2	4.6	0.1	0.09	< 0.01	0.25
Meal duration (min per meal)	45	1	36	1	< 0.01	< 0.01	0.09
Total meal time (min per day)	293	7	255	5	< 0.01	< 0.01	0.35
Total feeding time (min per day)	240	6	209	5	< 0.01	< 0.01	0.14
Meal size (kg per meal)	4.4	0.1	5.8	0.1	< 0.01	0.25	0.75
Feed intake ² (kg per day)	28.6	0.6	41.7	0.5	< 0.01	0.20	0.72
Feeding rate (g per min feeding)	127	7	211	5	< 0.01	< 0.01	0.27

Table 1. Effect of dry period (DP) and parity (Par) on feeding behaviour of cows in the 6 weeks before calving (least squares means and SE).

¹Week and DP \times Week were always significant (P < 0.05)

²Feed intake refers to fresh feed intake from the basal ration (i.e. concentrate intake is not included). Cows with a short DP were provided with a DP ration, whereas cows with no DP were provided with a lactation ration.

Table 2. Effect of dry period (DP) and parity (Par) on feeding behaviour of cows in the 7 weeks after calving (least squares means and SE).

	Short DP		No DP		P-value ¹		
Item ²	mean	SE	mean	SE	DP	Par	DP×Par
Cows (No.)	42		80				
Meals per day	7.9	0.2	8.2	0.1	0.19	0.13	0.60
Visits per meal	3.5	0.1	3.8	0.1	0.19	< 0.01	0.33
Meal duration (min per meal)	29	1	28	1	0.41	< 0.01	0.07
Total meal time (min per day)	227	7	227	5	0.96	< 0.01	0.11
Total feeding time (min per day)	183	5	179	4	0.53	< 0.01	0.02
Meal size (kg per meal)	4.6	0.1	4.9	0.1	0.10	0.36	0.71
Feed intake ² (kg per day)	35.7	0.9	39.1	0.6	< 0.01	0.31	0.89
Feeding rate (g per min feeding)	204	7	229	5	< 0.01	< 0.01	0.04

¹Week was always significant (P < 0.01), whereas DP × Week was never significant (P > 0.05) 2 Feed intake refers to fresh feed intake from the basal ration (i.e. concentrate intake is not included). All cows were provided with the same lactation ration.

Looking at the diurnal pattern of feeding, cows spent more time having meals during daytime than during the night, with the highest proportion of cows having meals after fresh feed delivery, peaking around noon (Figure 2). Before calving, cows with a short DP spent more time having meals during daytime than cows with no DP (Figure 2a). After calving, the diurnal pattern of meals was similar for cows with a short DP and cows with no DP (Figure 2b).



Figure 2. Daily pattern of percentage of time spent in a meal (i.e. time spent feeding and in withinmeal intervals) for cows with a short DP and cows with no DP, at 4 weeks before calving (panel a) and 4 weeks after calving (panel b).

3.2 The effect of a short or no dry period on lying behaviour and steps

The number of lying bouts per day was not affected by DP treatment or period relative to calving (P > 0.05; Table 3). Young cows (parity 1 before calving) had 13.2 (SE: 0.5) lying bouts per day, whereas older cows (parity > 1) had 11.4 (SE: 0.5) lying bouts per day (P < 0.01).

Daily lying time was affected by a DP treatment × period interaction. At 4 weeks before calving, daily lying time was 1.1 h longer for cows with a short DP than for cows with no DP (P = 0.01). At 4 weeks after calving, however, daily lying time was 0.9 h shorter for cows with a short DP than for cows with no DP (P = 0.03). The change in lying time between the period before calving and the period after calving was more extreme for cows with a short DP (-3 h) than for cows with no DP (-1 h).

Before calving, time spent lying dipped during milking for cows with no DP, whereas this was not the case for cows with a short DP (Figure 3a). After calving, lying patterns were similar for cows with a short DP and cows with no DP (Figure 3b).

Table 3. Lying behaviour and number of steps of cows with a short DP and cows with no DP, 4 weeks before expected calving (period 1) and 4 weeks after calving (period 2; least squares means and SE).

		Short	DP	No DP		P-value	¹		
	Period	mean	SE	mean	SE	DP	Par	Period	DP×Period
Cows (No.)		28		53					
Lying bouts (No. per day)	1	11.8	0.6	12.6	0.4	0.46	0.01	0.45	0.36
	2	12.4	0.7	12.5	0.5				
Lying time (h per day)	1	13.7ª	0.3	12.6 ^b	0.2	0.82	0.70	< 0.01	< 0.01
	2	10.7ª	0.3	11.6 ^b	0.2				
Steps (No. per day)	1	663ª	43	1130 ^b	31	< 0.01	< 0.01	< 0.01	<0.01
	2	1193	58	1250	42				

¹DP×Parity interaction was never significant (P > 0.05)

^{a,b}Different letters indicate differences between means for a short DP and no DP in the same period (P < 0.05).



Figure 3. Daily pattern of percentage of time spent lying for cows with a short DP and cows with no DP, at 4 weeks before calving (panel a) and 4 weeks after calving (panel b).

The number of steps per day was affected by a DP treatment × period interaction (P < 0.01; Table 3). Before calving, cows with a short DP had 41% lower step counts than cows with no DP (P < 0.01). After calving, the number of steps did not differ between DP treatments (P > 0.05), and was similar to the number of steps of cows with no DP during the period before calving.

Before calving, cows with a short DP were weighed in the milking parlour on Tuesdays. To assess the impact of this additional exercise on daily number of steps, step counts were compared between days of the week. On average, 220 (SE: 29) more steps were recorded for cows with a short DP on Tuesdays than on Wednesdays through Mondays (P < 0.01; Figure 4). Cows with a short DP also had lower step counts during weekends than on weekdays. Cows with no DP had no increased step count on Tuesdays during the period before calving. Their step count was highest on Mondays, which was the day animals were regrouped (although focal cows were not moved in this period).



Figure 4. Number of steps recorded per day of the week for cows with a short DP and cows with no DP in the recording period 4 weeks before calving (least squares means and SE). Different letters within the same DP treatment indicate differences between means (P < 0.05).

3.3 Associations between behaviour, milk yield, and energy balance

Mean milk yield of cows with a short DP was 37.9 kg (SD: 6.0) per day for cows in parity 2 and 36.8 kg (SD: 6.3) per day for older cows; mean milk yield of cows with no DP was 29.5 kg (SD: 5) per day for cows in parity 2 and 35.3 kg (SD: 8.1) per day for older cows (overall range 15 – 50 kg). Mean energy balance of cows with a short DP was -191 kJ per kg^{0.75} (SD: 150) per day for cows in parity 2 and -179 kJ per kg^{0.75} (SD: 190) per day for older cows; mean energy balance of cows with no DP was 44 kJ per kg^{0.75} (SD: 113) per day for cows in parity 2 and -94 kJ per kg^{0.75} (SD: 193) per day for older cows.

Dry matter intake and basal ration intake had a low positive correlation with milk yield (Table 4). No correlations with milk yield were found for other variables of feeding behaviour (feeding rate, feeding and meal duration, and number of visits and meals). A low negative correlation was found between daily lying time and milk yield. Steps and number of lying bouts were not correlated with milk yield.

Low positive correlations were found between energy balance and number of meals, and between energy balance and number of visits (Table 4). Feeding rate and total feeding time were not correlated with energy balance, but were highly negatively correlated with each other (r: -0.71; P < 0.01).

Low positive correlations were found between daily lying time and energy balance (r: 0.28; P = 0.01), dry matter intake (r: 0.26; P = 0.02), and basal ration intake (r: 0.32; P < 0.01). There were no correlations, however, between total feeding time or total meal time and daily lying time. Number of steps had low positive correlations with energy balance (r: 0.27; P = 0.02), daily meal duration (r: 0.38; P < 0.01), and daily feeding duration (r: 0.29; P: 0.01); and a moderate correlation with the number of visits to the feeder (r: 0.54; P < 0.01).

Table 4. Descriptive statistics of milk yield, energy balance, feeding behaviour (n=122), lying behaviour (n=81) and number of steps (n=81) variables, and Pearson correlation coefficients with milk yield and energy balance (EB).

	Variable		Pearson Correlation ¹		
Variable ² Milk (kg per day) EB (kJ per kg ^{0.75} ×day) DMI (kg DM per day) Basal ration intake (kg per day) Feeding rate (g per min) Meals (No. per day) Visits (No. per day) Visits (No. per day) Total meal time (min per day) Total feeding time (min per day) Lying time (h per day) Lying bouts (No. per day)	Mean	SD	Milk	EB	
Milk (kg per day)	34.1	7.3			
EB (kJ per kg ^{0.75} ×day)	-80	187	-0.78		
DMI (kg DM per day)	21.8	2.2	0.21	0.32	
Basal ration intake (kg per day)	38.0	6.5	0.21	0.26	
Feeding rate (g per min)	212	55	n.s.	n.s.	
Meals (No. per day)	7.8	1.2	n.s.	0.25	
Visits (No. per day)	29	10	n.s.	0.22	
Total meal time (min per day)	232	53	n.s.	0.19	
Total feeding time (min per day)	187	42	n.s.	n.s.	
Lying time (h per day)	11.3	1.8	-0.22	0.28	
Lying bouts (No. per day)	12.4	3.6	n.s.	n.s.	
Steps (No. per day)	1233	327	n.s.	0.27	

¹Pearson correlation coefficients are given when correlations were significant (P < 0.05); n.s. = no significant correlation (P \ge 0.05)

²Energy balance (EB); dry matter intake from basal ration and concentrate (DMI).

4 Discussion

The objective of the current study was to assess the effects of a short and no DP with associated management on feeding, lying, and number of steps of dairy cows in late gestation and early lactation.

Compared with cows with no DP, cows with a short DP spent a longer time feeding but had a reduced feed intake (kg and NE) before calving. Other experimental studies with different DP lengths also reported lower feed intake (DM) before calving for cows with a conventional or short DP than for cows with no DP (Rastani et al., 2005; Van Knegsel et al., 2014b). In the current study, the DP was accompanied by a DP ration, as is common in commercial dairy farming (US: Rastani et al., 2005; Canada: Santschi et al., 2011a; the Netherlands: Steeneveld et al., 2013). It is unlikely, however, that the reduced feed intake was due to the change from the lactation ration to the DP ration, because this reduction was also observed for cows with a short DP without a ration change (Rastani et al., 2005). This lower feed intake of dry cows likely reflects the lower energy requirement of dry cows. The lower feeding rate during the DP may be related to the high amount of fibre and the lower palatability of the DP ration (Baumont, 1996; Friggens et al., 1998).

Calving and associated changes in management had a large impact on feeding behaviour, irrespective of DP treatment. In the first week after calving, cows had more frequent, but shorter, meals, and increased feeding rates, overall resulting in a lower feed intake than in subsequent weeks. This suggests that the impact of calving, removal of the calf, and changes in routine, housing, and social grouping is a distruption in its own right, irrespective of the DP management system of the period that preceded it. Cows had about 7 meals per day before calving, and 8 meals per day after calving, irrespective of DP treatment. The use of separate meal criteria for feeding behaviour before calving and after calving did not influence this difference: conclusions were similar when a single meal criterion was used for both periods.

After calving, feed intake remained lower for cows with a short DP than for cows with no DP, despite being fed the same diet. Rastani et al. (2005) also reported a lower feed intake for cows with a short DP than for cows with no DP in the first 3 weeks after calving, whereas Van Knegsel et al. (2014b) did not find a difference in feed intake between cows with a short DP and cows with no DP in the first 14 weeks after calving. It is unclear why cows with a short DP had a lower feeding rate than cows with no DP after calving. This might be related to rumen adaptation after a change in diet (Martens et al., 2012), the onset of lactation, the severity of the negative energy balance, or the change of social environment after calving. Further studies are needed in order to disentangle the impact of these factors. The higher energy intake and lower milk yield of cows with no DP compared with cows with a short DP may reduce the risk of metabolic diseases and improve welfare in early lactation (Ingvartsen, 2006).

Cows with a short DP had a lower daily average step count before calving than cows with no DP. This could be a direct consequence of the absence of the milking procedure, because going through the milking parlour (to be weighed) increased the step count of cows with a short DP by 220 steps. Excluding the day of weighing, cows with a short DP performed on average 624 steps per day, and cows with no DP performed 1117 steps per day. Twice-daily milking, therefore, could explain 89% of the difference $((2 \times 220)/(1117 - 624))$ in step count between cows with a short DP and cows with no DP, which suggests that the difference in steps was mainly due to a difference in walking distance. Stepping could also occur without walking, e.g. as a restless behaviour in the milking parlour (Gygax et al., 2008). However, with stepping rates of less than 1 step per minute during the preparation and milking phases (Gygax et al., 2008), this is unlikely to contribute much to the observed difference in steps per day. Pen size was smaller for dry cows than it was for lactating cows. Due to year-round calving and the capacity of 60 cows in the trial at one time, the dry cow group mostly consisted of 6 or fewer cows. The density was maintained at 7 m^2 per cow, which would result in 42 m² for a group of 6 dry cows versus 378 m² for a group of 54 lactating cows. This could have further reduced the number of steps of cows with a short DP. Previous research showed that lactating cows walked more in larger pens (Telezhenko et al., 2012).

It could be questioned whether the reduced number of steps during the DP is beneficial, or whether the reduced physical activity might be a risk factor for cow health. Walking distance of housed cows is already limited compared with walking distance of grazing cows. For example, studies reported walking distances of 233 m per day for housed cows versus 2170 m for cows on pasture (Olmos et al., 2009), and step counts of 1506 versus 4064 steps per day (Dohme-Meier et al., 2014). Studies showed that exercise is beneficial for health in early lactation and for fitness of lactating and dry dairy cows (Gustafson, 1993; Davidson and Beede, 2009). In humans, women who continued to exercise regularly throughout pregnancy had a lower incidence of operative delivery, and had shorter active labour than women who discontinued their exercise (Clapp, 1990).

Before calving, the daily lying time of cows with no DP (12.6 h) was lower than for cows with a short DP (13.7 h), but higher than previously reported lying times of dry cows of 11.7 and 12.2 h per day (Huzzey et al., 2005; Schirmann et al., 2011). These lying times likely reflect the overall response to the environment in late gestation, as opposed to a short-term response to changes in management, because cows were regrouped and rations were switched 11 days before the measurement period,

and cows were dried off 4 days before the measurement period. Cows in both DP treatments spent less time lying after calving than before calving. Other studies found that lying time was lower in early lactation than later in lactation (Munksgaard et al., 2005; Bewley et al., 2010). Due to the short lying time, hormonal changes, and a negative energy balance, cows in early lactation are particularly susceptible to lameness (Cook and Nordlund, 2009). The no DP treatment increased daily lying time after calving by 0.9 h compared with the short DP treatment, which might reduce the risk of developing lameness in early lactation.

Daily lying time had a low negative association with milk yield in early lactation. It has been suggested that cows with higher yields and a more severe negative energy balance have to spend more time feeding, creating a trade-off between feeding and resting (Roche et al., 2009; Bewley et al., 2010). In the current study, however, cows with a short DP and cows with no DP spent less time feeding and less time lying after calving than before calving. For cows with a short DP, the reduction in feeding and lying time may be related to the twice-daily milking after calving, compared with no milking before calving, that reduced their time budget for other behaviours. This was not the case for cows with no DP, however, because they were milked both before and after calving. Moreover, daily feeding time was not associated with daily lying time, or with milk yield. Therefore, lying time was probably not constrained by feeding time. Norring et al. (2012) also found a negative association between daily lying time and milk yield at 8 weeks in milk, with no associations between milk yield and feeding time. Løvendahl and Munksgaard (2016) found a positive correlation between milk yield and feeding time and a negative correlation between milk yield and lying time in primiparous cows. Other factors may explain why a higher milk yield was associated with a shorter lying time in early lactation. Considering that level of milk yield relates to udder pressure (Bertulat et al., 2013), cows with higher milk yields may experience discomfort when lying down and therefore lie down less. There was a low positive association between energy balance and daily lying time in this study. A prolonged negative energy balance might also cause discomfort due to exhaustion or (subclinical) metabolic disorders, which might reduce lying behaviour (Webster, 2000; Roche et al., 2009).

Not including a DP in the management of the cow in the weeks before calving could improve cow welfare. It does not require cessation of lactation, or ration and group changes commonly associated with a DP. During late gestation, cows with no DP spent more than 12 h per day lying, without the reduction in steps that was seen in cows with a short DP. In early lactation, cows with no DP had a higher feed intake, improved energy balance, and increased lying time compared with cows with a short DP. It should be kept in mind, however, that no DP may not be a strategy for all

cows: some cows may benefit from a DP to treat intramammary infections, and some cows may dry off spontaneously. Also, the impact of being dry cannot be separated from the impact of group and ration changes in the current study. The impact of DP management might be reduced through technical solutions. For example, separation gates can be used to separate dry cows out at the feed bunk and at the milking parlour, and thereby facilitate that they remain in the herd. Moreover, a short DP can be applied without a change in ration (Rastani et al., 2005). An experimental design in which dry and lactating cows remain in the same herd is necessary to assess the impact of being dry as such.

5 Conclusions

Cows with a short DP with associated changes in ration and social group appeared to get more rest than cows with no DP: they had a lower step count and longer lying and feeding times in late gestation. The differences in number of steps and feed intake seemed direct consequences of not being milked. Cows with no DP also had longer lying times (exceeding 12 h per day) before calving than in early lactation, despite the twice-daily milking. After calving, cows with no DP had longer lying times and ate more than cows with a short DP. Omission of the DP may improve cow welfare through absence of DP-related changes in management (i.e. cessation of lactation, ration and group changes), increased walking activity in late gestation, and a better feed intake and longer lying time in early lactation.

Chapter 4

Relationship between metabolic status and behaviour in dairy cows in week 4 of lactation

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Abstract

The aim of this study was to analyse relationships between metabolic status (based on plasma metabolites), and feeding behaviour, lying behaviour, motion index, and steps of dairy cows in week 4 postpartum after a 0-d or 30-d dry period. Data from 81 Holstein-Friesian cows were collected using computerised feeders, accelerometers, and from analyses of EDTA plasma samples for freefatty acid (FFA), β -hydroxybutyrate (BHB), glucose, insulin, insulin-like growth factor 1 (IGF-1), and growth hormone (GH) concentrations. Cluster analysis of plasma metabolite and metabolic hormone concentrations was used to categorise cows as having poor, average, good, or very good metabolic status. Clusters with poor, average, and good metabolic status were compared. Cows with a poor or average metabolic status tended to have greater FPCM yield than cows with a good metabolic status. Furthermore, cows with a poor metabolic status had a lower energy balance and DMI than cows with an average or good metabolic status and had a lower number of meals than cows with a good metabolic status. Daily number of visits to the feeder and lying time tended to be related with metabolic status, whereas feeding rate (kg/min), daily meal time (min/day), number of lying bouts per day, steps, and motion index were not related with metabolic status. In conclusion, better metabolic status in dairy cows in early lactation was associated with a greater DMI, increased feeding activity, and a tendency to more time spent lying, compared with a poor metabolic status. These results suggest that compromised metabolic status is reflected in altered cow behaviour in week 4 of lactation.

1 Introduction

Diseases like ketosis, retained placenta, metritis, mastitis, milk fever, displaced abomasum, lameness, and impaired fertility, result in a high disease incidence in in dairy cows early lactation (Drackley, 1999; Ingvartsen et al., 2003). Disease in early lactation may affect milk production performance in later lactation including total lactation yield (Fourichon et al., 1999). Changes in lying, walking, and feeding behaviour of dairy cows have been associated with diseases (Edwards and Tozer, 2004) such as lameness (González et al., 2008), metritis (Urton et al., 2005), or a displaced abomasum (Van Winden et al., 2003). Diseases in early lactation are often related with the negative energy balance (EB) of dairy cows during this period (Collard et al., 2000; Ingvartsen et al., 2003). The negative EB in early lactation is caused by insufficient energy intake to support the high milk yield. A negative EB is related with an altered metabolic status, indicated by lower glucose, insulin, and insulin-like growth factor 1 (IGF-1) concentrations in plasma, and greater plasma free-fatty acid (FFA), β -hydroxybutyrate (BHB) and growth hormone (GH) concentrations (Fenwick et al., 2008).

Plasma metabolites and hormones can be indicators of metabolic status (Butler et al., 2003), but blood sampling is more invasive than monitoring behaviour. Behaviour such as feeding time and feeding rate, rumination time, and steps are increasingly collected using sensors to indicate, for instance, heat or the moment of calving (Reith et al., 2014). A better insight into relationship between metabolites and metabolic hormones with behaviour may improve the interpretation of behaviour with respect to metabolic status in early lactation. A previous study reported that cows with a lower plasma FFA concentration had a greater walking activity postpartum, compared with cows with a greater plasma FFA concentration (Adewuyi et al., 2006). In addition, cows with ketosis had a lower feed intake, feeding time, meal time, and fewer meals and visits to the feeder than cows without ketosis (González et al., 2008). To our knowledge, relationships between behaviour and other plasma metabolites and metabolic hormones have not been described previously.

Shortening or omitting the DP improves the EB and metabolic status through a lower milk yield and an increased or similar feed intake after calving (Rastani et al., 2005; Van Knegsel et al., 2014b). A previous study showed that a better EB after short or no DP was weakly correlated with longer lying time, more feeding behaviour and a higher feed intake (Kok et al., 2017). It can be hypothesised, however, that metabolic status may better reflect cow health and feelings than the energy balance, because metabolic status better reflects the degree to which the animal can cope with the catabolic state that is typical for early lactation (Van Knegsel et al., 2014a). The aim of this study was to analyse associations between metabolic status, based on plasma metabolites and hormones, and lying and feeding behaviour, motion index and steps of dairy cows in the fourth week postpartum. To address this aim, metabolic status and behaviour were recorded in an experiment with dairy cows in early lactation after a o-d or 30-d DP. The current study monitored dairy cows in week 4 after calving to limit the direct effect of the calving process and start of lactation on behaviour, and maximise the contrast in energy balance of cows with different dry period lengths (Van Knegsel et al., 2014b). Moreover, lying time was expected to be lowest in week 4 of lactation (Maselyne et al., 2017).

2 Materials and methods

2.1 Animals and housing

The Institutional Animal Care and Use Committee of Wageningen University & Research approved the experimental protocol in compliance with the Dutch law on Animal Experimentation (protocol number 2014125). The experiment was originally designed to study effects of dry period length on metabolic status (Van Hoeij et al., 2017). The experiment was conducted at the Dairy Campus research herd (Lelystad, The Netherlands) between January 27th 2014 and August 26th 2015. The research herd was composed of 400 lactating Holstein cows. Cows were selected based on 1) being bred with a Holstein sire, 2) expected calving interval <490 days, 3) daily milk yield >16 kg at 90 days before the expected calving date, and 4) no clinical mastitis or high SCC (\geq 250,000 cells/mL) at the final two test-days before drying off. All cows were housed in the same freestall barn, which had concrete slatted floors, and cubicles (1.25 m × 2.20 m) fitted with rubber mattresses (4 cm thick) covered with sawdust. Stocking density was maintained at 7 m² per cow, with one cow per cubicle. Cows were milked twice daily at ~06:00 hours and ~17:00 hours. Lying behaviour, steps, and motion index of cows were recorded for 6 complete days (Friday till Wednesday) in the fourth week after calving (week 4) after calving, because of limited sensor availability and changing of sensors on Thursdays.

2.2 Experimental design

In total, 130 cows entered the experiment, including 6 cows that entered twice. To obtain a balanced distribution of cows across treatments, cows were blocked according to expected calving date, milk yield in the previous lactation, and parity $(2, \ge 3)$ in the subsequent lactation. Within each group of

3 cows, 2 cows were assigned randomly to a DP length treatment of 0 days (0-d DP) and 1 cow to a DP length treatment of 30 days (30-d DP). Within the group of cows with a 0-d DP, cows were assigned randomly to either a low level of concentrate based on the energy requirement for their expected milk yield (LOW) or a standard (STD) level of concentrate based on the energy requirement for the expected milk yield of cows after a 30-d DP (Van Knegsel et al., 2014b). Cows with a 30-d DP were fed a STD level of energy, based on the requirement for their expected milk yield. This resulted in the following 3 treatment groups: cows with a 30-d DP fed the STD level of concentrate required for their expected milk yield [30-d DP(STD)], cows with a 0-d DP fed the same STD concentrate level as cows with a 30-d DP [0-d DP(STD)], and cows with a 0-d DP fed a LOW concentrate level [o-d DP(LOW)]. Preliminary statistical analyses showed no effect of concentrate level within the o-d DP treatment on feeding behaviour, lying behaviour, steps, or motion index. Concentrate level was therefore excluded from further analyses in this study. Lying behaviour, steps, and motion index were only measured for 81 of 130 cows. The final dataset for this study consisted of milk production, EB, body weight, feed intake, plasma metabolite and metabolic hormone concentrations, and lying behaviour, steps, and motion index data of 81 unique cows in week 4 postpartum (n = 53 for 0-d DP and n = 28 for 30-d DP). Basal lactation ration, concentrate composition, and feeding strategy were reported previously (Van Hoeij et al., 2017).

2.3 Measurements

Measurement of behaviours was described earlier (Kok et al., 2017). In short, feeding behaviour was measured in week 4 postpartum. Basal lactation ration was provided and its daily intake was measured individually using roughage intake control (RIC) feeders (Insentec, Marknesse, The Netherlands). The stocking density was 2 cows per trough. The actual quantity of concentrate dispensed (kg/d) was recorded by the computerised feeder *(Manus VC5, DeLaval, Steenwijk, the Netherlands)*. For each visit to a feeder, RIC feeders recorded cow identity, the start time and end time (hh:mm:ss) of the visit, and the start weight and end weight of the feed in the feeder to the nearest 0.1 kg. Visits were clustered into meals based on the interval length between visits (Yeates et al., 2001; Tolkamp et al., 2002), with a threshold of 20.9 minutes between meals (Kok et al., 2017). Feeding behaviours used for analyses were the average daily duration for 6 days of meals (meal time, min/d), average daily number of visits (visits, n/d), the average daily number of meals (meals, n/d), and the secondary variables daily feed intake (kg DM/d) and feeding rate (kg/min) that were derived from these variables.

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

Milk yield was recorded daily in week 4 postpartum. Milk samples for fat, protein and lactose analysis (ISO 9622, Qlip, Zutphen, The Netherlands) were collected four times per week (Tuesday afternoon, Wednesday morning, Wednesday afternoon, and Thursday morning) and were analysed as a pooled sample per cow per week. Fat-and-protein-corrected milk (FPCM) was calculated as:

FPCM = (0.337 + 0.116 × fat percentage + 0.06 × protein percentage) × milk yield (CVB, 2012).

Body weight (BW) was recorded daily before each milking and averaged for week 4 of lactation. Energy balance was calculated per week according to the Dutch NE system for lactation (VEM) (Van Es, 1975), as the difference between energy intake and energy requirements for maintenance and milk yield (1,000 VEM = 6.9 MJ of NE). According to the VEM system, the daily requirement for maintenance is 42.4 VEM/kg^{0.75} BW per day and the requirement for milk yield is 442 VEM/kg FPCM (Van Es, 1975). Energy intake and EB are expressed in kJ/ kg^{0.75} BW per day (Van Es, 1975).

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

2.4 Statistical analyses

The natural logarithm of the plasma FFA, BHB, and GH concentration were calculated to approximate normal distribution of these variables and were used in all statistical analyses. To evaluate normality of residuals, a normality test was used in data distribution (PROC UNIVARIATE), and skewness between -1 and 1, kurtosis between -2 and 2, and a non-significant Shapiro-Wilk test were used as criteria for normality. To analyse correlations of plasma metabolites and hormones (FFA, BHB, glucose, insulin, IGF-1, and GH) with DMI, feeding behaviour, lying behaviour, steps, and motion index, a Pearson correlation was used [PROC CORR; SAS 9.3, SAS Institute Inc. (2011)]. Pearson correlation analysis was also used to analyse correlations among the different plasma metabolites and hormones.

Because in the current study most metabolites and hormones were correlated (Table 1b), as a second step, cluster analysis with the Ward method was performed using plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration as explanatory variables (PROC CLUSTER). Based on the cubic clustering criterion, pseudo F statistic, and pseudo t-squared, cows were clustered in 4 clusters using the tree procedure (PROC TREE). The cluster with the very good metabolic status included only 6 cows that had a 0-d DP, a 12 kg lower average milk yield, and a lower feed intake than other clusters. Because of the limited number of animals and the extremely low milk production in this cluster, the cluster with a very good metabolic status was excluded from the analysis. Clusters with poor, average, and good metabolic status were compared.

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

 Y_i = Cluster_i + ε_i , where cluster_i indicates the mean of cluster i (i = good, average, or poor) and ε_i indicates the random residual. To evaluate DMI, feeding behaviour, lying time, steps, and motion index for cows with different metabolic status, the general linear model was extended with DP length (o-d DP or 30-d DP) and parity (2 or \geq 3) as dependent variables:

 y_{ijkl} = Cluster_i + DP_j + Parity_k + ε_{ijk} , where DP_j indicates the DP length (i= o-, or 3o- DP) and Parity_k indicates the parity of the cow (j= parity 2, or \ge 3).

The model was analysed using a backward elimination procedure with a stay-in *P*-value of <0.05 in the type III Wald test.

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3 Results

3.1 Correlations between plasma metabolites, hormones and behaviour

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

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

3.2 Relation between metabolic status and behaviour

Cows were clustered for metabolic status based on their plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration in week 4 postpartum. This resulted in 4 groups of cows with a poor, average, good, or very good metabolic status. Cows with a poor, average, or good metabolic status had a 0-d or 30-d DP length, and parity 2 or \geq 3 (Table 2). All 6 cows with a very good metabolic status had a 0-d or d DP length and were not included in further analyses. Cows with a good metabolic status had lower plasma FFA concentration (Figure 1a), and greater plasma glucose (Figure 1c) and IGF-1 concentration (Figure 1e) than cows with average or poor metabolic status (P < 0.01) (Table 3). Cows with an average metabolic status had lower plasma FFA and greater plasma glucose and IGF-1 concentrations than cows with a poor metabolic status. Cows with a good metabolic status tended to have a lower plasma BHB (Figure 1b) and greater plasma insulin (Figure 1d) than cows with a

poor metabolic status (P < 0.10). Plasma IGF-1 increased with metabolic status (adjusted R²: 0.82, P < 0.01; Figure 1e). Energy balance increased with metabolic status (P < 0.01). Cows with a good metabolic status tended to have lower FPCM yield than cows with a poor or average metabolic status (P < 0.10). Body weight did not differ between cows with different metabolic status.

Table 1. Pearson correlation coefficients (P < 0.05) of plasma metabolites with fat-and-protein corrected milk (FPCM) yield, dry matter intake (DMI), energy balance (EB), feeding rate, number of meals and visits, meal time, lying time and bouts, steps, and motion index (a). Pearson correlation coefficients (P < 0.05) among plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentrations (b).

a)		FFA	BHB	Glucose	Insulin	IGF-1	GH
aj		(mmol/L) ¹	(mmol/L) ¹	(mmol/L)	(µU/mL)	(ng/mL) ¹	(µg/L)1
FPCM (kg/	d)1	0.49**	0.31**	-0.37**	-0.47**	-0.41**	0.26*
DMI (kg D	M/d)1	-0.49**	Ns	0.23*	Ns	0.22*	-0.34**
EB (kJ / kg	^{0.75} ∙d)¹	-0.78**	-0.41**	0.48**	0.33*	0.55**	-0.45**
Feeding ra	ite (kg/min)	Ns	Ns	0.30**	Ns	Ns	-0.25*
Meals (n/d	l)	-0.34**	Ns	Ns	Ns	0.34**	-0.24*
Visits (n/d)	-0.39**	-0.2*	0.23*	Ns	0.22*	Ns
Meal time	(min/day)	-0.38**	Ns	Ns	Ns	Ns	Ns
Lying time	(hrs/d)	-0.43**	Ns	Ns	Ns	0.32**	Ns
Lying bout	:s (n/d)	Ns	Ns	Ns	Ns	Ns	Ns
Steps (n/d	l)	-0.32**	-0.25*	Ns	Ns	Ns	Ns
Motion ind	ex	-0.37**	-0.28*	Ns	Ns	Ns	Ns
ь)	FFA	BHB	Glucose	Insul	in IC	GF-1	GH
D)	(mmol/L) ¹	(mmol/L) ¹	(mmol/L	_) (µU/ı	mL) (r	ng/mL)1	(µg/L)1
FFA ¹		0.33**	-0.48**	-0.40)** -().52**	0.40**
BHB ²			-0.52**	-0.24	ŀ* N	S	Ns
Glucose				0.39*	** 0.	.53**	-0.27*
Insulin					Ν	S	Ns
IGF-1							-0.39**

 $^{*}P < 0.05, ^{**}P < 0.01, Ns = not significant$

¹FFA= free fatty acids, analysed using the natural logarithm of FFA, BHB= β -hydroxybutyrate, analysed using the natural logarithm of BHB, IGF-1 = insulin-like growth factor 1, GH = bovine growth hormone, analysed using the natural logarithm of GH, FPCM = fat-and-protein corrected milk, DMI = dry matter intake, EB = energy balance.

Table 2. Number of cows in different clusters based on the plasma FFA, BHB, glucose, insulin, IGF-1 and GH concentration.

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

	Adjusted R ²		Metabolic status		SEp	P- Value
		Poor	Average	Good	-	
Cows		9	41	25		
FFA (mmol/L) ²	0.30	0.46 ^c	0.16 ^b	0.10 ^a		<0.001
		(0.28 – 0.75)	(0.13 – 0.20)	(0.07 – 0.13)		
BHB (mmol/L) ²	0.07	1.00	0.72	0.63		0.06
		(0.72 – 1.39)	(0.62 – 0.84)	(0.52 – 0.77)		
Glucose (mmol/L)	0.23	3.21ª	3.72 ^b	3.96 ^c	0.18	<0.001
Insulin (µU/mL)	0.07	7.7	15.0	14.9	3.8	0.09
IGF-1 (ng/mL) ²	0.82	52ª	101 ^b	151 ^c	6.5	<0.001
GH(µg/L) ²	0.04	4.68	4.52	3.81		0.29
		(3.48 – 6.29)	(3.94 – 5.19)	(3.15 – 4.60)		
FPCM ³	0.07	38.8	39.0	35.6	2.5	0.06
EB (kJ / kg ^{0.75} ·d) ³	0.22	-242ª	-124 ^b	6 ^c	66	< 0.001
Body weight (kg)	0.03	638	674	662	27	0.30

Table 3: Plasma metabolite and metabolic hormone concentrations in week 4 postpartum for cows with different metabolic status after a 0 or 30 day dry period. Metabolic status was based on the plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration. Values are presented as LSM and pooled SE (SEp).

^{a-c}Values with different superscripts differ (P < 0.05).

 2 FFA = Free fatty acids, BHB = β -hydroxybutyrate, IGF-1 = insulin-like growth factor 1, GH = growth hormone. FFA, BHB, and GH were log transformed for analyses, but are shown as actual values with confidence interval.

³FPCM = fat-and-protein corrected milk. EB = energy balance.

Cows with good or average metabolic status had greater DMI and basal ration intake than cows with a poor metabolic status (P < 0.01) (Table 4). Cows with a good metabolic status had more meals per day than cows with an average or poor metabolic status (P < 0.05). Cows with a good metabolic status tended to have a longer lying time than cows with a poor metabolic status (P < 0.10). Irrespective of metabolic cluster, cows with a 0-d DP had greater DMI (21.9 ± 0.3 vs 20.5 ± 0.5 kg/d), basal ration intake (15.0 ± 0.3 vs. 13.5 ± 0.4 kg DM/d), and visits to the feeder (29.0 ± 1.5 vs 24.2 ± 1.9 /d) than cows with a 30-d DP (P < 0.05). Cows of parity 2 had a lower feeding rate (0.20 ± 0.01 vs. 0.24 ± 0.01 kg/min), and more visits to the feeder (29.7 ± 1.7 vs. 23.1 ± 1.6 /d), longer meal times (233 ± 9 vs. 195 ± 9 min/d), and more steps (1280 ± 57 vs 1085 ± 56 /d) and motion index (5234 ± 233 vs 4464 ± 230) (P < 0.01) than cows with parity ≥ 3 .



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

		Me	etabolic st	atus	SEp		P-value	1
	Adjusted R ²	Poor	Average	Good	_	С	DP	Р
Cows		9	41	25				
Dry matter intake (kg DM/d)	0.25	19.5 ^b	22.0ª	22.4ª	0.9	< 0.01	< 0.01	0.44
Basal ration intake (kg DM/d)	0.30	12.5 ^b	15.1ª	15.3ª	0.9	< 0.01	< 0.01	0.41
Feeding rate (kg/min)	0.22	0.20	0.22	0.23	0.03	0.44	0.11	< 0.01
Meals (n/d)	0.15	7.17 ^b	7.38 ^b	8.24ª	0.49	0.02	0.62	0.64
Visits (n/d)	0.33	23.7	28.5	31.7	4.1	0.08	0.04	< 0.01
Meal time (min/d)	0.25	200	224	225	22	0.39	0.57	< 0.01
Lying time (h/d)	0.12	10.3	10.9	11.9	0.8	0.06	0.26	0.32
Lying bouts (n/d)	0.07	11.6	12.0	13.2	1.5	0.40	0.84	0.16
Steps (n/d)	0.18	1100	1231	1324	134	0.21	0.73	0.01
Motion	0.20	4409	4945	5386	542	0.15	0.51	0.01

Table 4. Behaviour in week 4 postpartum of cows with different metabolic status after a 0 or 30 day dry period. Metabolic status was based on the plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration. Values are presented as LSM and pooled SE (SEp).

^{a-c}Values with different superscripts differ (P < 0.05).

 ${}^{1}C$ = Cluster, DP = Dry period length, P = Parity.

4 Discussion

The aim of this study was to analyse relationships between metabolic status, based on plasma metabolites and metabolic hormones, with feeding behaviour, lying behaviour, steps, and motion index of dairy cows in week 4 postpartum. First, Pearson correlation analysis was used to correlate metabolites and metabolic hormones with behaviour. In particular, plasma FFA concentration was related to the behavioural indicators. Dry matter intake, number of meals and visits to the feeder, meal times, lying times, steps, and motion index were all lower at a greater plasma FFA concentration. Plasma BHB, glucose, insulin, IGF-1, and GH concentration were related to only 3 or fewer behavioural indicators, and the strength of these correlations was generally lower than between FFA concentration and behavioural indicators. This seems to indicate that cows with a greater FFA concentration in particular, have a lower feed intake, partly explained by lower feed intake related behaviour and lower activity. Our results are in line with previous studies that found that cows with a greater plasma FFA concentration have lower feed intake (Waterman et al., 1972; Lean et al., 1992), and lower walking activity (Adewuyi et al., 2006). Because in the current study most metabolites and hormones were correlated (Table 1b), as a second step, cluster analysis was performed to cluster cows for metabolic status based on their plasma FFA, BHB, glucose, insulin, IGF-1, and GH concentration. Cows were clustered in 4 groups for poor, average, good, or very good metabolic status. Plasma IGF-1 concentration seemed to have the largest impact on clustering $(R^2 = 0.82)$, followed by plasma FFA concentration $(R^2 = 0.30)$ and glucose $(R^2 = 0.23)$. Correlations with individual metabolites and metabolic hormones and behaviour, however,

indicated that plasma FFA concentration had more and stronger correlations with feeding behaviour, lying behaviour, steps, and motion index than IGF-1. This might indicate that high plasma FFA is more related to behaviour and feelings in week 4 of lactation than plasma IGF-1, possibly because cows already recover from the catabolic state at this time.

To our knowledge no other studies have directly related metabolic status with behavioural parameters. However, previous studies evaluated relationships between disease events in early lactation and metabolic status, and reported plasma metabolites for the diseased and non-diseased cows. These studies reported that cows with a metabolic disease, metritis, or mastitis had lower metabolic status as reflected by a greater plasma FFA, BHB, and haptoglobin, and lower plasma calcium concentration, than non-diseased cows (Soriani et al., 2012). Additionally, cows with a metabolic disease in previous studies had lower daily feeding time (Urton et al., 2005), decreased activity and rumination (Soriani et al., 2012; Stangaferro et al., 2016), compared with non-diseased cows. In another study, cows with ketosis had lower feed intakes, feeding times, meal times, and fewer meals and feeder visits than cows without ketosis (González et al., 2008). The lower reported DMI, and number of meals and visits to the feeder in these earlier studies are in accordance to our findings. In contrast, in our study, steps, motion index, and meal time were not different among cows with different metabolic status. It should, however, be noted that in previous studies cows were diseased and likely showed sickness behaviour, whereas cows in our study were not clinically diseased. The current study illustrates how behaviour measures like number of meals per day and lying time can be an indicator for metabolic status, even in case of no clinical disease.

On average, cows with a good metabolic status had a lower plasma FFA concentration and a greater plasma glucose and IGF-1 concentrations compared with cows with an average or poor metabolic status. Better metabolic status was associated with greater DMI, basal ration intake, and number of meals, and tended to be associated with a longer daily lying time. These behaviours can be automatically recorded and may be reliable indicators of metabolic status. Number of visits to the feeder can be measured as proximity to the feed bunk using sensors, and can be processed into number of meals. However, these sensors are unlikely to be used at present in commercial settings. Meanwhile, lying time can be recorded with commercially available sensors that are widely used for estrus detection (Rutten et al., 2017). Lying time may, therefore, be a useful indicator for metabolic status, although the relation should be studied further, as well as potential interactions with other factors such as stocking density. Reduced lying time may be indicative of restlessness or discomfort, e.g. due to udder pressure (Huzzey et al., 2005; Bertulat et al., 2017).

In the current study, plasma FFA concentration had stronger correlations and plasma IGF-1 concentration had similar correlations with behaviour in week 4 of lactation, compared with correlations between EB and behaviour in our previous study (Kok et al., 2017). These results imply that plasma FFA has a stronger relation with behaviour than calculated EB. It could be hypothesised, therefore, that plasma metabolites like FFA may better reflect cow health and feelings than the energy balance itself, because metabolic status better reflects the degree to which the animal can cope with the catabolic state typical in early lactation. In addition, it could be hypothesised that the relation between behaviour and plasma metabolites may even be stronger in cows with a conventional DP due to a more severe NEB and poorer metabolic status, than in cows with a o-d or 30-d DP.

Relations between metabolic status and behaviour can be different for other weeks in early lactation. Daily lying time, for example, was shown to decrease in the weeks after calving, with the lowest lying time in week 4, after which lying time gradually increased and then levelled off at about 12.5 hours per day (Maselyne et al., 2017). Plasma FFA concentration, in contrast, peaks in the first week after calving (Chen et al., 2015a). Together, these two patterns imply that associations between plasma FFA concentration and daily lying time are different over time. Variable relations between metabolic status and behaviour may be explained by the duration of the catabolic state, and interactions with other motivations. For example, when cows were given access to feed and a lying place for only 12 hours per day for a period of 2 weeks, they prioritised lying time over feed intake and consequently lost weight (Munksgaard et al., 2005). The authors hypothesised that, if the experiment had lasted longer, a more severe weight loss could shift these behavioural time budgets towards feeding at the cost of lying time.

The application of different DP lengths was used to create variation in metabolic status between cows in early lactation. A potential drawback of this method could be that short or no DP affect behaviour independent of metabolic status. However, the model to evaluate the effect of metabolic status on behaviour corrected for effects of DP length and parity. Correlations between metabolites and metabolic hormones and behaviour were performed without DP length and parity included as covariates in the model. Including DP length and parity in a general linear model to evaluate effects of a plasma metabolite on behaviour (results not shown) resulted in similar relations between plasma metabolites and behaviour compared with the correlations presented in table 1a.

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

5 Conclusions

Better metabolic status, as indicated by plasma metabolites and metabolic hormones, in dairy cows in week 4 of lactation after a short or no DP was associated with a greater DMI, increased feeding activity, and a tendency to more time spent lying, compared with a poor metabolic status. A compromised metabolic status was reflected in altered cow behaviour in week 4 of lactation.

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Chapter 5

Effective lactation yield: a measure to compare milk yield between cows with different dry period lengths

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Abstract

To compare milk yield between cows or management strategies, lactations are traditionally standardised to 305-d yields. The 305-d yield, however, gives no insight in the combined effect of additional milk yield before calving, decreased milk yield after calving and a possible shorter calving interval in the case of a shortened dry period. We aimed to develop a measure that enables the comparison of milk vield between cows with different dry period lengths. We assessed the importance of accounting for additional milk yield before calving and for differences in calving interval. The 305-d yield was compared with a 365-d yield, which included additional milk yield in the 60 d before calving. Next, an effective lactation yield was computed, defined as the daily yield from 60 d before calving to 60 d before the next calving, to account for additional milk yield before calving and for differences in calving interval. Test-day records and drying-off dates of 15 commercial farms were used to compute the 305-d, 365-d, and effective lactation yield for individual cows. We analysed 817 second parity lactations preceded by no, a short (20 to 40 d), or a conventional (49 to 90 d) dry period. Compared with cows with a conventional dry period, the 305-d yield of cows with no dry period was 7.0 kg fat-and-protein-corrected milk (FPCM) per d lower, and the 305-d yield of cows with a short dry period was 2.3 kg of FPCM per d lower. Including additional milk yield before calving in the 365-d yield reduced this difference to 3.4 kg of FPCM per cow per d for cows with no dry period and to 0.9 kg of FPCM per cow per d for cows with a short dry period. Compared with cows with a conventional dry period, median days open were reduced by 25 d for cows with no dry period and by 18 d for cows with a short dry period. Accounting for these differences in calving interval in the effective lactation yield further decreased yield reductions for cows with no or a short dry period by 0.3 kg of FPCM per cow per d. At herd level, estimated 365-d yield losses for cows with no or a short dry period differed from effective lactation yield losses by 0.4 to -0.8 kg of FPCM per cow per d. Accounting for additional milk yield before calving had a major and consistent impact on yield comparisons of cows with different dry period lengths. The impact of correcting for calving interval was more variable between farms and will especially be important when calving interval is affected by dry period length.
1 Introduction

Milk yield of cows is an important determinant of the economic and environmental impact of management strategies in dairy farming. Milk yield directly relates to farm revenues (Santschi et al., 2011a), and environmental impacts per kg of milk often decrease when milk yield levels increase (Wall et al., 2010; Van Middelaar et al., 2014). To compare milk yield between cows, lactations are traditionally standardised to 305-d yields (Ashton, 1956; Patton et al., 2006; Windig et al., 2006).

Recently, shortening the dry period has been suggested as a management strategy to reduce the negative energy balance in early lactation and to increase fertility in dairy cattle (Andersen et al., 2005; Van Knegsel et al., 2013). Shortening the dry period results in additional milk yield before calving, whereas milk yield after calving is reduced (Annen et al., 2004; Santschi et al., 2011b; Van Knegsel et al., 2013, 2014b). The additional milk yield before calving can be accredited to the choice of dry period length. The 305-d yield does not include this additional milk yield, and, therefore, is less suitable to assess the impact of dry period length on milk yield.

To compare milk yield between cows with different dry period lengths, various lactation lengths and summations of milk yield before and after calving have been used (Annen et al., 2004; Santschi et al., 2011b; Steeneveld et al., 2014). Steeneveld et al. (2014), for example, accounted for additional milk yield by adding the yield from the 60 d before expected calving to the 305-d yield after calving.

Shortening the dry period may also improve fertility (Gümen et al., 2005; Watters et al., 2009; Chen et al., 2015b), although not all studies found this (Pezeshki et al., 2007; Santschi et al., 2011c). An improved fertility can partly compensate for milk losses related to a shortened dry period if the calving interval is shortened (Inchaisri et al., 2010b). To correct for calving interval, while accounting for additional milk yield before calving, a measure of milk yield similar to a whole lactation yield (i.e. milk yield from calving to next calving) is required. Santschi et al. (2011a) combined additional milk yield before calving and the lactation yield after calving in an expression of kg milk per cow per year. However, because this measure included milk yield until drying off, it could lead to double counting of the additional milk yield if multiple consecutive lactations are assessed.

The first aim of this study was to develop a measure that would enable the comparison of milk yield between cows with different dry period lengths. The second aim was to assess the importance of accounting for additional milk yield before calving and for differences in calving interval when evaluating the effect of dry period length on milk yield.

2 Materials and methods

2.1 Definition of yield measures

Three measures of milk yield were compared in this study: conventional 305-d yield, a 365-d yield and an effective lactation yield. First, the conventional 305-d yield sums milk yield from calving to 305 DIM. Second, as an equivalent of the conventional 305-d yield that accounts for additional milk yield before calving, a 365-d yield was defined as the sum of the milk yield in the 60 d before calving and the 305-d yield after calving. It was assumed that the conventional dry period lasts 60 d (Bachman and Schairer, 2003). Milk produced from 60 d before calving until calving was therefore considered additional milk due to the decision to shorten the dry period. Third, the effective lactation yield was defined as milk yield from 60 d before calving to 60 d before next calving, to adjust milk yield for length of calving interval. The effective lactation thus corresponds to the period from one dry period decision to the next dry period decision, as opposed to the period from calving to next calving. Milk yield in the last 60 d before next calving was considered to be attributable to the next dry period decision and was therefore excluded from the present effective lactation. Like the conventional whole lactation yield, the duration of the effective lactation is equal to the calving interval. To facilitate comparison, all 3 measures of milk yield were standardised to kilograms fatand-protein-corrected milk (FPCM) per day.

2.2 Application of yield measures

In total, 15 commercial Dutch farms that apply or recently applied a short dry period or no dry period on their farm provided their test-day milk records and drying-off dates. Half of the farms (A, B, C, D, E, G, and H) applied one dry period strategy at a time for all cows, whereas the other half of the farms (F, I, J, K, L, M, N, and O) selected cows with high yields and low SCC in late lactation for short, or no (mainly farms F and I) dry periods. The farms differed in herd size, milk yield level, housing system, milking frequency and diets. Diets mainly comprised grass, grass silage and maize silage complemented by concentrates. In total, 10 out of 15 farmers applied no dry period and 14 applied a short dry period during the period of analysis. Table 1 shows the number of cows included in the analysis, median calving intervals, and first-lactation 305-d yields per farm.

Test-day milk records were recorded every 4 to 6 weeks from January 2007 to September 2014 by the Dutch national milk recording organisation (CRV, Arnhem, the Netherlands). These milk records were combined with drying-off records to compute lactation lengths and dry period lengths.

To clearly illustrate the effect of different measures of milk yield, strict selection criteria were applied to the dataset. In short, lactations with implausible drying-off dates were excluded, and only second parity lactations preceded by specific dry period lengths and with regular milk records were included in the analysis. The final dataset included 817 cows with 17,333 milk records on their complete first and second lactation.

	First	parity	h		Sec	ond parity	/	
				No DP	S	Short DP	C	onv. DP
farm	305-d	n	CI	n	CI	n	CI	n
Α	7922	136	37	3 43	366	82	383	11
В	8947	61	36	2 42	2 336	3	405	16
С	7279	39	34	7 39) –	-	-	-
D	7048	70	35	7 36	5 360	25	363	9
E	5937	40	34	0 25	5 365	2	402	13
F	6335	86	38	5 19	357	39	360	28
G	7400	33	36	5 14	378	5	392	14
Н	7129	39	37	8 7	378	14	369	18
Ι	8255	60	35	7 7	346	14	381	39
J	9132	24	36	7 2	354	22	-	-
К	7950	85	-	-	371	39	414	46
L	9108	31	-	-	379	29	366	2
М	7009	58	-	-	365	27	375	31
N	7650	30	-	-	401	12	412	18
0	8424	25	-	-	435	10	432	15
Overall	7618	817	35	9 23	34 368	323	385	260

Table 1. First lactation 305-d yield (kg of FPCM per cow) and median calving interval in second lactation (CI) of the cows included in the analysis per dry period (DP) category¹ per farm.

¹Short dry period: 20 d to 40 d; conventional dry period: 49 d to 90 d

Drying-off records

Drying-off records were combined with lactation data based on cow identity, parity and calving date. These combinations were validated as follows. If milk records occurred after the date of drying off, the date was assumed to be incorrect and the lactation was excluded. If no drying-off date was present and the farmer stated not to practice continuous milking, the drying-off date was considered to be missing and the lactation was excluded. If continuous milking until parturition occasionally occurred, continuous milking was assumed if there was no drying-off date and no milk records were missing at the end of the lactation. If a farmer only applied continuous milking, all lactations were classified as such.

Dry period categories

Lactations were categorised into 3 dry period classes using the drying-off records: no dry period, with an assumed continuous lactation (n=234); a short dry period, with a drying-off date 20 d to

40 d before calving (mean: 33.3 d; SD: 5.2 d; n=323); and a conventional dry period, with a dryingoff date 49 d to 90 d before calving (mean: 58.3 d; SD: 8.9 d; n=260). Lactations with intermediate (i.e. from 1 d to 19 d, and from 41 d to 48 d) and more extreme dry period lengths were excluded (n=422) to create clear contrasts.

Completeness

Milk records with missing values for milk yield, fat content, or protein content were excluded. Three additional requirements were defined to improve the dataset, which resulted in the exclusion of 349 incomplete lactations. First, the first milk record for each first and second parity lactation had to occur earlier than 50 DIM. Second, the first and second parity lactation of each cow had to have at least 5 milk records and the period between these records could not exceed 90 d. Third, the calving interval from first to second and second to third calving could not exceed 600 d.

Standard lactation curves

Milk yield was converted to FPCM yield, as follows (CVB, 2012):

FPCM (kg) = milk (kg) × (0.337 + 0.116 × fat content (%) + 0.06 × protein content (%))

Subsequently, a Wilmink lactation curve was fitted on the 17,333 milk records (in kg of FPCM):

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

where y_t is the yield of a cow at t DIM, and a, b, c, and k are parameters that relate to the level of production (a), persistency after the peak yield (b) and slope towards and moment of peak yield (c and k) (Wilmink, 1987). The model was fitted using maximum likelihood in SAS (PROC NLMIXED; version 9.3, SAS Institute Inc., Cary, NC). The initial model had a fixed effect for parity (1 or 2) and in the case of parity 2 also for dry period category (no, short, conventional) on parameters a, b, and c; and included a random effect on parameters a, b, and c to account for repeated measures per lactation (817 cows \times 2 lactations). No random effect on k was included, to mitigate model convergence problems. Significance of fixed effects was assessed with a Wald test. The least significant fixed effect was removed from the full model until the smallest Bayesian information criterion was reached. The resulting lactation curves for each parity \times dry period combination, presented in the appendix (Table 5.a), were used as standard lactation curves to interpolate and extrapolate milk yields of individual cows at regular intervals.

Interpolation and extrapolation

First, milk yield in kg of FPCM was interpolated at intervals of 20 d from 10 DIM to the end of each lactation, and on 0 DIM and 305 DIM, using

$$y_i = g_i + (x_i - x_1) \times (y_2 - y_1 - (g_2 - g_1)) / (x_2 - x_1) + (y_1 - g_1),$$

where y_i is the to be estimated milk yield at x_i DIM; x_1 , x_2 , and x_i are the DIM at the moments of milk recording and the to be estimated day x_i (that is between x_1 and x_2 DIM); y_1 and y_2 are the measured milk yields at x_1 and x_2 DIM; g_1 , g_2 , and g_i are the predicted milk yields at x_1 , x_2 and x_i DIM according to the standard lactation curves (CRV, 2002).

Milk yields before the first milk record and after the last milk record were extrapolated, using

$$y_i = g_i + (y_1 - g_1),$$

where y_1 is the actual and g_1 is the predicted milk yield at the nearest milk record. Negative predictions of milk yield were set to zero.

In case cows in the second parity were dried off before they reached 305 DIM or 60 d before third calving (e.g. because of a short calving interval or because the farmer dried the cows off early), the lactation was extrapolated to estimate milk yield without bias due to choices regarding the next dry period.

Cumulative yields

Cumulative milk yields were approximated by summing the measured and estimated milk records over the lactation interval, using

$$\sum_{i=1}^{n-1} [(INTi - 1) \times y_i + (INTi + 1) \times y_{i+1}]/2,$$

where y_i is the recorded or estimated milk yield at *i*DIM, INT_i is the interval between this milk yield and the next in days; and n is the total amount of milk records (CRV, 2002; ICAR, 2009). Milk records were summed from calving until 305 DIM, until 60 d before next calving and until next calving. To compute the additional milk yield before calving, the milk yield until 60 d before second calving was subtracted from the total milk yield in the first parity lactation. The additional milk yield in the first parity, and the milk yield until 305 DIM and until 60 d before next calving in the second parity were used to compute the 305-d, 365-d, and effective lactation yield.

2.3 Effect of dry period length

A mixed model using restricted maximum likelihood was used to assess the impact of dry period category on 305-d, 365-d, and effective lactation yield in the second parity lactation (PROC MIXED). Herd was included as a random effect, because multiple animals in the same herd cannot be regarded as independent units of observation. Moreover, a random herd × dry period category interaction effect was included to model possible differences in the effect of dry period length on milk yield between farms. To standardise milk yield levels among cows and herds, the individual 305-d yield in the first lactation was used as a covariate for lactation potential (Kuhn and Hutchison, 2005; Pezeshki et al., 2007; Cermakova et al., 2014). An interaction effect of dry period category and 305-d yield in the first lactation was included in the initial model. Only significant fixed effects based on Kenward-Roger approximate F-tests were retained in the model (P < 0.05). Random effects were retained in the model if their inclusion improved the model based on a likelihood ratio test (P < 0.05). Pairwise comparisons of milk yield per dry period strategy were performed using Wald tests. For illustrative purposes, milk yields were also predicted per dry period category per farm, using the mean values per dry period category (LSMEANS) and best linear unbiased predictions (ESTIMATE) of random effects per dry period category per farm (Robinson, 1991). These predictions were used to calculate the impact of no or a short dry period as compared with a conventional dry period on milk yield for all yield measures. In addition, the difference in impact between the 305-d and 365-d yield, and between the 365-d and effective lactation vield were computed.

The same mixed model and procedure were used to assess the effect of dry period length on calving interval. The skewed distribution of calving interval was converted to a normal distribution by transformation to the natural logarithm of days open, where days open was defined as calving interval minus 280 d. Mean values (LSMEANS) of the lognormal distribution were transformed back to median days open (Median(X) = $\exp(\mu)$; (Johnson et al., 1994)).

To visually assess the effect of using an effective lactation yield versus a 365-d yield, the individual differences between the two yield measures were computed and plotted against calving interval.

3 Results and discussion

3.1 Milk yield measures

305-d yield

Mean 305-d yields in the first lactation varied from 19.5 to 29.9 kg of FPCM per cow per d (5,937 to 9,132 kg of FPCM in 305 d) between farms (Table 1), with an overall mean yield of 25 kg of FPCM per cow per d. In the second lactation, the mean 305-d yield was 30.8 kg of FPCM per cow per d for cows with a conventional dry period, whereas it was 28.4 kg of FPCM per cow per d with a short dry period and 23.8 kg of FPCM per cow per d with no dry period (Table 2).

We detected no interaction between dry period length and lactation potential, and the interaction term was removed from the model to explain 305-d yield (Table 3). Therefore, the expected decrease in milk yield for cows with a short or no dry period is a fixed amount of kg of FPCM per cow per d, regardless of lactation potential. Hence, it might be more informative to express the effect of dry period length on milk yield in kg reduction instead of % reduction of milk yield, unless cows of equal or standardised lactation potential are compared.

An experimental study observed daily yields of 35.9 kg of FPCM per cow after a conventional dry period, 33.9 kg of FPCM per cow after a short dry period, and 26.2 kg of FPCM per cow after no dry period in second parity Holstein cattle during 44 weeks of lactation (Van Knegsel, 2014). The reduction of 2 kg of FPCM per cow per d after a short dry period is similar to the current results. Applying no dry period, however, reduced milk yield with almost 10 kg of FPCM per cow per d in the experimental study of van Knegsel (2014), compared with 7 kg of FPCM per cow per d in this observational study. Whereas over 40% of cows with no planned dry period dried off spontaneously in some experiments (Rémond et al., 1997; Van Knegsel, 2014), the farmers in this study indicated that few cows dry themselves off, which might partly explain the difference in milk losses.

365-d yield

Additional milk yield (mean (SD)) in the 60 d before second calving was 77 (73) kg of FPCM for cows with a conventional dry period, whereas it was 478 (161) kg of FPCM for cows with a short dry period and 992 (308) kg of FPCM for cows with no dry period. Other studies reported similar additional milk yields of 454 to 544 kg milk per cow for short dry periods of 30 d to 35 d and of 846 to 1176 kg milk per cow for no dry period in primiparous cows (Annen et al., 2004; Watters et al., 2008; Santschi et al., 2011b; Steeneveld et al., 2013; Van Knegsel et al., 2014b).

	Conv. D	Conv. DP		Short DP			No DP		
Yield measure	FPCM	SE	FPCM	SE	%	FPCM	SE	%	
305-d	30.8ª	0.58	28.4 ^b	0.57	-7.6	23.8 ^c	0.62	-22.8	
365-d ²	26.0 ^{a*}	0.50	25.1ª*	0.49	-3.4	22.6 ^b	0.54	-13.1	
effective lactation ³	25.4ª	0.53	24.9ª	0.52	-2.2	22.4 ^b	0.57	-12.0	

Table 2. Least squares mean milk yield (kg fat-and-protein-corrected milk per cow per day, FPCM) of second-parity cows per dry period (DP) category¹ for 3 measures of milk yield.

 a,b,c Different letters indicate different means in the same row (P < 0.05)

¹Short DP: 20 d to 40 d; conventional DP: 49 d to 90 d. Relative differences between milk yield of cows with a short or no DP versus a conventional DP are given for each measure (%).

 $^2\mbox{Milk}$ yield in the 60 d before second calving plus the 305-d yield after calving

³Milk yield from 60 d before second calving until 60 d before next calving

*Asterisks indicate a tendency of a difference between means in the same row (0.05 < P < 0.10)

Table 3. Effects of dry period length and yield level on 305-d, 365-d, and effective lactation yields (kg of fat-and-protein-corrected milk per cow per day).

	305-d		365-d ¹			effective lactation ²			
Variable	β	SE	Р	β	SE	Р	β	SE	Р
constant ³	15.7	1.13	-	12.3	0.95	-	12.7	0.95	-
dry period ⁴									
no vs. conv.	-7.0	0.58	<0.001	-3.4	0.50	<0.001	-3.1	0.51	< 0.001
short vs. conv.	-2.3	0.51	0.002	-0.9	0.44	0.06	-0.6	0.45	0.22
yield level ⁵	1.98	0.13	<0.001	1.80	0.11	<0.001	1.67	0.10	< 0.001

¹Milk yield in the 60 d before second calving plus the 305-d yield after calving ²Milk yield from 60 d before second calving until 60 d before next calving ³Population mean of the conventional dry period is the reference in the parameterisation ⁴Short dry period: 20 d to 40 d; conventional (conv.) dry period: 49 d to 90 d

⁵305-d yield in the first lactation (tonnes of fat-and-protein-corrected milk)

Including the additional milk in the 365-d yield measure considerably reduced the difference in daily milk yield between conventional and short or no dry period. Changing from the 305-d to the 365-d yield reduced the milk losses for cows with a short dry period from 2.3 to 0.9 kg of FPCM per cow per d, and for cows with no dry period from 7.0 to 3.4 kg of FPCM per cow per d (Table 3). Similarly, (Schlamberger et al., 2010) et al. (2010) found a decrease in 305-d milk yield of 6.1 kg milk per cow per d when no dry period was applied to multiparous Brown Swiss cows, which was reduced to 1.9 kg per cow per d when the 56 d additional milk yield before calving was taken into account.

Shoshani et al. (2014) concluded that the additional milk yield resulted in a higher milk yield for second-parity cows with a dry period length of 40 d as opposed to 60 d. In our study, 365-d milk yield of cows with a short dry period (mean: 33.3 d; SD: 5.2 d) was 0.9 kg of FPCM per cow per d lower than of cows with a conventional dry period, although some farms had increased 365-d yields (see appendix Table 5.b). The difference between our findings and the findings from Shoshani et al. could result from the different 'short' dry period lengths, as dry periods shorter than 40 d have a more detrimental effect on milk yield after calving (Kuhn et al., 2005).

Effective lactation yield

Compared with cows with a conventional dry period, median days open were 18 d fewer for cows with a short dry period and 25 d fewer for cows with no dry period, with a relative ratio of days open (95% confidence interval) of 0.77 (0.67-0.87) for a short dry period and 0.83 (0.74-0.94) for no dry period (P < 0.01). In other studies, mean days open were 52 d fewer after no dry period compared with a conventional dry period (Gümen et al., 2005), median calving interval tended to be 17 d shorter after a short dry period (Watters et al., 2009), and mean calving interval was 19 d shorter after a short dry period (Santschi et al., 2011a). Some studies did not find an effect of dry period length on days open or calving interval (Pezeshki et al., 2008; De Feu et al., 2009), but this could be due to the relatively low number of cows (61 and 40). Shorter calving intervals when applying a short or no dry period could be caused by a better energy balance, earlier onset of ovarian cyclicity, and more regular estrus cycles in early lactation (Gümen et al., 2005; De Feu et al., 2009; Watters et al., 2005).

Switching from 365-d yield to effective lactation yield, thus correcting for shorter calving intervals with a short or no dry period, decreased the reduction in milk yield for cows with no and a short dry period by 0.3 kg of FPCM per cow per d (Table 3). The effective lactation yield was not different between cows with a short and a conventional dry period, whereas the effective lactation yield of cows with no dry period was 3.1 kg of FPCM per cow per d lower than that of cows with a conventional dry period.

3.2 Individual and herd variation

The effect of changing from a 305-d to a 365-d yield was consistent among herds (Figure 1). Including the additional milk reduced the reduction in milk yield with 3.0 to 4.1 kg of FPCM per cow per d for cows with no dry period, and with 1.3 to 1.8 kg of FPCM per cow per d for cows with a short dry period (Table 4).

The difference between the 365-d yield and the effective lactation yield was small compared with the difference between the 365-d yield and the 305-d yield. One reason for this result is that the 365-d yield by definition is identical to the effective lactation yield when the calving interval is 365 d, while the median calving interval for each dry period category is close to this value (359 d to 385 d). When individual differences between the 365-d yield and the effective lactation yield are plotted against calving interval, however, the difference between the two measures becomes apparent

(Figure 2). Assuming that the effective lactation yield accurately reflects actual yield, the 365-d yield overestimates milk yield per day when the calving interval exceeds 365 d, while it underestimates milk yield per day when the calving interval is shorter than 365 d. The individual difference between 365-d yield and effective lactation yield ranged from -3.2 to 7.4 kg of FPCM per cow per d.

	No DP			Short DP ²			
farm	305-d	365-d	eff.	305-d	365-d	eff.	
A	-7.0	-3.6	-3.1	-3.0	-1.5	-0.9	
В	-5.5	-1.4	-0.8	-1.6	0.2	1.0	
С	-	-	-	-	-	-	
D	-6.8	-3.2	-3.1	-3.2	-1.7	-1.6	
E	-6.1	-3.0	-2.5	-2.3	-1.1	-0.8	
F	-7.6	-4.0	-4.4	-2.7	-1.4	-1.4	
G	-8.7	-4.7	-4.5	-2.4	-0.9	-0.8	
Н	-7.9	-4.2	-3.9	-2.6	-1.1	-0.8	
Ι	-7.6	-3.9	-3.4	-3.2	-1.7	-1.1	
J	-	-	-	-	-	-	
К	-	-	-	-2.1	-0.6	0.0	
L	-	-	-	-2.5	-0.9	-0.8	
Μ	-	-	-	-1.3	0.0	0.2	
Ν	-	-	-	-0.8	0.3	0.5	
0	-	-	-	-3.2	-1.6	-1.4	

Table 4. Change in predicted¹ 305-d, 365-d, and effective lactation yield (eff.) for cows with no or a short dry period² compared with a conventional dry period per farm (kg of FPCM per cow per day).

¹Using SAS statements LSMEANS for fixed and ESTIMATE for random effects ²Short dry period: 20 d to 40 d; conventional dry period: 49 d to 90 d.



Figure 1. Change in reduction in fat-and-protein-corrected milk yield due to no (no fill) or a short (solid fill) dry period (DP) compared with a conventional DP when switching from a 305-d yield to a 365-d yield (diamonds) and from a 365-d yield to an effective lactation yield (triangles). Farm A to O are represented in alphabetical order from left to right, excluding farms C and J (farms C and J have no conventional DP with which to compare the effect of no DP or a short DP; see Table 5.b).



Figure 2. Individual differences (one dot per cow) between 365-d yield and effective lactation yield plotted against calving interval. FPCM = fat-and-protein-corrected milk.

At herd level, the difference in yield reduction due to no or a short dry period between the 365-d yield and effective lactation yield ranges from 0.4 to -0.8 kg of FPCM per cow per d (Figure 1). Assuming that the effective lactation yield accurately reflects the actual yield, using the 365-d yield would lead to an underestimation of milk yield of up to $(0.784 \times 365 =)$ 286 kg of FPCM per cow per year for the entire herd. Figure 3 illustrates that this underestimation of milk yield due to no or a short dry period is larger at farms where the calving interval was considerably shortened. For farm B, for example, correcting for a 30 d shorter calving interval in the case of a short dry period reduced milk losses for the effective lactation yield by 0.784 kg of FPCM per cow per d compared to the 365-d yield.

The Dutch mean calving interval for second parity cows was 413 d in 2013-2014 (CRV, 2014), whereas in this study, mean calving interval for second parity cows with a conventional dry period was 401 d. Differences between the 365-d yield and the effective lactation yield may therefore be more pronounced for an average Dutch farm, where calving intervals deviate more from 365 d.

3.3 365-d yield versus effective lactation yield

Conclusions on the effect of dry period length on milk yield depend on the measure of milk yield. Both proposed measures of milk yield, i.e. the 365-d and effective lactation yield, include the additional milk yield before calving, which had a major impact on milk yield comparisons between cows with different dry period lengths. Milk losses due to no dry period declined from 23% to 12% by switching from the 305-d yield to the effective lactation yield. This 11% difference in estimated milk losses could entirely change the economic prospects of the strategy. For example, labour income of a modelled Dutch dairy farm without milk quota increased with €23,000 to €40,000 per year when milk losses due to no dry period decreased from 23% to 13% (Heeren et al., 2014).



Figure 3. Change in reduction in milk yield due to no (no fill) or a short (solid fill) dry period compared with a conventional dry period when switching from a 365-d yield to an effective lactation yield, in relation to the reduction in median calving interval compared with a conventional dry period per herd. FPCM = fat-and-protein-corrected milk.

Although comparisons between dry period strategies on average were similar for the 365-d yield and the effective lactation yield, the effective lactation yield was more accurate for individual animals and in case calving interval is shortened by a short or no dry period. Individual yields are required for any analysis that aims to optimise cow-specific dry period lengths. Therefore, the effective lactation yield seems more suitable to decide which dry period length is optimal for an individual cow or herd.

A disadvantage of the effective lactation yield, however, is that a next (expected) calving is required to approximate the yield of the current effective lactation. If cows are culled based on low milk yield or infertility (Rajala-Schultz and Gröhn, 1999; Brickell and Wathes, 2011), the effective lactation yield may be biased towards cows that have a lower reduction in milk yield after a short or no dry period. This potential bias was not evaluated in the current study, in which only completed lactations were included. The 365-d yield does not require a next calving, thus also culled animals can be evaluated using this measure. Depending on the aim of the study, another option besides exclusion could be to include the final lactation of dairy cows as an effective lactation until the moment of culling (Santschi et al., 2011b).

4 Conclusions

We proposed the 365-d yield and the effective lactation yield to compare individual milk yields between cows with different dry period lengths. Both measures include the additional milk yield before calving. The effective lactation yield additionally corrects for calving interval, which especially impacts milk yield comparisons when calving interval is shortened as a consequence of a short or no dry period. The effective lactation yield was illustrated for second parity cows and can now be applied to evaluate the effect of dry period length on milk yield over multiple consecutive lactations.

Chapter 6

Effect of dry period length on milk yield over multiple lactations

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Abstract

Shortening or omitting the dry period (DP) can improve the energy balance of dairy cows in early lactation through a decrease in milk yield after calving. Little is known about the impact of a short or no DP on milk yield over multiple lactations. Our objectives were 1) to assess the impact of DP length over multiple lactations on milk yield, and 2) to assess if the prediction of milk yield in response to DP length could be improved by including individual cow characteristics before calving. Lactation data (2007 to 2015) of 16 Dutch dairy farms that apply no or short DP were used to compute cumulative milk yield in the 60 days before calving (additional yield) and in the 305 days after calving (305-d yield), and the mean daily yield over the interval from 60 days before calving to 60 days before next calving (effective lactation yield). DP categories were: no (0 to 2 wk), short (3 to 5 wk), standard (6 to 8 wk), and long (9 to 12 wk). The effect of current DP and previous DP on yields was analysed with mixed models (n=1420 lactations). The highest effective lactation yield of fat-and-protein-corrected milk (FPCM) was observed for cows with a standard current DP (27.6 kg per day); there was a daily decrease of 0.6 kg for a long DP, 1.0 kg for a short DP, and 2.0 kg for no DP. Previous DP did not significantly affect the effective lactation yield. Thus, cows can be managed with short or no DP over consecutive lactations without a change in quantity of milk losses. Cows that received no DP for consecutive lactations had a lower additional yield before calving (-172 kg FPCM), but a higher 305-d yield (+560 kg FPCM), compared with cows that received no DP for the first time. This could lessen the improvement of the energy balance in early lactation when no DP is applied a second time compared with the first time. For the second objective, a basic model was explored to predict effective lactation yield based on parity, DP length, and first parity 305-d yield (n= 2866 lactations). The basic model was subsequently extended with data about recent yield, days open, and somatic cell count. Extending the model reduced the error of individual predictions by only 6%. Therefore, the basic model seems sufficient to predict the effect of DP length on effective lactation yield. Other individual cow characteristics can still be relevant, however, to make a practical and tailored decision about DP length.

1 Introduction

A dry period (DP) of 42 d to 60 d is common practice in dairy cow management (Arnold and Becker, 1936). The conventional DP facilitates the replacement of senescent mammary epithelial cells (Capuco et al., 1997) and maximises milk yield in the next lactation (Kuhn et al., 2005). However, the DP is a challenge for the cow due to the process of drying off and the accompanied transitions in management (Ingvartsen, 2006; Zobel et al., 2015). In addition, the high milk yield with limited feed intake after a conventional DP results in a negative energy balance in early lactation that may last several months (Rastani et al., 2005; Van Knegsel et al., 2014b). This negative energy balance is associated with metabolic disorders and reduced fertility (Collard et al., 2000; Butler, 2003). To improve the energy balance, health, and fertility, and to ease the transition period, the DP can be shortened or omitted (Andersen et al., 2005; Gümen et al., 2005; Rastani et al., 2005; Chen et al., 2015b).

Effects of short or no DP on milk yield in the subsequent lactation have been documented for experimental and commercial farms (Rastani et al., 2005; Santschi et al., 2011a; Steeneveld et al., 2013; Van Knegsel et al., 2014b). Meta-analyses showed that milk yield after calving (over periods of varying duration) decreased by 4.5% for a short DP (4 to 5 wk) and by 19.1% for no DP, whereas protein content of the milk increased by 0.06% for a short DP and by 0.25% for no DP (Van Knegsel et al., 2013). These milk losses after calving, however, were compensated partly, sometimes completely, by the additional milk yield before calving (Van Knegsel et al., 2013).

The additional milk yield before calving (when the former lactation is extended) increases with a shorter DP and depends on the parity of the cow (Van Knegsel et al., 2014b). Moreover, calving interval can be shortened by short and no DP (Gümen et al., 2005; Santschi et al., 2011b), which increases mean daily milk yield after calving, and can further compensate milk losses (Kok et al., 2016). To account for additional yield before calving and for differences in calving interval, the measure "effective lactation yield" was developed to compare milk yield between cows with different DP lengths (Kok et al., 2016). The effective lactation yield was defined as the mean daily yield over the interval from 60 d before calving to 60 d before next calving, and was applied to young cows (parity 2). The 305-d yield of young cows was reduced by 23% after no DP compared with a standard DP, whereas the effective lactation yield was reduced by only 12% (Kok et al., 2016).

Adoption of a short or no DP on commercial farms is currently hindered by uncertainty of the impact on milk yield over multiple lactations and differences in response between cows (Santschi et al., 2011a; Van Knegsel et al., 2013). So far, it is unclear how milk yield is affected when the DP

is shortened or omitted for multiple consecutive lactations. The first omission or shortening of the DP reduces peak milk yield after calving with no or limited effects on persistency (Schlamberger et al., 2010; Santschi et al., 2011a; Chen et al., 2016a), which likely results in less additional milk at the end of that lactation. When the DP is shortened or omitted a second time, milk yield after calving could be reduced, remain the same, or increase compared with the first time the DP was shortened or omitted (scenarios are visualised in figure 1). A further reduction of milk yield could result from increased carryover of senescent, less-functional, mammary epithelial cells into the next lactation (Capuco et al., 1997; Annen et al., 2007, 2008; Collier et al., 2012). Milk yield could stabilise or increase if cows adapt to continuous milking (Rémond and Bonnefoy, 1997), perhaps through increased renewal of mammary epithelial cells during lactation (Capuco et al., 2001).



Figure 1. Scenarios for milk yield over time when a standard (solid line) or no (dashed line) dry period is applied before 3rd and 4th calving. Additional milk before calving (shaded area) is higher when no dry period is applied for the first time (1) than for the second (2) time. When no dry period is applied a second time, yield after calving could decrease further (scenario A), stabilise (scenario B) or increase up to level after a standard dry period (scenario C). FPCM = fat-and-protein-corrected milk.

Regarding individual responses to short or no DP, cow characteristics, such as milk yield and persistency in late lactation, can improve the prediction of additional milk yield before calving (Steeneveld et al., 2014). It is unknown if such variables improve prediction of effective lactation yield in response to DP length. Individual prediction of overall milk yield could facilitate decisions about DP length at cow-level (Grummer and Rastani, 2004).

Our objectives were 1) to assess the impact of DP length over multiple lactations on milk yield on commercial dairy farms, and 2) to assess if the prediction of milk yield for individual cows in response to DP length could be improved by cow characteristics before calving. The effective lactation yield was used for both objectives. In addition, milk yield before and after calving were analysed separately over multiple lactations, because timing of milk yield can affect the energy balance of the cow.

2 Materials and methods

2.1 Data and data processing

This study used data from 16 commercial Dutch dairy farms that recently (mostly in 2010 and 2011) changed their DP management from conventional to short or no DP (Kok et al., 2016). Dry cows were generally housed in a group of non-lactating cows, and fed a DP ration, whereas cows with no DP remained in the lactating herd. Milk yield and composition were recorded every 4 to 6 weeks, from January 2007 through September 2015, by the Dutch national milk recording system (CRV, Arnhem, the Netherlands). Test-day milk records were matched with drying off records, provided by the farmers, by cow identity, parity, and calving date. Matched data were validated (described in Kok et al. (2016)), and used to compute lactation length and DP length.

Milk records with missing values for milk yield, fat content, or protein content were excluded, because all were required to compute fat-and-protein-corrected milk (FPCM). FPCM was computed as: milk (kg) \times [0.337 + 0.116 \times fat content (%) + 0.06 \times protein content (%)] (CVB, 2012). To improve data quality, each lactation was included only when the following 4 criteria were met: a first record before 50 days in milk (DIM); at least 5 records in total; a maximum period of 90 d between records; and at least 1 record after 215 DIM or less than 90 d before drying off. Lactations after a DP that exceeded 12 wk (about 5%) were excluded from the analyses. The final dataset included 2,074 first, 2,176 second, and 3,924 third and higher parity lactations. Standard lactation curves per parity were estimated from test-day records until 600 DIM for kg milk, fat, protein, lactose, and FPCM, using the Wilmink curve (Wilmink, 1987). The full mixed model in SAS (version 9.3; SAS Institute Inc., Cary, NC) to obtain Wilmink curves for yield was:

Yield (DIM) = parity + DIM + expDIM + DIM × parity + expDIM × parity

with parity classes 1, 2, and \geq 3, DIM at the test-day, and expDIM computed as e^(-k × DIM). Moreover, the model included random effects on intercept, DIM, and expDIM for repeated measures per cow lactation (8,174 lactations; 89,400 records), assuming unstructured covariance (type = UN). Parameter k in expDIM was determined with a grid search, in which k was varied between 0.01 and 0.10, with steps of 0.01. We selected the value for k that resulted in the smallest deviance; this is the maximum likelihood estimator for k. Only significant fixed effects based on Kenward-Roger approximate F-tests were retained in the model (P < 0.05; Kenward and Roger, 1997).

Next, individual yield records were interpolated and extrapolated using the estimated standard lactation curves, and subsequently summed to compute cumulative yields per cow lactation

(method described in Kok et al., 2016; CRV, 2002; ICAR, 2009). Per cow lactation, the following yields were computed: yield in the 60 d before calving (additional yield), 305-d yield, and effective lactation yield of fat, protein, lactose, milk, and FPCM. The cumulative effective lactation yield, from 60 d before calving to 60 d before subsequent calving, was subsequently divided by the calving interval and expressed as effective lactation yield in kg per day (Kok et al., 2016). To facilitate comparison between 305-d yield and effective lactation yield, 305-d yield was also expressed in kg per day.

2.2 Analysis 1: impact of dry period length on milk yield over multiple lactations

The analysis was performed using 1420 lactations with known current DP length, previous DP length, and first lactation production of the cow (Table 1). DP categories were no (0 to 2 wk; 19%), short (3 to 5 wk; 21%), standard (6 to 8 wk; 47%), and long (9 to 12 wk; 13%). In the no DP category, 89% of the lactations had no DP (o d), whereas 11% of the lactations had a DP of 1 to 17 d. We assessed the impact of the fixed effects previous DP, current DP, parity class (3, or \geq 4 after calving; NB: parity 2 cows have no previous DP), and their interactions on effective lactation yield, additional yield before calving, and 305-d yield after calving, using mixed models and restricted maximum likelihood. Inclusion of the fixed effects was based on Kenward-Roger approximate Ftests, using backward elimination (P < 0.05). Moreover, herd was included as a random effect, and first parity 305-d yield (kg FPCM) was included as a fixed covariate in the models. Fat, protein, lactose, milk, and FPCM effective lactation yields, additional yields, and 305-d yields were analysed. Moreover, additional yield was analysed separately for each DP category, because variances differed between categories (Levene's test on residuals). Pairwise comparisons were performed using Wald tests (Cox and Hinkley, 1974). When the current DP \times previous DP interaction was significant (P < 0.05), the effect of the previous DP was compared for each current DP length separately. When previous DP affected yield within the current DP category, data was presented separately for each previous DP category. When previous DP did not affect yield within the current DP category, data were clustered per current DP category. Next, comparisons between the different resulting categories were made, using the ESTIMATE statement to specify contrasts.

The same model structure and approach were used to assess the effect of previous and current DP on (the natural logarithm of) days open, defined as calving interval minus 280 d (Kok et al., 2016). Mean values (LSMEANS) of the lognormal distribution were transformed back to median days open (Median(X) = $exp(\mu)$; Johnson et al., 1994).

icit (analysis 2; ii per parity category).										
	Analy	/sis 1 (n=	1,420)	Analysis 2 (n=2,866)						
Current dry period	No	Short	Standard	Long	Par 2	Par ≥ 3				
No	191	39	111	16	292	357				
Short	63	89	112	31	357	295				
Standard	74	107	371	52	686	604				
Long	18	37	74	35	111	164				

Table 1. Sample sizes (no. of lactations) for analyses of effective lactation yields of fat, protein, lactose, milk, and FPCM (analysis 1; n per previous dry period) and for the basic model for effective lactation yield (analysis 2; n per parity category).

2.3 Analysis 2: impact of cow characteristics on prediction of effective lactation yield

A mixed model with DP category, parity class (2, or \geq 3 after calving), and first parity 305-d yield (tonnes of FPCM) as fixed effects and a random herd effect was used to explain effective lactation yield (kg FPCM per cow per day) in the subsequent lactation (basic model). Second parity cows (n=1446; see Table 1) were included in this analysis, because analysis 1 showed no effect of previous DP length on effective lactation yield of FPCM. Moreover, parity 3 and parity \geq 4 cows were clustered, because their effective lactation yield of FPCM was not significantly different.

To assess whether the precision of prediction of individual effective lactation yield (kg FPCM per cow per day) could be improved, 6 variables that would be available at the moment of the DP decision in practice were extracted from test-day records. Three of these variables, to reflect each cow's actual yield and udder health, were extracted from the last test-day record before 70 d before calving (i.e. available before the DP decision is made): kg FPCM, natural logarithm of SCC (transformation to normalise data), and a binary value that reflected SCCs \leq 250,000 or > 250,000 cells per mL on the test-day. The fourth variable, to reflect persistency, was the change in yield between the last (before 70 d before calving) and the before-last test-day (kg FPCM per day). The final 2 variables, as indicators of yield level and fertility, were the 305-d yield (kg FPCM per day) and (natural logarithm of) days open of the lactation preceding the lactation of interest.

The 6 variables were added as fixed effects to the basic mixed model, including interaction effects with DP and parity class (extended model). Potential explanatory variables and their interactions were tested for their predictive value with approximate F-tests (Kenward and Roger, 1997). F-tests were constructed based on leave-one-out (similar to the use of type II sums of squares in conventional ANOVA), but also on backward and forward elimination (P < 0.05). Results of these different approaches were basically the same. The final extended model that was selected using backward elimination is shown in the results.

To assess the precision of prediction of effective lactation yield for individual cows, residuals of the basic and extended models were assessed. Residuals consisted of the random herd effect and the individual error because, in practice, herd effects are unknown when a decision to shorten DP length is first made. To facilitate interpretation of the mixed model analyses, Pearson correlations were calculated between variables in the final model.

3 Results and discussion

3.1 Analysis 1: impact of dry period length on milk yield over multiple lactations

Effective lactation yield

The highest effective lactation yield of fat-and-protein-corrected milk (FPCM) was observed for cows with a standard current DP (27.6 kg per cow per day); there was a daily decrease of 0.6 kg for a long DP, 1.0 kg for a short DP, and 2.0 kg for no DP (Table 2). These yields were achieved over an overall median calving interval of 383 days. Median calving interval, analysed as days open, was 8 to 18 days shorter for cows with no DP than for cows with a short, standard, or long DP (Table 3; parity \geq 3). We found no effect of previous DP or parity on effective lactation yield for FPCM, fat, protein, lactose, and milk (P \geq 0.05), except that third parity cows produced 16 g fat per day less than older cows (P < 0.05). The effective lactation yield reflects the average milk yield per day and corrects for differences in calving interval (Kok et al., 2016). Therefore, the maximum average daily milk yield per lactation was obtained with a standard DP, despite a longer average calving interval. Further research is necessary to assess the overall effect of DP length on farm performance. For example, applying a short or no DP might lower involuntary culling through improved fertility, thus increasing the average age and reducing replacement costs.

Additional yield

No DP resulted in the highest additional yield over the 60 days before calving, with 857 kg FPCM (SE: 48.5) for third parity cows and 791 kg FPCM (SE: 48.2) for older cows. Additional yields for cows with a short DP (501 kg FPCM; SE: 34.9) or standard DP (187 kg FPCM; SE: 10.2) did not differ between parities. Other studies reported similar additional yields (over the 56 days before calving) for multiparous cows (Annen et al., 2004; Schlamberger et al., 2010; Van Knegsel et al., 2014b). The different quantities of additional yield can be explained by an increased number of days in lactation in case of fewer days dry; and by a higher lactation persistency in younger cows

(second parity before calving) than older cows (Wood, 1969). Additional yield was lower for cows that previously had no DP than for cows that previously had a short, standard, or long DP. Compared to a standard previous DP, no previous DP reduced additional yield by 172 kg FPCM in case of no DP, 85 kg FPCM in case of a short DP, and 51 kg FPCM in case of a standard DP (Figure 2A). This reduction could be expected: omitting the DP was previously found to reduce peak milk yield with limited effects on persistency (Schlamberger et al., 2010), which results in a lower daily milk yield after calving, and, consequently, a lower additional yield in the current DP (Figure 1).

305-d yield

After calving, the lowest mean daily 305-d yield of FPCM was observed for cows with no DP (28.8 kg), and it was lower for cows with a short DP (30.9 kg) than for cows with standard (32.7 kg) or long (33.1 kg) DP (Table 4; Figure 2B). A short DP reduced 305-d yield by 1.8 kg FPCM per day compared to a standard DP. Similar milk yield reductions of 1.1 kg per day (until 17 weeks postcalving) and 1.9 kg per day (until 210 DIM) have been reported after a short DP of 28 and 30 days, respectively (Annen et al., 2004; Pezeshki et al., 2008).

Table 2. Effect of current dry period¹ on effective lactation yield² (least squares means and SE) of fat, protein, lactose, milk, and FPCM.

	Fat		Protein	Protein		Lactose		Milk		FPCM	
Dry period	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	
No	1082 a	28	871 ^a	25	1031 a	30	23.2 ^a	0.6	25.6 ª	0.7	
Short	1115 ^b	28	895 ^b	25	1102 ^b	30	24.5 ^b	0.6	26.6 ^b	0.7	
Standard	1159 ^c	27	911 ^c	24	1157 ^c	30	25.7 ^c	0.6	27.6 ^c	0.6	
Long	1132 ^b	29	886 ^{ab}	25	1143 ^c	31	25.4 ^c	0.6	27.0 ^b	0.7	

^{a-c}Different letters within the same column indicate different means (P < 0.05; n=1,420 lactations). ¹Previous dry period did not affect the effective lactation yield of fat, protein, lactose, milk, or FPCM ²Fat, protein, and lactose in grams per cow per day; milk and FPCM in kilograms per cow per day

Table 3. Effect of dry period length on days open (DO) of parity 2 (included in analysis 2 only; n = 1446) and parity $\geq 3 \text{ cows } (n=1420)^1$.

	parity 2			parity ≥ 3		
Dry period	median DO	P1	P99	median DO	P1	P99
0 - 2 wk	86 ^a	25	305	94 a	29	347
3 - 5 wk	90 ^a	31	353	102 ^b	30	376
6 - 8 wk	100 ^b	32	338	105 ^b	41	331
9 - 12 wk	114 ^c	41	394	112 ^b	38	375

^{a-c}Different letters within the same column indicate different means (P < 0.05).
¹Data are presented as median DO [backtransformed from least squares means of ln(DO)], and 0.01 percentile (P1) and 0.99 percentile (P99) of the data.



Figure 2. Effect of previous dry period (legend) on additional yield in the 60 d before calving (A; n=2010 lactations), 305-d yield (B; n=2010 lactations) and effective lactation yield (C; n=1420 lactations) for cows with no, a short, a standard, or a long dry period. Data are presented as least squares means and SE. In A, different letters within the same current dry period category indicate differences between means; in B and C, different letters indicate differences between means. FPCM = fat-and-protein-corrected milk.

The length of the previous DP affected only FPCM yield of cows that had no current DP (Figure 2B): cows that previously had a standard DP produced 560 kg less than cows that previously had no DP, and 572 kg FPCM less than cows that previously had a short DP. The 305-d milk yield after one omission of the DP (after a standard previous DP), was 5.6 kg milk per day lower than after a standard current DP (25.0 vs. 30.6 kg milk per day, see Table 4), similar to yield reductions of 5.0 and 7.7 kg milk per day reported in literature (Mantovani et al., 2010; Schlamberger et al., 2010). The reduction in milk yield likely results from reduced renewal of mammary epithelial cells when

the DP is omitted, which results in an increased carryover of senescent, less-functional cells into the next lactation (Capuco et al., 1997; Annen et al., 2007, 2008; Collier et al., 2012). The 305-d milk yield after a second omission of the DP was higher than after the first omission of the DP (intermediate between scenario B and C in Figure 1; 26.9 kg milk per day), which compensated the reduction in additional yield before calving compared with the first omission of the DP (Figure 2C). It can be hypothesised that the lower milk yield and improved energy balance after one omission of the DP (Gümen et al., 2005; Van Knegsel et al., 2014b) facilitate more renewal of mammary epithelial cells throughout lactation (Capuco et al., 2001). More renewal of mammary epithelial cells throughout lactation can be expected to result in a higher secretory activity after a second omission of the DP, despite the absence of the DP. Because the current study is based on commercial milk records only, these physiological questions could not be adressed. Higher yields after a second omission of the DP might also be due to a selection effect: farmers could give a DP to cows with lower yields after a first omission of the DP and omit the DP multiple times for cows with higher yields. However, a lower additional yield and an increased 305-d yield after the second omission of the DP were also reported in an experimental study (n=17 cows with no DP; Chen et al., 2016a). Cows with a long previous DP and no current DP had yields similar to cows after multiple omitted DP, but this result is based on few lactations (n=16; Table 1).

Protein, lactose, and milk yields after no current DP were also found to be lower after a standard previous DP, as compared with no or a short previous DP (Table 4). No such interaction between current and previous DP was found for 305-d fat yield. There was an effect of previous DP length on fat yield: omission of the previous DP increased fat yield in the current lactation compared with a short or a standard previous DP, irrespective of current DP length. Fat yield after omission of the previous DP, irrespective of a standard previous DP, and 10 kg (SE: 4 kg) higher compared to a standard previous DP, and 10 kg (SE: 4 kg) higher compared to a standard previous DP, and 10 kg (SE: 4 kg) higher compared to a standard previous DP, and 10 kg (SE: 0.19) less milk, and 0.5 kg per day (SE: 0.18) less FPCM than older cows (P < 0.05).

A main reason to apply short and no DP strategies is to improve the energy balance, and related metabolic health and fertility, of dairy cows in early lactation (Collier et al., 2004; Grummer et al., 2010). Energy balance in early lactation was greater for cows with no DP than for cows with a short DP, and greater for cows with a short DP than for cows with a conventional DP (Rastani et al., 2005; van Knegsel et al., 2014b). A reduction in yield precalving and an increase in yield postcalving, when no DP is applied multiple times, is expected to lessen the improvement of the energy balance in early lactation. Chen et al., (2016b) indeed reported a more negative energy balance in the 9

weeks after the second short or omitted DP than after the first short or omitted DP. No DP likely results in the least negative energy balance in early lactation, even over multiple lactations, because this strategy results in the greatest reduction in milk yield (Table 4), combined with a similar or increased feed intake compared with a standard DP (Rastani et al., 2005; Van Knegsel et al., 2014b). A short DP can also be applied without changes in ration and thereby ease the transition period (Rastani et al., 2005). A short DP results in smaller milk losses than no DP. It can be questioned whether these smaller milk losses sufficiently improve the energy balance, metabolic health, and fertility of cows. Further research is needed to elucidate the impact of short and no DP on health, disease incidences, and longevity (Van Knegsel et al., 2013); and to assess the overall effect of DP length on farm performance.

Table 4. Effect of dry period length and previous dry period length on 305-d yields¹ of fat, protein, lactose, milk, and fat-and-protein-corrected milk (FPCM), presented as least squares means and SE.

Drv	Previous	Fat		Protein		Lactose	2	Milk		FPCM	
period	dry period	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No	All	366 a	10								
	No			298 ^b	9	366 ^b	12	26.9 ^b	0.8	29.3 ^b	0.8
	Short			302 ^b	10	371 ^b	14	27.3 ^b	0.9	29.4 ^b	0.9
	Standard			284 a	9	339 a	12	25.0 ª	0.8	27.5 a	0.8
	Long			299 ^{abc}	12	362 ^{ab}	18	26.6 ab	1.2	29.2 abc	1.2
Short	All	391 ^b	10	317 ^c	9	395 ^c	11	28.9 ^c	0.8	30.9 ^c	0.8
Standard	All	422 ^c	9	327 ^d	9	420 d	11	30.6 ^d	0.7	32.7 ^d	0.8
Long	All	424 ^c	10	328 ^d	9	428 ^d	12	31.2 ^d	0.8	33.1 ^d	0.8

^{a-c}Different letters within the same column indicate different means (P < 0.05; n=2010 lactations). ¹Fat, protein, and lactose in total 305-d yield (kg); milk and FPCM in kg per day

3.2 Analysis 2: impact of cow characteristics on prediction of effective lactation yield

The basic model to predict effective lactation yield (kg FPCM per cow per day) consisted of a random herd effect, the covariate first parity 305-d yield, parity, DP, and a DP × parity interaction (Table 5). Compared with a standard DP, the FPCM effective lactation yield of parity \geq 3 cows was reduced by 2.2 kg per day in case of no DP (2.0 kg in analysis 1) and 1.1 kg in case of a short DP (1.0 kg in analysis 1). Second parity cows, despite fewer days open (Table 3), had lower effective lactation yields than older cows, and a greater FPCM loss (2.8 kg per day) when no DP was applied as compared to a standard DP (P < 0.05; Figure 3). Van Knegsel et al. (2014b) also reported a greater reduction in milk yield for second parity cows than for older cows when the DP was omitted. One explanation could be that continued mammary development between the first and second lactation is impaired when the DP is omitted, resulting in a greater reduction in milk yield for second parity cows than for older cows (Collier et al., 2012).



Figure 3. Effect of dry period (legend) and parity (par) category on effective lactation yield (n=2866 lactations). Data are presented as least squares means and SE. Different letters within the same parity category indicate differences between means. FPCM = fat-and-protein-corrected milk.

The main question in analysis 2 was whether the prediction of individual cows' effective lactation yield after different DP lengths could be improved by including individual cow characteristics into the model. If variation between cows in response to DP length could be predicted more precisely, this could be used for tailored decisions about DP length (Grummer and Rastani, 2004).

The 2 test-day records before the DP decision, that provided individual cow characteristics for the extended model, occurred at 88 (SD: 12) d and 121 (SD: 15) d before calving. The variables for SCC at the last test-day (as binary and continuous variable) did not improve predictions and were eliminated from the final extended model ($P \ge 0.05$). The final extended model did include calving interval and yield variables (Table 5). A lower persistency between the last 2 test-days, higher 305-d and last test-day yields, and more days open in the previous lactation were all related to a higher effective lactation yield in the subsequent lactation. These relations were irrespective of DP length, except that a higher yield at last test-day resulted in a smaller increase in effective lactation yield for cows with no DP than for cows with a standard DP.

The positive relations of recent yield with effective lactation yield may be expected because cows with a high yield at the last test-day and a high 305-d yield before calving are likely high-yielding cows in an extended lactation and after calving. The correlation of yield level across lactations was our motivation to include first parity 305-d yield in the basic model as a covariate of lactation yield potential. First parity 305-d yield was less important in the extended model than in the basic model, implying that the inclusion of other variables made this variable partially redundant. Indeed, previous 305-d yield was identical to, or highly correlated with, first parity 305-d yield (identical for parity 2 and r: 0.61 for parity \geq 3), and yield at the last test-day also correlated with first parity 305-d yield (r: 0.67 for parity 2 and r: 0.33 for parity \geq 3).

	Basic	model		Extende	ed model	
Effect	β	SE	Р	β	SE	Р
Constant ³	17.1	0.68	< 0.01	8.8	0.97	< 0.01
Level ⁴	1.4	0.06	< 0.01	0.8	0.08	< 0.01
Parity						
2 versus ≥ 3	-1.7	0.17	< 0.01	-0.6	0.69	0.35
Dry period			< 0.01			0.89
No versus standard	-2.2	0.23	< 0.01	0.1	0.58	0.84
Short versus standard	-1.1	0.22	< 0.01	-0.2	0.58	0.74
Long versus standard	-0.5	0.27	0.07	0.1	0.60	0.83
Dry period × parity ⁵			0.02			0.01
No \times parity 2	-0.6	0.30	0.04	-0.8	0.29	< 0.01
Short × parity 2	0.3	0.29	0.30	0.1	0.29	0.67
Long × parity 2	0.6	0.41	0.16	0.2	0.41	0.65
Extra Cow Characteristics						
Persistency before last test-day ⁶				-2.8	0.71	<0.01
Yield at last test-day				0.16	0.02	< 0.01
Previous 305-d yield				0.10	0.02	<0.01
Previous In(days open)				1.25	0.14	<0.01
Yield at last test-day $ imes$ parity						
2 versus ≥ 3				0.07	0.03	0.02
Previous 305-d yield × parity						
2 versus ≥ 3				-0.07	0.03	0.03
Yield at last test-day \times dry period						<0.01
Yield at last test-day × no				-0.11	0.03	<0.01
Yield at last test-day \times short				-0.04	0.03	0.10
Yield at last test-day × long				-0.01	0.03	0.63
SD of residual (n lactations)	3.56	(2866)		3.33	(2803)	

Table 5. Final basic model¹ and extended model² for effective lactation yield [kg fat-and-proteincorrected milk (FPCM) per cow per day; coefficient and SE]; and the SD of the residuals for predictions per lactation for each model.

¹Basic model: based on dry period, parity, and first parity 305-d yield

²Extended model: based on variables of the basic model and individual cow characteristics before calving

³Population mean of cows in parity 3 and older, with a standard dry period

⁴First parity 305-d yield in tonnes of FPCM

⁵Compared with cows in parity 3 and older, with a standard dry period

⁶Last test-day before 70 d before calving

The negative relation between persistency before calving and effective lactation yield seems to be in contrast with the positive relation between yield before calving and effective lactation yield. Although there was no interaction between persistency and parity (2 versus \geq 3) to explain effective lactation yield, this relation may be explained partly by younger cows being more persistent and at the same time having lower (effective lactation) yields than older cows (Santschi et al., 2011a). Moreover, the positive relation between days open and effective lactation yield may be caused by a weak positive correlation (r: 0.18) of (the natural logarithm of) days open with first parity 305-d yield, which could be explained by impaired fertility in cows with higher yield levels (Butler, 2003). Compared with the basic model, the SD of the residuals of the extended model was reduced by only 6%. The extended model did not add much insight and, therefore, the basic model seems sufficient to predict the effect of DP length on effective lactation yield. The correlations between different yield variables may explain why additional yield variables barely improved the fit of the model.

Although additional information on cow characteristics did not improve predictions of effective lactation yield, variables such as SCC, milk yield, and persistency around 3 months before calving can be relevant in a tool to select the best DP strategy for a dairy cow for other reasons than effective lactation yield. For example, high yield and high persistency before calving can pose a risk for cow welfare when drying off (Rajala-Schultz et al., 2005; Zobel et al., 2015), and at the same time indicate that the cow would be capable of a continuous lactation. In contrast, high yield levels and low persistency at 3 months before expected calving could indicate that drying off at a month before calving, when yield is likely much lower, is suitable, whereas no DP is not feasible. Finally, high SCC could be indicative of an intramammary infection, which could require a DP to facilitate treatment with an intramammary antibiotic.

4 Conclusions

Shortening or omitting the dry period (DP) can improve the energy balance of dairy cows in early lactation through a decrease in milk yield after calving. Cows submitted to short DP produced less milk than cows submitted to standard DP, but the quantity of this loss did not change when a short DP was applied over consecutive lactations. Consecutive omissions of the DP also decreased milk production as compared with a standard DP. Consecutive omission of the DP reduced the additional milk produced before calving and increased the milk production after calving, compared with the first omission of the DP. Individual cow characteristics did not improve the prediction of individual response of yield to DP length based on parity and first lactation 305-d yield, but may be relevant to make a practical decision about DP length. Further study is needed to assess the impact of short or no DP on farm performance; the reduced milk yield may be compensated by improved health and fertility, which could increase cow longevity.

Chapter 7

Effects of dry period length on production, cash flows and greenhouse gas emissions of the dairy herd: A dynamic stochastic simulation model

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Abstract

Shortening or omitting the dry period of dairy cows improves metabolic health in early lactation and reduces management transitions for dairy cows. The success of implementation of these strategies depends on their impact on milk yield and farm profitability. Insight in these impacts is valuable for informed decision-making by farmers. The aim of this study was to investigate how shortening or omitting the dry period of dairy cows affects production and cash flows at the herd level, and greenhouse gas emissions per unit of milk, using a dynamic stochastic simulation model. The effects of dry period length on milk yield and calving interval assumed in this model were derived from actual performance of commercial dairy cows over multiple lactations. The model simulated lactations, and calving and culling events of individual cows for herds of 100 cows. Herds were simulated for 5 years with a dry period of 56 (conventional), 28 or 0 days (n = 50 herds each). Partial cash flows were computed from revenues from sold milk, calves, and culled cows, and costs from feed and rearing youngstock. Greenhouse gas emissions were computed using a life cycle approach. A dry period of 28 days reduced milk production of the herd by 3.0% in years 2 through 5, compared with a dry period of 56 days. A dry period of 0 days reduced milk production by 3.5% in years 3 through 5, after a dip in milk production of 6.9% in year 2. On average, dry periods of 28 and 0 days reduced partial cash flows by €1,249 and €1,632 per herd per year, and increased greenhouse gas emissions by 0.8% and 0.5%, respectively. Considering the potential for enhancing cow welfare, these negative impacts of shortening or omitting the dry period seem justifiable, and they might even be offset by improved health.

1 Introduction

A dry period (DP) of 6 to 8 weeks is common practice in dairy cow management (Arnold and Becker, 1936). The DP facilitates the renewal of udder tissue and results in maximum milk yield after calving (Capuco et al., 1997; Kuhn et al., 2005). The DP starts with the forced cessation of milk production (drying off) and is often accompanied by ration and group changes. These procedures may cause pain (due to udder pressure), hunger, and frustration, and may therefore impair welfare of high-producing dairy cows in the period before calving (Zobel et al., 2015). Moreover, the high milk yield and limited feed intake in the first months of lactation result in a negative energy balance (Rastani et al., 2005; Van Knegsel et al., 2014b). This negative energy balance is associated with metabolic disorders and reduced fertility and thus impaired animal welfare (Collard et al., 2000; Butler, 2003).

Shortening or omitting the DP of dairy cows can improve cow welfare through fewer management changes (Zobel et al., 2015; Kok et al., 2017a) and better metabolic health in early lactation (Andersen et al., 2005; Rastani et al., 2005). Both shortening and omitting the DP improved the energy balance through a reduced milk yield, and a similar or increased feed intake in the subsequent lactation (Rastani et al., 2005; Van Knegsel et al., 2014b). The implementation of short or no DP, however, will depend on the impact of these management strategies on factors such as herd level milk yield and farm profitability.

The effect of shortening or omitting the DP on milk yield at the herd level cannot be easily extrapolated from yield losses at the cow level. Effects of DP length also depend on herd composition, because milk yield of heifers is unaffected by DP length, whereas second parity cows experience greater reductions in milk yield than older cows (Kok et al., 2017b). Moreover, effects of DP length on milk yield are dynamic: yield reductions due to omission of the DP decreased when no DP was applied over multiple subsequent lactations (Chen et al., 2016a; Kok et al., 2017b). Also, the reduction in milk yield when the DP is shortened or omitted can be compensated partly by shorter calving intervals (CI) (Kok et al., 2016), that could result from improved fertility (Gümen et al., 2005; Chen et al., 2015b).

The economic impact of DP length at the farm level depends on more factors than changes in total milk yield. Compared with a conventional DP, shortening and omitting the DP were found to increase milk protein content, whereas fat content appeared unaffected (Van Knegsel et al., 2013), which increases revenues when the payment system is based on milk solids. Omission of the DP improved metabolic health and reduced veterinary costs in a study on commercial dairy farms

(Köpf et al., 2014), although results from experimental studies on effects of DP length on disease incidence remain unclear (Van Knegsel et al., 2013). An improvement in cow fertility could reduce economic losses (Inchaisri et al., 2010b) and involuntary culling rates (Mohd Nor et al., 2014). Heeren et al. (Heeren et al., 2014) showed that a reduction in culling rate from 37% to 24% could financially compensate an assumed reduction in milk yield of 13% due to omission of the DP.

Some studies evaluated economic impacts of shortening or omitting the DP on commercial farms (Santschi et al., 2011a; Köpf et al., 2014), and some modelled the economic impact of varying DP lengths at the herd level using either experimental (Sørensen et al., 1993) or commercial data (Heeren et al., 2014). These evaluations, however, were based on comparisons of the first lactation after a change in DP length, and did not assess dynamic long-term effects on milk yield or fertility. Insight in the expected milk production at the herd level over time is valuable for informed decision-making on DP length management by farmers.

A change in DP length management might not only affect farm profitability, but also the environmental impact of milk production. One of the major global environmental challenges is climate change (Steffen et al., 2015), induced by emissions of greenhouse gases (GHG). Dairy cattle are responsible for about 30% of the GHG emissions produced by the global livestock sector (Gerber et al., 2013), and for about 30-40% of the emissions produced by the European livestock sector (Lesschen et al., 2011; Weiss and Leip, 2012). A major part of the GHG emissions along the milk production chain relate to the production and utilisation of feed (Lesschen et al., 2011; Gerber et al., 2013). Shortening or omitting the DP could be accompanied by a change in ration, because a DP ration may no longer be necessary (Rastani et al., 2005), and a lower daily milk yield could be matched by a reduction in energy density of the lactation ration (Garnsworthy, 2004). These dietary changes can have an important influence on the level of GHGs produced (Van Middelaar et al., 2013). Moreover, changes in milk yield and fertility might affect efficiency of milk production and, therefore, may affect GHG emissions per unit of milk produced (Garnsworthy, 2004; Van Middelaar et al., 2014). Shortening or omitting the DP also improves metabolic health and could lengthen the productive life of dairy cows, which would dilute the GHG emissions related to the rearing phase (Van Middelaar et al., 2014). To our knowledge, no evaluations of the impact of DP length on GHG emissions of milk production have been made.

The aim of this study was to investigate how shortening or omitting the DP of dairy cows affects technical and economic results at the herd level, and GHG emissions per unit of milk, using a dynamic stochastic simulation model. The effects of DP length on milk yield, CI, and cow fertility in this model were based on actual performance of commercial dairy cows over multiple lactations.

2 Materials and methods

2.1 Cow simulation model

A dynamic stochastic simulation model was developed in R version 3.3.1 (R Core Team, 2016) to assess how DP length affects milk production, calving, and culling at the dairy herd level over time. The model generates an average Dutch herd with 100 cow places. Each of the cow places contains one individual cow at a time, that is simulated per lactation (Figure 1). Each lactation starts with the birth of a calf, either from a healthy cow that remained in the herd or from a replacement heifer, and ends with next calving or culling of the cow. Instead of fixed daily or weekly time steps, the time steps in the developed simulation model are of a variable duration. A new time step starts when a cow calves or is culled, and when a new calendar year starts. The use of calendar years in the time steps enables the aggregation of simulated data per herd per year. When the current lactation ends before the calendar year, the whole lactation is one time step. When the current lactation exceeds the remaining number of days in the calendar year, and another that starts in the next year and ends at calving or culling. Per time step per cow place, the model records the produced milk, calves, and culled cows, and computes the associated energy requirements.

To simulate lactations of cows in cow places over time, lactation curves, CI, and culling (probability and timing) were modelled. Input values for each DP length were derived from milk production data (2007-2015) from 16 Dutch dairy farms that deliberately shorten or omit the DP since 2010/2011 and applied conventional DP (≥ 6 weeks) before (Kok et al., 2016, 2017b). The modelling and input values for milk production, CI and culling are described in more detail below.

Milk production

Lactation curves were used to simulate milk production of cows after a DP of 56, 28, or 0 days. Individual milk production (MP) in kg of cow *i* in parity *j* with DP category *l* at each day in milk (DIM) was calculated as:

 $MP_{ijl} = a_{jl} + b_j \times DIM + c \times exp(-k \times DIM) + RPL_i \times ADY_{jl},$

where RPL_i is the relative production level of cow *i*; ADY_{jl} is the average daily 305-d yield in kg milk of a cow in parity *j* with DP category *l*, and a, b, c, and k model the shape of the lactation curve (Wilmink, 1987).

The RPL was drawn from a normal distribution with a mean of o and standard deviation of 0.1, to

reflect natural variation in milk production from about 80% to 120% of the average lactation (Inchaisri et al., 2010b). All other parameters in the lactation curve were fixed (Table 1).



Figure 1. Schematic representation of the simulation model of lactations within cow places. Each cow place starts with a cow with an individual production level and parity, with a previous dry period of 56, 28, or 0 days. At the start of each lactation, cows are stochastically assigned to a healthy lactation and continuation to the next lactation, or to being culled (for general reasons or due to fertility issues) and replaced by a new heifer. Stochastic events are marked with an asterisk. Output of milk, calves and culled cows from these processes and the associated energy requirements of the cows are recorded.

Table 1. Model inputs for individual lactation curves per dry period length. The average daily 305-d milk yield (ADY); parameters a and b of the Wilmink lactation curves; and fat, protein, and lactose content of the milk per parity class per dry period (DP) category. Parameter c was -16.1 and parameter k was 0.06.

Parity	DP length (days)	ADY (kg)	а	b	Fat (%)	Protein (%)	Lactose (%)
1	-	23.9	31.6	-0.0447	4.48	3.55	4.62
2	56	28.9	40.6	-0.0708	4.50	3.59	4.53
	28	25.9	37.6	-0.0708	4.64	3.75	4.55
	0	22.1	33.8	-0.0708	4.81	3.93	4.51
>2	56	30.5	44.1	-0.0835	4.51	3.51	4.48
	28	27.7	41.3	-0.0835	4.49	3.62	4.48
	56-0ª	24.4	38.0	-0.0835	4.60	3.71	4.41
	0-0 ^a	27.0	40.6	-0.0835	4.53	3.62	4.41

^a56-0: no DP in the current lactation after a DP of 56 days in the previous lactation; 0-0: no DP in the current lactation after no DP in the previous lactation.
Production level (parameter a) was assumed to be affected by parity class and DP category (Van Knegsel et al., 2014b; Kok et al., 2017b); persistency (parameter b) was assumed to be affected by parity only (Chen et al., 2016a); parameter c was assumed not to be affected by parity or DP length (best model fit based on BIC values); and parameter k was set to 0.06 (Kok et al., 2017b). To compute values for parameters a, b, and c, Wilmink lactation curves (Wilmink, 1987) were fitted on the raw test-day milk records per parity class per DP category, using a mixed model in SAS version 9.3 (SAS Institute Inc.; Kok et al., 2016, 2017b) (Appendix Table 7.a). The model included random effects on a, b, and c for repeated measures per cow lactation assuming unstructured covariance (Kok et al., 2017b). Milk records were grouped in the parity classes 1, 2, and >2 to model the difference in persistency and effect of DP length on parity, and in DP categories standard DP (6-12 weeks), short DP (3-5 weeks), and no DP (0-2 weeks), to represent the model DP lengths of 56, 28, and o days. Because the effect of no DP on milk production depends on the previous DP length (Kok et al., 2017b), the last category was split up in two subcategories: no DP preceded by a standard DP, and no DP for multiple lactations.

Average protein, fat, and lactose content of the produced milk were calculated per parity class per DP category, and used to parameterise the milk composition of the simulated lactation curves. Previous research already indicated interaction effects of parity and DP length for these variables (Van Knegsel et al., 2014b). Milk yield of each cow was computed per cow space per time step, using the integral of the MP function. If the individual daily milk production reached o kg before the designated moment of dry-off, occurrence of the spontaneous dry-off was recorded.

Calving interval

The model randomly assigned a CI to each lactation based on parity class and DP category, except when the cow was culled due to fertility issues. It was assumed that DP length affected CI, because a reduction in CI due to shortening or omitting the DP has been reported on commercial farms (Santschi et al., 2011a; Köpf et al., 2014; Kok et al., 2016). The CI data in this model were taken directly from the same dataset as the milk production data, clustered per parity class per DP category (Table 2) (Kok et al., 2016, 2017b). Calving intervals exceeding 518 days were discarded, to reflect that attempts of insemination would cease 34 weeks after calving (Rutten et al., 2014) to reduce economic losses due to longer CI (Inchaisri et al., 2010b).

Culling

Within a cow space, each lactation of a cow is stochastically assigned to one of three categories: healthy, culled due to fertility issues (fertility culling), or culled for other reasons (general culling).

When a cow is culled, she is replaced by a heifer that is assumed to calve and to enter the herd the following day. This is a simplified version of the assumption that some heifers enter the herd before a cow is culled (overstocking), whereas others replace culled cows with a possible delay, thus leaving a cow space empty for some time (Rutten et al., 2014).

The probability of fertility culling varied based on parity class and DP category (Table 2). It was assumed that CI in the unfiltered dataset that exceeded 518 days would result in fertility culling in the model (Rutten et al., 2014). Therefore, the probability of fertility culling per parity class per DP category was set equal to the percentage of CI exceeding 518 days in the unfiltered dataset. This was about 8% of the lactations for cows with a standard DP. Cows assigned to fertility culling did not become pregnant and were culled when their milk production dropped below 15 kg per day (Rutten et al., 2014).

The probability of general culling was constant across parities and DP lengths, and was set at 0.22 per lactation to create an overall culling rate (fertility culling and general culling) of about 30% for cows with a standard DP (Mohd Nor et al., 2014). General culling occurred at a certain fraction of completion of a cow's assigned CI, drawn from a distribution with a positive skew and a median fraction of 0.17 (beta distribution with parameters a = 1.3, b = 5 (Rutten et al., 2014)).

Table 2. Model inputs for calving intervals and fertility culling per dry period length. Distribution of calving interval (CI) records (median days, 5 and 95 percentiles, n) used as model input, and fraction of records exceeding 518 days (Pfertility culling), per parity class per dry period (DP) category. Records exceeding 518 days were excluded from the dataset before descriptives were computed.

Parity	DP length (days)	Median CI	Р5	P95	n	Pfertility culling
1	-	374	327	477	2,348	0.080
2	56	381	330	487	1,116	0.075
	28	365	325	482	495	0.052
	0	359	316	464	342	0.039
>2	56	385	333	489	1,850	0.078
	28	378	328	480	629	0.074
	0	370	321	473	573	0.037

2.2 Simulation and model outputs

The model herd started on day 1 with 100 cows, with a fixed number of cows from parity 1, 2, 3, 4, and >4 (30, 21, 15, 10, and 24, respectively) to reflect a 30% culling rate. The model was run for 5 years with a standard DP of 56 days to introduce variation in initial herds. For each DP length (56, 28, and 0 days), 50 herds were simulated, to get insight in the degree of variation in technical performance due to stochasticity. At the start of the 6th year, average herd composition of the 150

herds was equal to the input herd composition (29.8, 20.7, 14.9, 10.4, and 24.2 cows in parity 1, 2, 3, 4, and >4, respectively), with SD of 3.0 to 5.1 cows per parity class. The 6th year was used as a baseline situation (year 0), and scenarios with a DP of 28 or 0 days were implemented from the start of the 7th year (year 1 after change in DP length). Each herd was simulated for 5 years following implementation of the new DP length in year 7. Preliminary data showed that additional herds hardly changed the average and range of model results.

Sensitivity analysis

A sensitivity analysis was performed to assess the effect of general culling rate and of assumed effects of DP length (on milk production, CI, and fertility culling) on model results. The probability of general culling was 0.22 in the model, resembling an overall culling rate of about 30% for cows with a standard DP. Culling rates for Dutch dairy farms, however, commonly vary from 20% to 35% between farms (Mohd Nor et al., 2014). A change in culling rate could affect the effect of DP length on milk yield through a different herd composition, and through more lactations being terminated in early lactation. To assess this impact, the probability of general culling was changed to 0.12, 0.17, and 0.27 in the sensitivity analysis, creating overall culling rates of 20%, 25%, and 35% for cows with a standard DP, respectively.

Dry periods of 28 and 0 days were assumed to reduce milk production, shorten CI, and reduce fertility culling compared with a DP of 56 days. Reductions in milk production varied between 2.8 to 6.8 kg milk per day in the model input, depending on parity and DP length. In the sensitivity analysis, the impact of a greater or lesser reduction in milk production was assessed. To assess the impact of shorter CI and reduced fertility culling in case of a DP of 28 or 0 days, two more scenarios were assessed in which CI or fertility culling was not affected by DP length (i.e. input values from the DP of 56 days were used).

Energy requirements and ration composition

Energy requirements for maintenance, milk production, growth (for parity 1 and 2), and gestation were computed per time step according to the Dutch net energy evaluation system in VEM (1,000 VEM = 6.9 MJ of net energy) (Van Es, 1975), using the parity, weight, milk production, and pregnancy status of the cow (CVB, 2012). Maintenance requirements are 42.4 VEM per kg^{0.75} of body weight (CVB, 2012). Body weight linearly increased from 540 kg at first calving to 595 kg at second calving, and to 650 kg at third calving. Cows had fixed energy requirements for growth in parity 1 (660 VEM per day) and parity 2 (330 VEM per day) and in the last 4 months of pregnancy (450, 850, 1,500, and 2,700 VEM per day, respectively) (CVB, 2012). It was assumed that the

lactating cows were grazing for 8 hours per day in the summer period for 170 days per 365 days (CBS, 2014), and that grazing increased energy requirements for maintenance by 6.7% (CVB, 2012). It was assumed that dry cows were housed indoors, which is, based on the experience of the authors, generally the case.

Feed requirements were computed using an average Dutch ration for (lactating and dry) dairy cows in the summer and winter period (Table 3) (CBS, 2014). Roughage consisted of grass, grass silage and maize silage, and was supplemented with byproducts and concentrate (CBS, 2014; Mostert et al., 2018a). In case of a DP of 28 or 0 days, a second ration was composed, in which the energy content of the average Dutch ration was reduced to simulate a potential change in feeding management (Garnsworthy, 2004; Van Hoeij et al., 2017). This was done by first computing the ration for an average day for a cow with a DP of 56 days, based on her average energy requirements per day (CVB, 2012; Vellinga et al., 2013). Subsequently, the amount of concentrate was reduced to match the average energy requirements per day of cows in herds with DP of 28 or 0 days. To keep a comparable intestinal digestible protein to net energy ratio in the ration, standard concentrate was exchanged for protein-rich concentrate (Tamminga et al., 1994). Effects of a DP of 28 or 0 days are presented for the average Dutch ration; the impact of the potential reduction in concentrate is presented separately. Because the average daily energy requirements were very similar for herds with a DP of 28 or 0 days from year 3 after the change in DP length onwards, the alternative ration was computed using the average energy requirement of herds with a DP of 28 or 0 days from year 3 to year 5, based on a reduction of 2.2 MJ per cow per day in winter and 1.8 MJ in summer compared with herds with a DP of 56 days.

2.3 Calculation of partial cash flows

A partial cash flow analysis was performed to assess economic consequences of shortening or omitting the DP at the herd level. This analysis included revenues from sold milk, calves, and culled cows, and costs from buying or producing feed and rearing youngstock (Table 4).

Milk revenues were according to the Dutch payment system based on milk solids (value of protein:fat:lactose of 10:5:1), using the average Dutch milk price over the period 2008-2016 (FrieslandCampina, 2016). Revenues for surplus calves and culled cows, as well as the costs of raising a heifer were computed from yearly values over the period 2008-2016 taken from Wageningen Economic Research (Wageningen Economic Research, 2017a). It was assumed that 50% of the calves were male and 50% of the calves were female; and that the number of female

calves retained for replacement equalled 113.4% of the number of culled cows, to account for 13.4% mortality during the rearing phase; and that 7% of surplus calves died on farm (KWIN-V, 2014). Feed costs were calculated from Dutch feed prices per feedstuff (KWIN-V, 2014). Partial cash flows were computed per herd per year, and expressed as difference in partial cash flow compared with a DP of 56 days.

Table 3. Composition and specifications of the average Dutch ration for dairy cows and of a ration reduced in concentrate designed for herds with a DP of 28 or 0 days, split in a winter ration (195 days per year) and a summer ration (170 days per year).

	Average Du	utch ration ^a	Reduced conce	entrate ration
	Winter	Summer	Winter	Summer
Composition (% of DM)				
• Grass	0.0	39.0	0.0	39.5
Grass silage	55.1	25.2	55.9	25.6
Maize silage	13.7	10.9	13.9	11.0
 Wet by-products^b 	4.8	3.8	4.9	3.8
 Normal concentrate^c 	19.7	21.1	17.4	19.6
• Protein concentrate ^c	6.8	0.0	7.9	0.5
Net energy (MJ/ kg DM) ^d	6.5	6.8	6.5	6.8
GHG emissions (kg CO2e per t DM) ^e				
 Feed production 	468	470	463	466
Enteric fermentation	574	585	572	584

^aBased on (CBS, 2014)

^bWet by-products include brewers grain, potato peel, potato pulp, and maize gluten meal ^cProtein concentrate has more soybean hulls, palm kernel expeller, and distillers grains and solubles than standard concentrate per kg DM, and less maize and wheat middlings.

 $^{\rm d}$ Calculated with the Dutch net energy evaluation (VEM) system (Van Es, 1975) $^{\rm e}$ Based on (Vellinga et al., 2013)

Table 4. Costs and revenues of parameters used to compute partial cash flows.

	Value (€)
Milk revenues (per 100 kg solids) ^a	
Protein	576.48
• Fat	288.25
Lactose	57.65
Calves revenues (per animal) ^b	
Female calf	51.00
Male calf	109.00
Culled cows (per kg meat) ^{b,c}	2.32
Replacement heifer (per animal) ^b	969.00
Feed costs (per t DM) ^d	
Summer ration	167.80
Winter ration	202.30
 Summer ration low concentrate 	167.00
 Winter ration low concentrate 	202.00

^aThis results in €35.32 per 100 kg milk with average solids content (3.47% protein, 4.41% fat and 4.51% lactose), corresponding to the average Dutch milk price 2008-2016 (FrieslandCampina, 2016) ^bAverage of Dutch values from 2008-2016 (Wageningen Economic Research, 2017a) ^cAssumed dressing percentage of 60% (Rutten et al., 2014) ^dBased on (KWIN-V, 2014)

2.4 Calculation of greenhouse gas emissions

To assess the impact of shortening or omitting the DP on GHG emissions, a life cycle approach was used. Emissions of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) were computed for all processes along the milk production chain that were assumed to be affected by a change in DP length, including feed production, enteric fermentation, and manure management. Accounting for feed production (Vellinga et al., 2013), enteric fermentation (Vellinga et al., 2013), manure management (De Mol and Hilhorst, 2003; Velthof and Mosquera, 2011; Vries et al., 2011; RVO, 2015; Vonk et al., 2016) and mortality in the rearing phase (assuming an age at first calving of 24 months) (KWIN-V, 2014), GHG emissions related to the rearing of young stock were estimated to be 4,905 kg CO₂ equivalents per replacement heifer. GHG emissions of the dairy cows were computed from the model results using the same method. Emissions related to feed production included: production of inputs (e.g. fertiliser and machinery), cultivation, harvest, and processing of the feed products, and transport to farms (Vellinga et al., 2013). Economic allocation was used in case of a multiple output process (e.g. production of soybean meal also results in soybean oil), because feed ingredients and their co-products can be used in many pathways (e.g. feed, food, biofuel) and have distinct characteristics (nutritional values) which makes system expansion and physical allocation undesirable (International Dairy Federation, 2015; Mackenzie et al., 2017). Emissions related to enteric fermentation were calculated with feed specific emission factors (Vellinga et al., 2013).

Emissions related to manure management were calculated from the volume of manure and the nitrogen excretion. Nitrogen excretion was computed as the difference between nitrogen intake from feed and nitrogen retention for milk production, growth, and gestation (RVO, 2015). Moreover, it was assumed that during the grazing period, 1/3 of the manure was excreted during grazing (8 hours per day), and 2/3 was excreted in stables and subsequently stored; which resulted in different GHG emission factors (Appendix Table 7.b). Factors for N₂O, NH₃, NO_x and CH₄ emissions and NO₃⁻ leaching from manure on pasture and in the stable were taken from Dutch national inventory reports (De Mol and Hilhorst, 2003; Velthof and Mosquera, 2011; Vries et al., 2011; Vonk et al., 2016), and emission factors from NH₃, NO_x and NO₃⁻ to N₂O (i.e. indirect N₂O emissions) were taken from IPCC (Dong et al., 2006). All GHG emissions were converted to CO₂ equivalents, based on their equivalence factor in terms of CO₂ (100-year time horizon): 1 for CO₂, 28 for biogenic CH₄, 30 for fossil CH₄, and 265 for N₂O (Myhre et al., 2013). Total GHG emissions were expressed as CO₂ equivalents per kg fat-and-protein-corrected milk (FPCM). System

expansion was used to account for the production of meat from calves and cows (Van Middelaar et al., 2014). The production of meat from surplus calves (as white veal) and cows was assumed to substitute the production of other meat on the basis of kg edible product. The model accounted for additional GHG emissions related to rearing (calves), transport and slaughter (Van Middelaar et al., 2014), and for avoided GHG emissions related to the production of poultry, pigs and cows elsewhere (Mostert et al., 2018b).

3 Results

3.1 Technical results

The milk production, number of calves born and cows culled per herd (with 100 cows) per year are presented in Table 5. In the baseline year, all herds applied a DP of 56 days, and the average milk production per herd varied 0.4% between the DP strategies due to stochasticity (from 873,285 kg to 876,433 kg; n = 50 herds each). Herds that switched to a DP of 28 days had a higher average milk production in the first year the strategy was applied (+7,283 kg; +0.8%), and then seemed to stabilise at an average milk production of 845,987 kg per year from year 2 until year 5, which was 3.1% lower than herds with DP of 56 days (-37,869 kg per year). Herds that switched to a DP of 0 days also had a slightly higher average milk production than herds with a DP of 56 days in the first year the strategy was applied (+4,244 kg; +0.5%). In year 2, the average milk production was 812,275 kg, which was 6.9% lower than of herds with DP of 56 days (-60,117 kg). From year 3 until year 5, average milk production of herds with a DP of 0 days was 842,360 kg, which was 3.5% lower than herds with a DP of 56 days (-30,452 kg per year). Variation between herds was similar for different DP lengths (Figure 2A), with an average coefficient of variation of 1.4% on herd averages per year.

On average, 114 calves were born per herd per year in case of a DP of 56 days. From year 2, the number of calves born increased by 3 calves per year when a DP of 28 days was applied, and by 5 calves when a DP of 0 days was applied, compared with a DP of 56 days. Variation in the number of calves born between herds was similar for different DP lengths, with an average coefficient of variation of 5.0%. On average, 34 cows were culled per herd per year in case of a DP of 56 days. The number of culled cows appeared to be about 1 less when a DP of 0 days was applied, but variation between herds was large with an average coefficient of variation of 17.7%. In case of a target DP length of 0 days, some cows spontaneously dried themselves off, resulting in an average of about 1 day dry per cow per year.

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	DP	Year	0	Year	1	Year	2	Year	3	Year	4	Year	5
Output variable	(days)	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Milk (t)	56	873	13	872	12	872	10	871	11	872	12	875	10
	28	876	11	880	13	848	12	848	12	844	15	845	11
	0	875	10	877	11	812	10	839	13	843	12	845	12
FPCM ^a (t)	56	936	14	935	13	935	11	934	12	934	12	937	11
	28	939	12	946	14	914	12	914	13	910	16	911	11
	0	937	11	947	12	885	10	911	13	915	12	917	13
calves (n)	56	114	7	114	5	113	5	114	6	112	6	114	4
	28	114	6	114	6	115	6	117	6	116	6	117	5
	0	113	5	114	5	118	5	118	6	118	6	118	7
Cows culled (n)	56	34	7	34	6	33	5	34	7	32	6	34	5
	28	34	6	35	6	32	6	35	6	34	6	35	5
	0	33	5	35	7	32	6	32	6	32	5	32	6
Days dry ^b (n)	56	45	2	45	2	45	2	45	2	45	2	44	2
	28	44	2	22	1	23	1	23	1	23	1	23	1
	0	45	2	4	1	1	1	1	1	1	1	1	1
NE winter ^c (MJ)	56	122	1	121	1	121	1	121	1	121	1	122	1
	28	122	1	122	1	119	1	119	1	119	1	119	1
	0	122	1	122	1	117	1	119	1	119	1	120	1
NE summer ^c (MJ)	56	126	1	126	1	126	1	126	1	126	1	126	1
	28	126	1	127	1	124	1	124	1	124	1	124	1
	0	126	1	127	1	122	1	124	1	124	1	125	1

Table 5. Technical simulation results: average production, days dry and energy requirements per herd per year for herds with a dry period (DP) of 56 days in year 0, and a DP of 56, 28, or 0 days from year 1. Average values per herd (100 cows) per year and SD are presented (n = 50 herds per DP length).

^aFPCM = fat-and-protein-corrected milk

^bTotal days without milk production per cow per year

^cNE = Net energy requirement per cow per day

Effects of the model assumptions for general culling rate and for effects of DP length (on milk production, CI, and fertility culling) on average herd milk production are presented in Figure 3. For all DP lengths, a lower general culling rate resulted in a higher herd milk production (Figure 3A-C), but the impact of a change in general culling rate was smaller in case of a DP of 28 or 0 days. A reduction in general culling rate could not compensate milk losses due to a DP of 28 or 0 days. Assuming different milk reductions due to a DP of 28 or 0 days had a large impact on herd milk production, and lessening milk reductions by 2 kg per day in lactation resulted in higher herd milk production in fertility culling compared with a DP of 56 days (Figure 3D-F). Assuming no reduction in fertility culling compared with a DP of 56 days hardly reduced herd milk production in case of a DP of 28 or 0 days.



Figure 2. Impact of dry period length on milk production, partial cash flow, and greenhouse gas emissions. (A) Milk production per herd per year, (B) difference in partial cash flow, and (C) difference in greenhouse gas emissions per t fat-and-protein-corrected milk (FPCM) compared with mean of herds with a dry period of 56 days (reference line), over a period of 6 years for herds with a dry period of 56 days (white box plots), and herds that switched to a dry period of 28 days (light grey) or 0 days (dark grey) in year 1, following a dry period of 56 days in year 0.

3.2 Economic impact: partial cash flow

In the reference scenario, where DP length affected milk production, CI, and fertility culling, a DP of 28 or 0 days increased the average partial cash flow in the first year the strategy was applied (Figure 2B). From the second year onwards, however, both strategies resulted in a decreased cash flow compared with a DP of 56 days. In case of a DP of 28 days, losses from year 2 to year 5 averaged €2,608 per herd per year. In case of a DP of 0 days, losses were most severe in year 2 at €8,138 per herd, after which losses from year 3 to 5 averaged €1,705 per herd per year.

Results of the sensitivity analysis are expressed as change in cash flow in euros per year compared with herds with a DP of 56 days and a general culling rate of 22% (Table 6). For all DP lengths, a lower culling rate resulted in a higher partial cash flow. However, the difference in partial cash flow between 12% and 27% general culling was smaller for a DP of 28 or 0 days than for a DP of 56 days. Regarding the assumed effects of a DP of 28 or 0 days, partial cash flows were least sensitive to changes in the probability of fertility culling, quite sensitive to changes in CI, and most sensitive to changes in milk reduction. A reduction in concentrate in the ration in case of a DP of 28 or 0 days decreased feed costs by €132 per year at the herd level.

Table 6. Average difference in partial cash flow in euros per herd (100 cows) per year compared with a dry period of 56 days and 22% general culling for different parameter settings, following a change in dry period length to 28 or 0 days in year 1. Partial cash flows were computed as milk, meat, and calf revenues minus feed costs and youngstock costs.

	Year 1			Year 2			Year 3		
Parameter settings ^a	56	28	0	56	28	0	56	28	0
12% general culling	3,592	7,359	7,892	3,887	220	-7,757	4,313	1,083	1,050
17% general culling	2,405	5,142	5,769	2,085	-1,672	-8,450	2,578	-1,186	-576
22% general culling	REF	4,187	5,091	REF	-1,900	-8,138	REF	-1,827	-1,926
27% general culling	-2,074	1,807	2,760	-2,373	-3,733	-8,620	-2,050	-3,316	-2,936
Equal fertility culling		3,122	5,099		-2,547	-7,877		-2,829	-2,837
Equal calving interval		3,498	3,900		-4,340	-12,983		-3,390	-6,262
Milk yield +1 kg/ day		6,682	7,018		3,339	-1,960		3,598	4,330
Milk yield +2 kg/ day		9,617	10,975		10,142	5,503		11,047	12,192
Milk yield -1 kg/ day		342	2,284		-9,126	-15,178		-9,066	-9,040
Milk yield -2 kg/ day		-2,376	215		-14,174	-21,309		-14,625	-16,010

^aParameter settings were changed from the reference (REF) of 22% general culling to different general culling rates for all dry period lengths; and from the assumed reduction in fertility culling, shortening of calving interval, and quantity of milk reduction in case of a dry period of 28 or 0 days to: no effect of dry period length on fertility culling, no effect of dry period length on calving interval, or a 1 or 2 kg per day lesser or greater reduction in milk yield (assuming the same ration for all dry period lengths).

3.3 Environmental impact: greenhouse gas emissions

In the reference scenario with a DP of 56 days, GHG emissions of milk production were on average 943 kg CO_2 equivalents per t fat-and-protein-corrected milk (FPCM). On average over the 5 years, GHG emissions increased by 8 kg CO_2 equivalents per t FPCM in case of a DP of 28 days, and by 5 kg CO_2 equivalents per t FPCM in case of a DP of 0 days. These average increases were minor compared with the between-farm variation within DP strategies (Figure 2C). From year 3 onwards, GHG emissions per t FPCM were lower for a DP of 0 days than for a DP of 28 days.



Figure 3. Impact of model assumptions regarding culling and effects of dry period length on milk production. Average milk production per herd per year for different general culling rates with a dry period of 56 (A), 28 (B), or 0 days (C); and for 1 and 2 kg per day lesser or greater milk reductions, no effect on fertility culling, or no effect on CI compared with a dry period of 56 days (D) in case of a dry period of 28 (E) or 0 days (F). Results are shown for the year before and 5 years following a switch to a dry period of 28 or 0 days in year 1.

Table 7. Average change in greenhouse gas emissions in kg CO_2 equivalents per t fat-and-proteincorrected milk per herd (100 cows) per year compared with a dry period of 56 days and 22% general culling for different parameter settings following a change in dry period length to 28 or 0 days in year 1.

	Year 1	Year 1		Year 2	2			Year 3		
Parameter settings ^a	56	28	0	56	28	0	-	56	28	0
12% general culling	-42	-42	-45	-39	-29	-19		-43	-35	-41
17% general culling	-22	-25	-26	-19	-14	-3		-25	-16	-19
22% general culling	REF	-3	-5	REF	5	17		REF	9	3
27% general culling	23	16	21	30	33	36		25	29	24
Equal fertility culling		0	-7		10	26			11	13
Equal calving interval		0	-4		13	22			10	4
Milk yield +1 kg/ day		-5	-9		0	2			-3	-11
Milk yield +2 kg/ day		-13	-12		-6	-6			-14	-19
Milk yield -1 kg/ day		6	1		24	29			21	10
Milk yield -2 kg/ day		7	4		31	44			30	30

^aParameter settings were changed from the reference (REF) of 22% general culling to different general culling rates for all dry period lengths; and from the assumed reduction in fertility culling, shortening of calving interval, and quantity of milk reduction in case of a dry period of 28 or 0 days to: no effect of dry period length on fertility culling, no effect of dry period length on calving interval, or a 1 or 2 kg per day lesser or greater reduction in milk yield (assuming the same ration for all dry period lengths).

The sensitivity analysis showed that a lower culling rate resulted in lower GHG emissions per t FPCM for all DP lengths (Table 7). The effect of culling on GHG emissions was larger than any of the assumed effects of changes in DP length. Considering the assumed effects of a DP of 28 or 0 days, emissions seemed hardly sensitive to the change in CI, in case of a DP of 0 days quite sensitive to the probability of fertility culling, and most sensitive to changes in milk yield. A reduction in concentrate in the ration in case of a DP of 28 or 0 days reduced GHG emissions of milk production by 4 kg CO_2 equivalents per t FPCM.

4 Discussion

4.1 Technical results

The aim of this study was to investigate how shortening or omitting the DP of dairy cows affects technical and economic results at the herd level, and GHG emissions per unit of milk, using a dynamic stochastic simulation model. Considering the technical results, a change in DP length had a clear impact on milk yield, whereas the impact on number of calves born and cows culled was smaller than the variation between herds with the same DP. In the first year of application of a DP of 28 or 0 days, milk yield of the herd increased compared with the conventional DP of 56 days. This can be explained by the fact that all cows in the herd started the year in a lactation after a conventional DP, and this lactation was prolonged because of the shortened or omitted DP. The resulting additional yield was greater than the milk losses of cows that already entered their next lactation in year 1. Milk yields of herds with a DP of 28 days decreased by 3.1% from year 2 of the strategy, compared with a DP of 56 days. At this point, most multiparous cows started lactations following a DP of 28 days, and faced associated reductions in milk production. Milk yield of herds with no DP (o days) decreased by 6.9% in year 2 and by on average 3.5% per year from year 3 onwards, compared with a DP of 56 days. The higher milk yield from year 3 onwards can be explained by the milk yield input: cows in their second or later lactation after omission of the DP had a higher milk yield than cows in the first lactation after omission of the DP (Chen et al., 2016a; Kok et al., 2017b). From the third year onwards, most older cows will have lactations preceded by two omitted DP.

The decrease in milk production at the herd level in the current study (3.1% for a short and 3.5% for no DP) is much smaller than the reported milk losses in individual lactations following a shortened or omitted DP (4.5% for a short and 19% for no DP) (Van Knegsel et al., 2013), and smaller than calculated milk losses based on individual lactations after correcting for additional

milk yield before calving and improved fertility (3.1% in parity 2 and 4.0% in parity >2 for a short DP and 11% in parity 2 and 8.0% in parity >2 for no DP) (Kok et al., 2017b). Two factors that contribute to these lesser reductions in milk yield at the herd level are the presence of first parity cows and incomplete lactations due to culling. The lactation of a cow in first parity starts when the first calf is born, and therefore is not affected by a change in DP length. This means that roughly a third of the herd does not face reductions in milk production due to a short or no DP. Culling implies that lactations are terminated earlier in lactation. Before culling, cows with a DP of 28 or 0 days have realised a considerable additional milk production in the 8 weeks before calving, whereas cows with a DP of 56 days have been dry. This outweighs a lower milk production from calving until culling and results in a higher effective lactation yield (daily milk yield from 60 days before calving until the moment of culling) for cows with a shorter DP (Table 8; (Kok et al., 2016)). As a consequence, general culling had a larger impact on milk production in milk yield compared with a DP of 56 days.

Table 8. Average effective lactation yield in kg per day (ELY), calving interval (CI) and day of culling for lactations of cows that calved again (healthy), cows that were culled for fertility issues (fertility culling), and cows that were culled for other reasons (general culling). Effective lactation yield was computed as kg fat-and-protein corrected milk per day from 60 days before calving until 60 days before next calving or until culling.

		Healthy	,	Fertility	culling	General culling		
Parity	DP category	ELY	CI	ELY	Cull day	ELY	Cull day	
1	-	23.5	323	19.4	372	12.4	77	
2	56	24.4	331	23.7	359	18.6	81	
	28	22.7	319	22.9	323	19.1	75	
	0	21.0	309	21.7	267	21.0	73	
>2	56	25.4	335	25.2	350	19.9	80	
	28	23.7	326	24.0	317	20.0	78	
	56-0ª	22.1	322	23.2	276	21.8	78	
	0-0 ^a	23.9	319	24.2	308	21.3	77	

^a56-0: no DP in the current lactation after a DP of 56 days in the previous lactation; 0-0: no DP in the current lactation after no DP in the previous lactation.

The change in milk production over time after switching to no DP can be important knowledge for decision-making by dairy farmers, and illustrates the relevance of using a dynamic model. It is known from practice that some farmers have quit omitting the DP within 2 years because of a too low milk production (Steeneveld et al., 2013), whereas they might have continued – or never started – the strategy if they had been prepared for these dynamics.

The model also provides insight in days dry per cow per year, accounting for herd composition, CI, and culling. With the data used in this study, cows with a short and no DP lactated 22 and 44 days

per year more than cows with a standard DP, respectively. From this, effects of overall yield level on performance can be extrapolated: a 1 kg lower daily milk yield would result in 364 kg less milk per cow per year in case of no DP, and in 320 kg less milk per cow per year in case of a standard DP. In this way, the overall milk reductions of omitting the DP compared with a standard DP will be 44 kg per cow per year greater if production levels are 1 kg per day lower than the current scenario, and 44 kg per cow per year less if production levels are 1 kg per day higher than the current scenario. Thus, assuming that the impact of DP length on milk yield per day is absolute, the impact of shortening or omitting the DP on milk yield per year will be lower on herds with a higher average production level.

4.2 Economic impact: partial cash flow

The economic impact of shortening or omitting the DP at the herd level was assessed with revenues from sold milk, meat from culled cows and surplus calves, and costs associated with buying or producing feed, and rearing youngstock. Compared with a DP of 56 days, a DP of 28 days reduced partial cash flows by \bigcirc 1,249 per herd per year, and a DP of 0 days reduced partial cash flows by \bigcirc 1,632 per herd per year in the first 5 years of the strategy. This seems to be a limited burden compared with the average Dutch dairy farmer's family labour income from 2008 to 2016 of \bigcirc 42,322 (Wageningen Economic Research, 2017b). Santschi et al. (Santschi et al., 2011a) previously reported an increase in net annual income when a DP of 35 days was applied (for one lactation) instead of a DP of 60 days, resulting from an increase of 569 kg in annual milk production per cow. Depending on whether the quota or the number of cows was kept constant, this resulted in an increase of net annual income of \$41 (Can\$) or \$245 per cow. In the current study, annual milk production per cow was 379 kg lower for a DP of 28 days than for a DP of 56 days. Lowering reductions in milk yield after a DP of 28 of 0 days by 1 kg or 2 kg milk per day, however, increased partial cash flows compared with a DP of 56 days by \bigcirc 33 and \bigcirc 101 per cow in year 2.

Partial cash flows were sensitive to assumptions about CI and milk production levels. If a DP of o days did not result in a shortened CI, this further reduced partial cash flows by on average \pounds 4,498 per herd per year from year 2 onwards. A change in reductions in milk production of 1 kg per day in lactation changed the average partial cash flows by about \pounds 6,000 to \pounds 7,000 per herd per year. General culling rate had a small impact on partial cash flows. This result depends on the milk price, meat price and rearing costs. In case a mature cow (assumed weight of 650 kg) is culled and slaughtered, for example, revenues for meat are \pounds 905, which is only \pounds 64 below the assumed rearing

costs of the replacement heifer. In reality, costs of culling are likely higher due to costs of diseases prior to culling.

Effects of DP length on disease incidence are not clear yet from experimental and observational studies (Van Knegsel et al., 2013), and related veterinary costs were therefore not included in the model. Assuming that health and fertility will improve in case of short and no DP, as a consequence of the improved energy balance (Rastani et al., 2005; Van Knegsel et al., 2014b), this is the most conservative scenario. Partly, the effect of diseases on milk production was implicitly included in the current model, because milk production was based on actual milk records. Disease costs related to veterinary services or discarded milk, however, were not included. Köpf et al. (Köpf et al., 2014) reported €103 lower costs per lactation for treatment of diseases after no DP or spontaneous dryoff than after a DP of 56 days in German Simmental cows. Mostert et al. (2018a) estimated the costs of subclinical ketosis – with an incidence of 25% in the first 30 days after calving – to be €130 per case per year, of which 33% resulted from treatment and discarded milk. If shortening and omitting the DP not only improve metabolic status, but also reduce the incidence of (subclinical) metabolic disorders, such reductions in costs might easily offset the reductions in partial cash flow due to a short or no DP. In addition, costs related to reproductive treatments and fertility culling may be reduced when the DP is shortened or omitted. Multiple studies report shortened CI, that could be explained by an earlier onset of ovulation and normal overian cyclicity after calving (Gümen et al., 2005; Watters et al., 2009; Chen et al., 2015b; Kok et al., 2016). Gumen et al. (Gümen et al., 2005) also found that the number of services per conception was lower for cows with no DP (1.75) than for cows with a standard DP (3.00), with cows with a short DP being intermediate (2.44). Assuming €20 per service (Inchaisri et al., 2010b), shortening and omitting the DP could reduce reproductive costs in a herd of 100 cows by more than €1,000 and €2,000 per year, respectively.

4.3 Environmental impact: greenhouse gas emissions

The impact of DP length on GHG emissions related to milk production was assessed by calculating GHG emissions per t FPCM. In the current model, GHG emissions per t FPCM on average increased by 8 kg CO_2 equivalents in case of a DP of 28 days and 5 kg CO_2 equivalents in case of a DP of o days compared with a DP of 56 days. This increase seems small compared with the impact of culling: a reduction in culling rate of 15% reduced average GHG emissions by 56 to 70 kg CO_2 equivalents per t FPCM between years and DP length strategies. This is comparable to results reported by Van Middelaar et al. (Van Middelaar et al., 2014), who estimated that an increase in

lifespan of 270 days – which reduced culling by about 5% – reduced GHG emissions by 23 kg CO₂ equivalents per t FPCM. In case a change in DP length from 56 days to 0 or 28 days would reduce culling rate by 5%, GHG emissions of milk production would be lower for a DP of 28 days and lowest for a DP of 0 days, compared with DP of 56 days. Opposed to the economic impact, where replacement of a full-grown cow with a heifer costs merely €64, GHG emissions related to rearing a heifer (4,905 kg CO₂) are much larger than the amount of GHG of meat production that are substituted by slaughtering the cow (2,795 kg CO₂). If the improved metabolic health reduces the probability of culling in case of a short or no DP, and consequently lengthens the lifespan of dairy cows, the dilution of GHG emissions related to rearing would offset the negative impact on GHG emissions.

In the current study, the impact of DP length on disease incidence and treatment, and its effect on GHG emissions of milk production, was not included. The treatment of diseases is likely to increase GHG emissions per unit milk through discarded milk and removal of cows (Mostert et al., 2018b). Discarded milk due to the use of antibiotics was shown to contribute 30% to the impact of subclinical ketosis on GHG emissions (Mostert et al., 2018b). With a lower milk production per day, and perhaps fewer treatments per lactation in case of reduced disease incidence, less milk may be discarded in case of shortening or omitting the DP, which could reduce GHG emissions of milk produced.

The model used one average ration for all dairy cows, instead of a DP ration and a lactation ration, because the best estimate of the average Dutch ration is only available for all dairy cows together (CBS, 2014). The ration modification for cows with a DP of 28 or o days was based on the assumption that the reduction in energy requirement per day could be matched by a reduction in concentrate of 0.3 kg per cow per day. This amount is comparable to reducing the amount of concentrate by 1.8 kg per cow per day in early lactation and providing an additional 1.0 kg per cow per day in the 8 weeks before calving. Reducing the concentrate availability for cows after a DP of o days according to this scheme did not cause a further reduction in milk production, compared with cows with a DP of o days that were fed a standard concentrate level (Van Hoeij et al., 2017). The reduced concentrate ration reduced feed costs at the herd level (- €132 per herd per year) and GHG emissions of milk production (-4 kg CO₂ equivalents per t FPCM).

The model is a simplification of reality in which we aimed to incorporate and assess scientifically demonstrated effects of shortening or omitting the DP at the herd level. Stochastic elements were

related to individual lactation potential, CI, and the probability and the moment of culling. Lactation curves and CI were derived from data of commercial dairy farms.

Correlations among stochastic elements were not modelled. A relation between milk yield and culling would be difficult to quantify and requires many assumptions: physiologically, milk yield is related to metabolic status (Ingvartsen, 2006) and impaired metabolic status is related to increased culling in early lactation (Roberts et al., 2012); whereas due to management decisions, the probability of culling increases with lower productivity (Heuer et al., 1999; Pinedo et al., 2014). The probability of culling for fertility reasons was linked to DP length based on the commercial data, and the impact of a change in culling probability was assessed in the sensitivity analysis.

Further variation could be modelled through individual lactation curves, or through a variable delay in replacement of culled cows. Although the simplifications in the current model reduce variation between daily productions of individual cows and cow spaces, they are not expected to change the comparison of yearly productions between herds with different DP lengths.

The evaluation of partial cash flows and GHG emissions was performed with fixed numbers, based on average costs and revenues, Dutch national inventory reports on GHG emissions and IPCC emission factors. These parameter values, however, are variable and uncertain. The current study gives an indication of how shortening or omitting the DP will affect partial cash flows at the herd level and GHG emissions per unit of milk. Higher GHG emissions per unit feed or higher emission factors will increase GHG emissions per unit of milk produced for all DP lengths, but are unlikely to affect the overall comparison between DP lengths. For individual farms, however, farm-specific values should be used to come to a farm-specific conclusion.

Extensions to the model could be the incorporation of specific diseases and treatment of diseases to gain insight in the potential effect of DP length on discarded milk and the consequences for revenues and GHG emissions. Moreover, the model could be adapted to assess the impact of shortening or omitting the DP in seasonal calving systems, where fertility is of greater priority.

The current model results suggest that shortening or omitting the DP negatively affected partial cash flows and GHG emissions; however, considering the small effect size and the potential for enhancing cow welfare (Zobel et al., 2015; Kok et al., 2017a), these negative effects seem justifiable. Variation in effects of DP length on milk production and fertility between farms and overall production level may change these conclusions for individual farms (Santschi et al., 2011b; Kok et al., 2016). Besides an improvement in cow health, there could be other motivations to shorten or omit the DP. Dutch farmers appreciated the easier management with one ration for all cows, no

regrouping, and no drying-off procedure when the DP was omitted (Steeneveld et al., 2013). The perceived easier management is not necessarily reflected in reduced labour, because more cows have to be milked.

5 Conclusions

Shortening the dry period reduced milk production of the herd by 3.1% from the second year onwards, relative to a conventional dry period. Omitting the dry period reduced milk production of the herd by 3.5% from the third year onwards, after a dip in milk production of 6.9% in the second year. On average over 5 years, short and no dry periods reduced partial cash flows by \pounds 1,249 and \pounds 1,632 per herd per year, and increased greenhouse gas emissions per kg of milk by 0.8% and 0.5%, respectively, which might be offset by lower disease costs and reduced culling. Considering the potential for enhancing cow welfare, these negative impacts of a short or no dry period seem justifiable.

Chapter 8

Production, cash flows and greenhouse gas emissions of simulated dairy herds with extended lactations

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Abstract

The transition period is the most critical period in the lactation cycle of dairy cows. Extended lactations reduce the frequency of transition periods, the number of calves, and the related labour for farmers. This study aimed to assess the impact of 2 and 4 months extended lactations on milk yield and net partial cash flow at herd level, and on greenhouse gas (GHG) emissions per unit fatand-protein-corrected milk (FPCM), using a stochastic simulation model. The model simulated individual lactations for 100 herds of 100 cows with a baseline lactation length (BL), and for 100 herds with lactations extended by 2 months or 4 months for all cows (All+2 and All+4), or for heifers only (H+2 and H+4). BL herds produced 887 t (SD: 13) milk per year. The net partial cash flow, based on revenues for milk, surplus calves, and culled cows, and costs for feed, artificial insemination, calving management and rearing of youngstock, was k€174 (SD: 4) per BL herd per year. Extended lactations reduced milk yield of the herd by 4.1% for All+2, 6.9% for All+4, 1.1% for H+2, and 2.2% for H+4, and reduced the net partial cash flow per herd per year by k€7 for All+2, k€12 for All+4, k€2 for H+2 and k€4 for H+4 compared with BL herds. Extended lactations increased GHG emissions in CO₂-equivalents per t FPCM by 1.0% for All+2, by 1.7% for All+4, by 0.2% for H+2 and by 0.4% for H+4, but this could be compensated by an increase in lifespan of dairy cows. Subsequently, production level and persistency were increased to mimic lactations of cows managed for extended lactations. The increase in production level and persistency increased milk production of BL herds by 30%. Moreover, reductions in milk yield for All+2 and All+4 compared with BL herds were only 0.7% and 1.1% per year, and milk yield in H+2 and H+4 herds was similar to BL herds. The resulting net partial cash flows were equal to BL for All+2 and All+4 and increased by k€1 for H+2 and H+4 due to lower costs for insemination and calving management. Also, GHG emissions per t FPCM were equal to BL herds or reduced (0 to -0.3%) when lactations were extended. We concluded that, depending on persistency, extending lactations of dairy cows can have a positive or negative impact on the net partial cash flow and GHG emissions of milk production.

1 Introduction

The transition period around calving is the most critical period in the lactation cycle of dairy cows. It is characterised by a large number of changes in physiology and management routine, and by a high incidence of diseases and culling (Ingvartsen, 2006; Pinedo et al., 2014). To reduce the impact of the transition period, it has been proposed to extend lactation length (Knight, 2001; Dobson et al., 2007). With extended lactations, cows have fewer transition periods per unit time, farmers have less labour related with transition management, and the number of surplus calves is reduced (Knight, 2001).

Milk yield per cow per year and milk revenues were reduced in some studies when lactations were extended (Holmann et al., 1984; Strandberg and Oltenacu, 1989; Inchaisri et al., 2011), although other studies found no or opposite effects (Arbel et al., 2001; Lehmann et al., 2016). Production level and persistency had a great impact on the simulated economic consequences when first insemination was delayed and calving intervals increased from 13 to 14 months (Inchaisri et al., 2011). Extending lactations seemed more successful for heifers than older cows due to their greater lactation persistency (Arbel et al., 2001; Inchaisri et al., 2011), and in herds that were specifically managed for extended lactations (i.e. deliberate delayed insemination) (Lehmann et al., 2016). Cows in these herds may have production characteristics that better support an extended lactation length; similar milk yields per day of calving interval were realised for cows with calving intervals of 13 and exceeding 19 months (Lehmann et al., 2016).

Extending lactations of dairy cows could have economic consequences besides changes in milk revenues. A reduced frequency of transition periods could reduce labour and the veterinary costs related to diseases in the transition period (Liang et al., 2017), and involuntary culling (Pinedo et al., 2014). Moreover, later first insemination, when the cow has a lower milk production and a better energy balance, could increase the conception rate and thus lower the costs of artificial insemination (AI) (Butler, 2003; Inchaisri et al., 2010a). Fewer cows in peak production per unit time might also reduce the kg concentrates fed per kg milk produced, and lower the costs per unit of feed energy (Dekkers et al., 1998).

In addition, extending lactations could affect the environmental impact of milk production. Less frequent transition periods could reduce the number of cows culled per unit time (Lehmann, 2016). A lower culling rate would increase the lifespan of the cow, which dilutes the greenhouse gas (GHG) emissions of youngstock rearing and reduces the GHG emissions per unit milk (Van Middelaar et al., 2014; Kok et al., 2017c). Moreover, a possible reduction in disease incidence, or a reduction in

kg concentrates per kg milk could reduce GHG emissions per unit milk, whereas a possible reduction in milk yield per day could increase GHG emissions per unit milk (Van Middelaar et al., 2014). A reduction in the number of calves born and cows culled would reduce the ratio between produced meat and milk (Lehmann, 2016), which could increase GHG emissions from the alternative production of meat (Cederberg and Stadig, 2003).

The first aim of this study is to assess the impact of 2 and 4 months extended lactations on overall milk yield and cash flows at herd level, and on GHG emissions per unit milk in a stochastic simulation model. Simulations of milk production were based on empirical production data. The second aim of this study is to gain insight in the importance of production level and persistency for the impact of extended lactations on overall milk yield, cash flows and GHG emissions. For the second aim, a sensitivity analysis was performed, in which the peak yield and persistency of the lactation curves of cows with baseline lactations. Possible impacts of extended lactations on culling probability, as well as costs associated with AI and calving management were included in the analysis.

2 Materials and methods

This study used an adapted version of the model developed by Kok et al. (2017c). The model was designed to stochastically simulate Dutch dairy herds of 100 cows with different dry period lengths, and subsequently compute partial cash flows per herd and GHG emissions per unit of fat-and-protein-corrected milk (FPCM) produced. The model simulates individual lactations and calving intervals, with stochastic culling, comprising culling for fertility reasons and culling for other reasons (i.e. general culling). Partial cash flows per herd per year included revenues from milk, surplus calves, and culled cows, and costs for feed and rearing of youngstock. A life cycle approach from cradle to farm gate was used to compute GHG emissions per t FPCM. In the calculation of GHG emissions, system expansion was used to account for the production of meat from surplus calves and culled cows (Van Middelaar et al., 2014; Kok et al., 2017c; Mostert et al., 2018b).

Five different strategies for lactation length were evaluated in the herd simulation model. Cows in the reference scenario had a baseline lactation length (BL; Table 1). In the extension strategies, lactations were extended by either 2 months or 4 months for all cows (All+2 and All+4), or for heifers only (H+2 and H+4). Baseline lactation lengths and lactation curves were based on empirical data of cows with a conventional dry period length (\geq 42 days) from 16 Dutch dairy farms

(Kok et al. 2017c). Calving intervals in the extended lactation strategies were subsequently generated by shifting the baseline calving interval data by 60 or 120 days, to represent a deliberate delay of first AI. The shape of the lactation curves was deliberately derived from production data of cows with baseline lactations, to assess the impact of extending lactations with current production characteristics (base curves). In the sensitivity analysis, the shape of the lactation curves was derived from production data of 2 Danish dairy herds that were managed for extended lactations (managed curves) (Lehmann et al., 2016). This contrast was included as a proof of concept, to evaluate how much better lactation curves of cows that were specifically managed for extended lactations. The model was run for 100 herds of 100 cows per lactation length strategy. At the start of year 1, cows were at a variable moment in lactation; the new lactation length strategy was applied from the moment a new lactation started. Results are presented for the third year that extended lactations are applied, to show the stabilised long-term consequences of extending lactations.

Table 1. Mean, median, and 5 and 95 percentiles of calving intervals (CI) in days to simulate baseline lactation lengths and lactations extended by 2 months and 4 months.

	CI base	line lactatio	n length		CI +2 n	nonths	CI +4 months		
Parity	mean	median	Р5	P95	mean	median	mean	median	
1	384	374	327	477	444	434	504	494	
2	391	381	330	487	451	441	511	501	
>2	395	385	333	489	455	445	515	505	

Some further adjustments were made to the model of Kok et al. (2017c) to enable the evaluation of extended lactations. First, the shape of the lactation curve was adjusted to account for the (delayed) effect of gestation, and this new lactation curve was parameterised for every parity class (1, 2, >2). Second, model parameters regarding growth of parity 1 and parity 2 cows were adjusted for the increase in lactation length. Third, culling probability per lactation was adjusted for the increase in lactation length. Fourth, costs for AI and costs for calving management were added to the assessment of partial cash flows, to evaluate possible reductions associated with extended lactations. The adjustments are described in the next sections. Ration, revenues, and emission factors remained unchanged from the previous study (Kok et al., 2017c).

2.1 Lactation curves

The shape of the lactation curve was determined by the Wilmink lactation curve model (Wilmink, 1987), extended with a linear negative effect of gestation on milk production, that starts with a fixed delay after conception (Strandberg and Lundberg, 1991). Separating the gestation-related effect on persistency may be especially relevant when lactations are extended, because this effect on

persistency then starts later in lactation; simply extrapolating lactation curves to simulate extended lactations could underestimate milk production in late lactation. Individual milk production (MP) in kg of cow *i* in parity *j* at each day in milk (DIM) was calculated as:

$$MP_{ij} = a_j + b_j \times DIM + c_j \times exp(-k \times DIM) + RPL_i \times ADY_j + b_{gest} \times max[(Dgest_i - Ddelay), o]$$

where RPL_i is the relative production level of cow *i*; ADY_j is the average daily 305-d yield in kg milk of a cow in parity *j*; *a_j*, *b_j*, *c_j*, and *k* model the shape of the lactation curve (Wilmink, 1987); and *b_{gest}* models the linear negative effect of days in gestation (*Dgest*) from a fixed delay (*Ddelay*) after conception (Strandberg and Lundberg, 1991). Parameters relate to the level of production (*a_j*), persistency after the peak yield (*b_j* and *b_{gest}*), and slope towards and moment of peak yield (*c_j* and *k*).

The base lactation curves were parameterised using milk records of 16 Dutch dairy farms that were managed for a baseline lactation length and a conventional dry period (\geq 42 days) (Table 2; Figure 1A; Kok et al. 2017c). The managed lactation curves were parameterised using milk records of 2 Danish dairy farms that deliberately extended lactations of Holstein cows (Figure 1B; data from Lehmann et al. 2016). Base and managed curves were fitted on the raw test-day milk records using a mixed model in R. In addition to the fixed effects for a_j , b_j , c_j , and b_{gest} , the model included a random effect on a_j , b_j , and c_j for repeated measures per cow lactation within parity class, within herd, assuming an autoregressive covariance structure (AR1). A grid-search was performed to assess from which stage gestation affected yield, increasing *Ddelay* by 7 days from 84 days until 182 days after conception. The best model fit (based on lowest BIC value) was obtained for a delay of the effect of gestation of 175 days after conception for the base curves, and 168 days after conception for the base curves, and 168 days of lactation in the base curves, and in the last 56 days of lactation in the managed curves.

Table 2. Lactation curve parameters per parity class of cows with baseline lactation length (base) and cows managed for extended lactations (managed). Parameters relate to the level of production (a_j), persistency after the peak yield (b_j and b_{gest}), and slope toward and moment of peak yield (c_j). ADY_j is the average daily 305-d yield in kg milk of a cow with a calving interval of 390 days.

	base							managed						
Parity	aj	bj	Cj	b_{gest}^1	ADY_j	_	aj	bj	Cj	b_{gest} ¹	ADY_j			
1	30.8	-0.037	-14.7	-0.054	24.3		37.0	-0.017	-24.6	-0.105	32.9			
2	41.3	-0.072	-18.4	-0.054	29.3		50.7	-0.061	-27.2	-0.105	39.8			
>2	45.3	-0.085	-21.1	-0.054	31.2		52.0	-0.069	-30.2	-0.105	39.7			

 $^{1}b_{gest}$ effect on persistency starts after 175 days in gestation for the base curve, and after 168 days in gestation for the managed curve.



Figure 1. Lactation curves for parity 1 (solid line), 2 (dashed line) and >2 (dotted line) derived from cows with baseline lactation lengths (base; panel A) or managed for extended lactation lengths (managed; panel B), for calving intervals of 390 and 510 days. Lactation curves for different calving intervals differ in the moment that gestation linearly reduces persistency.

2.2 Growth

Kok et al. (2017c) assumed a fixed growth from 540 kg at first calving to 595 kg at second calving, to a mature body weight of 650 kg at third calving (CVB, 2012). Extending lactations, however, would under this assumption result in a slower growth. In the current model, therefore, growth was standardised to growth from 540 kg to mature weight in the 24 months following first calving. The energy requirements for growth were 660 VEM per day in the first 12 months, and 330 VEM per day in the second 12 months (CVB, 2012); the nitrogen fixation was 16.6 g N per kg body weight for the first 55 kg, and 22.5 g N per kg body weight for the second 55 kg (RVO, 2015).

2.3 Culling

Kok et al. (2017c) assumed a culling probability of 8% per lactation for fertility reasons, and 22% for other reasons (general culling). Extending lactations was assumed not to affect the culling probability for fertility reasons, whereas the probability of general culling per lactation was assumed to either be affected or unaffected. In case of an effect, culling probability per lactation was increased with a probability of 50/100,000 for each day the lactation was extended (Pinedo et al., 2014). This culling probability was derived from mid-lactation, where culling probability was not increased by transition diseases or fertility problems (Pinedo et al., 2014). The general culling probability per lactation was increased to 24.1% in case of extending the lactation with 60 days, and to 26.1% in case of extending the lactation with 120 days. In case of no effect, the probability of

general culling remained 22% per lactation, assuming that culling probability is largely determined by the transition period.

2.4 Insemination and calving management costs

It was assumed that extended lactations are the result of a deliberate delay of first insemination. This could improve conception rate, because cows are inseminated in a later lactation stage, which is less influenced by health and fertility issues typical for early lactation (Butler, 2003; Inchaisri et al., 2010a). Extended lactations will reduce the frequency of calving, and could consequently reduce labour and veterinary services associated with calving. Costs for AI and calving management, therefore, were included in computation of net partial cash flows. The number of inseminations per conception was assumed to be 1.89 for a baseline lactation and 1.69 for an extended lactation (Inchaisri et al., 2011). Costs associated with AI were assumed to be €20 per insemination (Inchaisri et al., 2010b). Moreover, costs for calving management were assumed to be €152 per calving, including costs for labour, disorders in the transition period, drug delivery, and dry-off treatment (Inchaisri et al., 2010b). Net partial cash flows were presented excluding and including costs for AI and calving management.

2.5 Sensitivity analysis

Base curves (Figure 1A) had a lower production level and persistency than managed curves (Figure 1B). In the sensitivity analysis, the peak yield and persistency of the lactation curves of cows with baseline lactation lengths were step-wise increased to mimic lactation curves of cows managed for extended lactations, to gain insight in the importance of peak yield and persistency for the impact of extended lactations. In 4 separate analyses, peak yield (a_j) was increased by 2.5, and 5.0 kg per day (peak+2.5 and peak+5) and persistency (b_j) was increased by 0.01, and 0.02 kg per day (slope+0.01 and slope+0.02). The importance of production level and persistency for consequences of extended lactation on milk production, cash flows, and GHG emissions was evaluated.

3 Results

3.1 Effect of extended lactations on production

The technical results per herd (of 100 cows) for all lactation length strategies are presented in Table 3, both for the model with base curves and the model with managed curves. This section describes the results for base curves only; results for managed curves are described in the sensitivity analysis. Moreover, unless explicitly stated, results refer to the model with general culling probabilities per lactation adjusted for lactation length.

Compared with BL herds, that produced 887 t milk per herd per year, extending lactations reduced milk yield of the herd. Extending lactations for all cows by 4 months (All+4) resulted in the largest reduction in milk yield (-61 t per herd per year; -6.9%), followed by All+2 (-36 t per herd per year; -4.1%). Extending lactations for heifers only resulted in a smaller reduction in milk yield, on average 10 t per herd per year for H+2 (-1.1%) and 20 t per herd per year for H+4 (-2.2%). Extending lactations from the BL strategy reduced the number of calves born and the number of days dry per herd per year. The reductions were larger when lactations were extended for all cows than for heifers only, and when lactations were extended for 4 months than for 2 months. The number of culled cows per herd per year was hardly affected by extending lactations when culling rates per lactation were adjusted for lactation length. When the general culling probability was maintained at 22% per lactation, extending lactations reduced the number of culled cows per year, with the largest reduction (-8 cows per year) in All+4 herds.

3.2 Effect of extended lactations on net partial cash flow

In BL herds, the average net partial cash flow was $k \in 174$ (SD: 4) per herd per year (Table 4). The net partial cash flows of herds with extended lactations were lower than that of BL herds (Table 5), and followed a similar pattern as the milk production of the herd (Figure 2A vs. 2E), with a small impact of the number of culled cows and calves born. Reduced costs for AI and calving management compensated $k \in 1$ to $k \in 5$ of the reduced revenues for milk, with the largest effect in H+4 herds.

Table 3. Milk yield, calves, cows culled, days dry, and net energy requirement (NE) for different lactation length strategies, with lactation curves derived from cows with baseline lactations lengths (base) or managed for extended lactations (managed). General culling probability per lactation was adjusted for lactation length (base and managed), or kept constant at 22% (base22% and man22%).

		base		base22	2%	manage	d	man229	6
Output variable	Strategy ^a	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Milk (t herd ⁻¹ y ⁻¹)	BL	887	13	885	11	1,156	16	1,153	15
	All+2	851	14	851	16	1,148	17	1,147	18
	All+4	825	16	823	18	1,143	17	1,142	18
	H+2	877	13	879	12	1,157	17	1,155	16
	H+4	867	15	867	14	1,156	18	1,157	15
Calves (n herd ⁻¹ y ⁻¹)	BL	114	6	114	6	104	7	104	6
	All+2	100	7	98	6	92	7	89	6
	All+4	90	7	85	7	83	7	78	8
	H+2	109	6	109	6	101	7	100	6
	H+4	105	7	104	6	98	6	96	6
Cows culled (n herd ⁻¹ y ⁻¹)	BL	34	6	34	6	29	6	30	5
	All+2	33	7	30	5	28	5	25	5
	All+4	32	6	26	6	29	6	23	5
	H+2	34	6	32	5	29	6	28	5
	H+4	33	7	31	6	29	6	28	5
Days dry (cow ⁻¹ y ⁻¹)	BL	45	2	45	2	42	2	42	2
	All+2	38	2	38	3	36	3	36	3
	All+4	33	3	34	3	30	3	31	2
	H+2	42	2	43	2	41	2	41	2
	H+4	41	2	41	2	38	2	39	2
NE (MJ cow ⁻¹ d ⁻¹)	BL	125	1	125	1	152	2	151	2
	All+2	121	1	121	1	150	2	150	2
	All+4	118	2	118	2	150	2	149	2
	H+2	124	1	124	1	152	2	151	2
	H+4	123	1	123	1	151	2	151	1

 $^{a}BL =$ baseline lactation length; All+2, All+4 = lactations of all cows extended by 2 and 4 months; H+2, H+4 = only lactations of heifers extended by 2 and 4 months.

Table 4. Average milk yield, net partial cash flows (NPCF) per herd excluding (Excl.) and including (Incl.) costs for AI and calving management, and greenhouse gas (GHG) emissions per t fat-and-proteincorrected milk (FPCM) for herds with baseline lactation lengths, for lactation curves^a differing in peak yield and persistency (slope) (n=100 herds; SD are similar to SD of table 3).

	Lactation curves ^a											
	base	slope										
			+2.5	+5	+0.01	+0.02						
Milk (t per herd per year)	887	1156	963	1040	935	984						
NPCF (k€ herd ⁻¹ y ⁻¹) Excl.	194	264	214	234	207	220						
NPCF (k€ herd ⁻¹ y ⁻¹) Incl.	174	245	194	214	187	200						
GHG emissions (kg CO_2 -e per t FPCM)	931	841	903	877	909	886						

^aLactation curves derived from cows with baseline lactation lengths (base) or managed for extended lactations (managed); and lactation curves where the peak yield (a_i) was increased by 2.5 (peak+2.5) or 5 (peak+5) kg per day, and where persistency (b_j) was increased by 0.01 (slope+0.01) or 0.02 (slope+0.02), compared with the base curve.

Table 5. Change in milk yield, days dry, net partial cash flows (NPCF) and greenhouse gas (GHG) emissions per unit milk for extended lactation strategies^a compared with baseline lactation length, for lactation curves^b differing in peak yield and persistency (slope). Results are presented as average impact for each extended lactation length strategy, compared with herds with a baseline lactation length strategy.

		Lactati	on curv	es ^b					
	Ctratagua	hace	base	man	man	peak	peak	slope	slope
	Sualeyy	Dase	22%	man	22%	+2.5	+5	+0.01	+0.02
Milk	All+2	-36	-35	-8	-6	-33	-29	-25	-17
(t herd ⁻¹ y ⁻¹)	All+4	-61	-62	-13	-11	-53	-53	-44	-24
	H+2	-10	-6	1	2	-7	-9	-6	-2
	H+4	-20	-18	0	4	-18	-17	-12	-6
NPCF Excl.	All+2	-10	-9	-2	-1	-9	-8	-7	-5
(k€ herd⁻¹ y⁻¹)	All+4	-17	-16	-4	-3	-15	-14	-12	-7
	H+2	-3	-1	0	1	-2	-3	-2	-1
	H+4	-5	-5	0	1	-5	-4	-3	-2
NPCF Incl.	All+2	-7	-6	0	2	-6	-5	-4	-2
(k€ herd⁻¹ y⁻¹)	All+4	-12	-11	0	2	-10	-10	-7	-2
	H+2	-2	0	1	1	-1	-2	-1	0
	H+4	-4	-3	1	3	-4	-3	-2	0
GHG emissions	All+2	10	0	-3	-10	3	3	3	-3
(Kg CO ₂ -eq per t FPCM)	All+4	16	-1	-1	-15	10	7	5	0
	H+2	2	-5	-1	-4	-1	4	3	1
	H+4	4	-2	0	-6	4	2	4	0

^aAll+2, All+4 = lactations of all cows extended by 2 and 4 months; H+2, H+4 = only lactations of heifers extended by 2 and 4 months.

^bLactation curves derived from cows with baseline lactation lengths (base) or managed for extended lactations (man); and base curves where the peak yield (a_i) was increased by 2.5 (peak+2.5) or 5.0 (peak+5) kg per day, and where persistency (b_j) was increased by 0.01 (slope+0.01) or 0.02 (slope+0.02). General culling probability per lactation was adjusted for lactation length, or kept constant at 22% (base22% and man22%).

3.3 Effect of extended lactations on greenhouse gas emissions

In BL herds, GHG emissions were 931 kg (SD: 16) CO_2 -equivalents per t FPCM. Extending lactations increased GHG emissions in CO_2 -equivalents per t FPCM by 1.0% for All+2, by 1.7% for All+4, by 0.2% for H+2 and by 0.4% for H+4. The impact of extended lactations on GHG emissions per unit milk showed a pattern opposite to that of milk yield of the herd, although differences in GHG emissions between lactation length strategies were smaller than the variation between farms (Figure 2A vs. 2F). When the probability of general culling was maintained at 22% per lactation, however, extending lactations resulted in a reduction of GHG emissions per t FPCM, which was largest for H+2 herds (-0.6%).



Figure 2. Total herd milk yield (A), days dry (B), number of calves born (C), number of cows culled (D), net partial cash flows excluding costs for AI and calving management (E), and greenhouse gas emissions (F) for the baseline lactation length (BL), all lactations extended by 2 (All+2) or 4 months (All+4), and only lactations of heifers extended by 2 (H+2) or 4 months (H+4). Each value represents a herd of 100 cows with lactation curves derived from cows with baseline lactation lengths (base curves) and culling probability adjusted for lactation length.

3.4 Sensitivity analysis: impact of production level and persistency

The milk yield of BL herds increased when production level and persistency were increased from base curves to managed curves, which increased energy requirements per cow and the net partial cash flow per herd, and reduced GHG emissions per t FPCM (Table 4). Using managed curves, annual milk production in the BL herds was 30% higher and energy requirements were 22% higher than using base curves. Also, BL herds with managed curves had fewer calves per year (104 vs. 114), fewer culled cows per year (28-29 vs. 32-42), and fewer days dry per year (42 vs. 45 days) than BL herds with base curves. In contrast to results with base curves, reductions in milk yield compared

with BL herds with managed curves were only 8 t (0.7%) and 13 t (-1.1%) per herd per year for All+2 and All+4 herds, and milk yield was similar to BL herds in H+2 and H+4 herds. Together with the reduction in costs for AI and calving management, this resulted in no change in net partial cash flow for All+2 and All+4 herds, and an increase in net partial cash flow for H+2 and H+4 herds compared with BL herds. Moreover, GHG emissions per unit milk were equal to BL herds or reduced (0 to -0.3%) when lactations were extended, and were further reduced (-0.4 to -1.8%) when the probability of general culling was maintained at 22% per lactation.

Extending lactations reduced milk yield compared with BL herds for all curves, except for H+2 and H+4 herds with managed curves (Table 5). Milk losses compared with the BL scenario were reduced to a lesser extent when peak yield increased than when persistency increased. Therefore, the impact of extending lactations remained negative with peak+5 curves, whereas H+2 and H+4 had net partial cash flows equal to BL herds with slope+0.02 and managed curves. Total milk yield, however, was increased to a greater extent when peak yield increased than when persistency increased to a greater extent when peak yield increased than when persistency increased. As a result, H+4 herds with peak+5 curves realised about 45 t milk per year more than H+4 herds with slope+0.02 curves.

4 Discussion

This study aimed to investigate how extending lactations of dairy cows by 2 or 4 months affects milk production and partial cash flows at herd level, and GHG emissions per unit milk, using a dynamic stochastic simulation model. The model simulated herds of 100 cows with lactation curves derived from cows with baseline (base curves) and from cows managed for extended lactation lengths (managed curves). Managed curves had a higher production level and persistency than base curves, and the impact of these features was assessed with lactation curves in which production level or persistency were step-wise increased from base curves towards managed curves.

Milk yield of BL herds averaged 8,870 kg per cow per year with base curves, and 11,560 kg per cow per year with managed curves. For base curves, annual milk yield of the herd decreased considerably when lactations of all cows were extended by 2 or 4 months (-4.1% and -6.9%), and to a lesser extent when lactations of heifers were extended by 2 or 4 months (-1.1% and -2.1%). A simulation study that postponed first insemination by 70 days also estimated a reduced annual milk yield, with a smaller reduction when only lactations of heifers were extended lactations were smaller when persistency was increased. For managed curves, annual milk yield of the herd decreased only 1.1%

when lactations of all cows were extended by 4 months, and extending lactations of heifers only did not lower milk production at herd level. Despite the same simulated calving intervals and culling rules, BL herds with base curves had more calves, culled cows, and days dry per year than herds with managed curves. This difference was caused by the prolonged presence of cows to be culled for fertility reasons (8% of lactations; cows were culled when yield became lower than 15 kg per day).

Table 6. Average milk yield per day (kg per day of calving interval) for cows of different parities with baseline (BL) or 2 or 4 months extended lactations, for lactation curves^a differing in peak yield and persistency (slope). Percentages indicate the change in milk yield per day compared with the BL strategy.

		Lactation curves ^a											
						peak		peak		slope		slope	
Parity	Strategy	base	%	man	%	+2.5	%	+5	%	+0.01	%	+0.02	%
1	BL	20.3		27.6		22.4		24.5		21.7		23.1	
	+2	20.0	-1.5	28.1	1.6	22.1	-1.1	24.3	-1.2	21.6	-0.3	23.3	0.8
	+4	19.4	-4.0	28.4	2.7	21.7	-3.2	23.8	-2.9	21.4	-1.2	23.4	1.2
2	BL	24.0		33.0		26.2		28.3		25.5		27.0	
	+2	22.9	-4.3	32.4	-1.8	25.0	-4.3	27.3	-3.5	24.7	-3.2	26.3	-2.4
	+4	21.5	-10.3	31.5	-4.4	23.9	-8.7	25.9	-8.5	23.5	-7.9	25.5	-5.5
>2	BL	25.4		32.8		27.6		29.7		26.9		28.4	
	+2	24.0	-5.6	32.0	-2.4	26.2	-5.0	28.4	-4.4	25.7	-4.3	27.5	-3.1
	+4	22.3	-12.3	31.0	-5.5	24.5	-11.1	26.7	-10.0	24.4	-9.3	26.4	-6.8

^aLactation curves derived from cows with baseline lactation lengths (base) or managed for extended lactations (man); and base curves where the peak yield (a_i) was increased by 2.5 (peak+2.5) or 5.0 (peak+5) kg per day, and where persistency (b_j) was increased by 0.01 (slope+0.01) or 0.02 (slope+0.02).

When milk yield is compared as production per day of calving interval, extending lactations by 2 months reduced milk yield of heifers by 1.5%, of second parity cows by 4.3%, and of older cows by 5.6% (Table 6). Under the best persistency scenario (i.e. slope+0.02), extending lactations increased milk yield of heifers, whereas milk yield of older cows remained reduced compared with the baseline lactation length. Extending lactations also increased milk yield per day of calving interval of heifers, and reduced milk yield of older cows in experimental studies with Swedish and Israeli Holstein cows (Rehn et al., 2000; Arbel et al., 2001). Despite the increase in milk yield per day for heifers, the annual milk yield of the entire herd decreased when lactations of heifers were extended. This can be explained by the lower milk production of heifers compared with older cows, and the increased ratio of heifers to older cows. Extending lactations of heifers using managed curves, however, did not reduce milk yield of the herd, because the reduced number of days dry and the increased production of heifers together compensated for the reduced presence of older cows. Our results for older cows seem to contradict a previous finding, where farmers who extended lactations of selected cows only were able to maintain milk yield per day of calving interval with

increasing lactation length (Lehmann et al., 2016). That finding may have been confounded with production level, because cows assigned to the longer lactations also had higher 305-d yields. Specifically extending lactations of high-producing heifers or highly persistent cows in the herd might be a strategy to reduce the impact of extended lactations on milk production. It should be considered, however, that predicting lactation persistency may be difficult in early lactation (Lehmann et al., 2017), and that extending lactations could therefore bring the risk of longer dry periods when cows spontaneously dry off (Rehn et al., 2000; Lehmann et al., 2016).

Similar to the effect on milk yield, extending lactations with base curves had a negative impact on the net partial cash flow, that was larger when lactations of all cows were extended than when lactations were extended for heifers only. Extending lactations of all cows or heifers by 2 months, accounting for costs related to AI and calving management, reduced the net partial cash flow by k€7 or k€2 per herd per year, or €70 or €19 per cow per year, respectively. These results are similar to previously estimated costs of delaying insemination by 70 days for all cows (€53 to €70 per cow per year), or for heifers only (€18 or €24 per cow per year) (Sørensen and Østergaard, 2003). In that estimate, it was assumed that milk production in the lactation after an extended lactation was up to 0.9% higher, due to a live weight closer to mature weight (Sørensen and Østergaard, 2003), whereas milk production was only affected by parity in the current study. A reduction in net partial cash flow of k€7 per year would be a considerable burden for a farmer, compared with the average annual family labour income of Dutch dairy farmers of k€42 between 2008 and 2016 (Wageningen Economic Research, 2017). In case of extending lactations by 2 months for heifers only, losses could be compensated if the culling probability per lactation would remain the same. Given that culling rate is highest in the transition period and in late lactation, a lower culling rate per year may be expected when lactations are extended (Pinedo et al., 2014). A positive relation between calving interval and average culling rate per year in Dutch dairy herds does not support this assumption (Mohd Nor et al., 2014), but results may be different when longer lactations are the result of deliberate management rather than an unwanted consequence (Lehmann, 2016). Moreover, reductions in net partial cash flow in case of extended lactations were smaller in herds with higher persistency. In case of the most persistent lactation curves evaluated in the current study, reduced costs for AI and calving management compensated for the reduced milk revenues when lactations of heifers were extended.

Estimated GHG emissions per unit milk increased when lactations were extended for base curves, by 1.0% when all lactations were extended by 2 months, and by 1.7% when all lactations were extended by 4 months. This increase was smaller than the estimated increase of 5.9% or 12.9%

when lactations were extended by 65 or 135 days, which was caused by an unexpected increase in enteric and manure emission of methane per head per year (Wall et al., 2012). If the culling probability per lactation would remain the same when lactations are extended, GHG emissions per unit milk would be reduced for all extended lactation strategies for base curves, despite the reduction in milk yield at herd level (-0.7% to -7%). This result was caused by a lower annual replacement rate, which reduced the GHG emissions from rearing replacement heifers. A simulation study of Australian dairy herds estimated that GHG emissions per unit milk (after mass allocation of emissions to milk and meat) would reduce when lactations were extended by 6 months, due to a 12% greater annual milk yield and a 9% lower replacement rate (Browne et al., 2015). In case of high persistency, GHG emissions per unit milk were similar for baseline and extended lactation lengths even when culling probability was adjusted for lactation length.

Increasing production level and persistency resulted in a great increase in milk yield per herd per year (30% from base curves to managed curves for BL herds), an increase in net partial cash flows ($k \in 27$), and a reduction in GHG emissions per unit milk (-90 kg CO₂-equivalents per t FPCM). These changes by far exceeded the changes due to extended lactations compared with a baseline lactation length. The impact on net partial cash flow and GHG emissions was evaluated using an average Dutch feed composition (CBS, 2014) with average costs and revenues (KWIN-V, 2014) and assuming no other changes, whereas changes in lactation curve and lactation length may be accompanied by changes in, for example, feed composition, milking frequency, or crops grown by the farmer (Dekkers et al., 1998; Sorensen et al., 2008; Van Middelaar et al., 2014). Therefore, the estimates of net partial cash flows and GHG emissions may not be accurate.

5 Conclusions

In conclusion, extending lactations by 2 or 4 months reduced milk production of the herd, except when only lactations of heifers were extended and lactation curves were very persistent. Whether the resulting net partial cash flow was reduced or increased compared with baseline lactation lengths depended on lactation persistency. In case of more persistent lactations, reduced revenues from milk could be compensated by reduced costs for AI and calving management. GHG emissions per unit milk increased when lactations were extended, except when lactations were very persistent or when the lifespan of cows increased by extending lactations.

Chapter 9

General discussion

1 Introduction

A high-producing dairy cow experiences a negative energy balance (NEB) for 3 months after calving (Rastani et al., 2005; Van Knegsel et al., 2014b), which is associated with impaired health and fertility (Lucy, 2001; Butler, 2003; Ingvartsen, 2006; Chen et al., 2015b). Moreover, the cessation of milking at the start of the dry period (DP) has become a challenge due to the high milk yield at dry-off (Zobel et al., 2015). Shortening and omitting the DP are strategies to improve cow health and ease the transition period around calving (Van Knegsel et al., 2013). Both strategies partly shift milk production from early lactation to the weeks before calving, and improve the energy balance, metabolic status and fertility in early lactation (Rastani et al., 2005; Van Knegsel et al., 2014b; Chen et al., 2015a; b). Consequences of shortening or omitting the DP for other aspects of cow welfare besides health, for the farmer and for global warming, however, are currently not well known.

The aim of this thesis, therefore, is to evaluate and integrate sustainability impacts of short or no DP in dairy cows, with a focus on cow welfare, cash flows and greenhouse gas (GHG) emissions. In this chapter, I will first discuss the assumed trade-off between metabolic status and milk yield, with associated consequences for cash flows and GHG emissions. Next, consequences of DP length for aspects of animal welfare, practical implications for farmers and options to improve sustainability will be discussed. Finally, the conclusions of this thesis are given.

2 Improved metabolic status at the cost of milk production

The assumed trade-off from the start of this project was that shortening or omitting the DP of dairy cows improves the energy balance, metabolic status and fertility at the cost of milk production. The assumption that milk production would be reduced was based on the large reduction in milk yield after calving across experimental studies, i.e. 5.9 kg per day (19%) after no DP and 1.4 kg per day (4.5%) after a short DP (Van Knegsel et al., 2013). These reductions would only be partly compensated by the extra milk produced in the extra milking days before calving (Rastani et al., 2005; Van Knegsel et al., 2014b). Previous comparisons of milk production between cows with different DP lengths did account for the extra milk produced before calving, but assessed only the first lactation after calving, and did not account for potential changes in calving interval (Annen et al., 2004; Rastani et al., 2005; Schlamberger et al., 2010; Steeneveld et al., 2013; Van Knegsel et al., 2014b). The improved fertility after short or no DP, however, may result in shorter calving intervals (Gümen et al., 2005), which could partly compensate milk losses because milk yield is
lower during late lactation (Inchaisri et al., 2010b). To compare milk yield between cows with different DP lengths, accounting for extra milk before calving and possible changes in calving interval, the 'effective lactation yield' was developed (Chapter 5).

2.1 Impact of dry period length on milk yield

The effective lactation yield was defined as the average fat-and-protein-corrected milk (FPCM) yield from 60 days before calving to 60 days before the next calving. This interval corresponds to the period from one DP length decision to the next, assuming that the standard DP length is 60 days, and thus accounts for changes in milk production and in calving interval that can be attributed to this decision. Compared with a conventional DP, no DP reduced the 305-d yield by 7.0 kg per day (23%), whereas the effective lactation yield was reduced by 3.0 kg per day (12%) (Chapter 5). A short DP reduced the 305-d yield by 2.4 kg per day (8%), whereas the effective lactation yield was reduced – numerically but not significantly – by 0.5 kg per day (2%) (Chapter 5). To understand the impact of DP length on milk production, therefore, we need to move beyond the traditionally used 305-d yields. A similar case has been made for comparing milk yield of lactations of different lengths, which also result in more milking days with lower yields during late lactation: these effects are not captured by 305-d yields (Lehmann et al., 2016). To compare long-term consequences of DP length for milk production, the developed concept of effective lactation yield needed to be applied to milk production data over multiple lactations.

In Chapter 6, the impact of DP length on effective lactation yields of second and greater parity cows is assessed over multiple lactations. It is hypothesised that milk yields could be reduced further after a second omission of the DP, because it would again prevent the regeneration of udder cells during the DP (Capuco et al., 1997). Alternatively, cows might adapt to continuous milking (Rémond and Bonnefoy, 1997), possibly through increased renewal of mammary epithelial cells during lactation (Capuco et al., 2001; Annen et al., 2008).

The analysis described in Chapter 6 was performed using data from 16 Dutch dairy farms that recently (mostly in 2010 and 2011) changed their DP management from conventional to short or no DP. In the dataset, milk records of 1,420 lactations could be matched with the 2 previous DP lengths. This dataset is exceptional for its large number of cows and lactations per cow in combination with the applied DP lengths. A drawback may be that the data is observational (as opposed to experimental), and that farmers continued the no DP strategy, or switched to short DP, based on perceived success. Therefore, the impact of short or no DP may be biased towards smaller

reductions in milk yield. However, the farmer that experienced the largest reduction in milk yield after calving when the project started (Steeneveld et al., 2013) still omits the DP for all cows.

Cows with a second omission of the DP had a higher milk yield after calving than cows in the same parity whose DP was omitted for the first time (Chapter 6), which is in agreement with experimental findings (Chen et al., 2016a). The higher milk yield after calving, however, did not result in a higher effective lactation yield, because these cows also produced a smaller amount of extra milk in the weeks before calving (Chapter 6). The effective lactation yield did not differ between the first and second omission of the DP; only the timing of milk production differed. This underlines the importance of the effective lactation yield when comparing milk yield of cows with a short or no DP. No DP, compared with a standard DP, reduced the effective lactation yield by 2.8 kg FPCM per day for second parity cows, and by 2.2 kg FPCM per day for older cows. Shortening the DP, compared with a standard DP, reduced the effective lactation yield by 0.8 kg FPCM per day for second parity cows, and by 1.1 kg FPCM per day for older cows (Chapter 6). Moreover, a standard DP cancelled all effects of the previous DP on milk yield (including solids) after calving (Chapter 6). Similarly, cows that dried off spontaneously more than 2 months before the next calving after an omitted DP returned to yields similar to cows with a standard DP (Chen et al., 2016a). These results indicate that shortening or omitting the DP indeed reduces the effective lactation yield of cows, with smaller reductions when the DP is shortened than when the DP is omitted. Moreover, second-parity cows have smaller milk losses than older cows with a short DP, but greater milk losses with no DP. These findings could be used for DP decisions for individual cows. To determine the trade-off between metabolic status and milk yield, however, we need to assess the impact of DP length on milk yield at the herd level. Milk yield at the herd level cannot be directly extrapolated from the results above, because it depends on herd composition (i.e. parity distribution) and herd dynamics (e.g. culling).

The impact of shortening or omitting the DP on milk yield at the herd level was estimated in Chapter 7 using a dynamic stochastic simulation model. This model simulated the entire herd, and thereby accounted for heifers and for cows that were culled. Milk production of a heifer starts after the first calving and is not affected by DP length (Figure 1). Cows that are culled have incomplete lactations, and the amount of milk that is realised in these lactations depends on the moment of culling and the timing of milk production. Modelled impacts of DP length were derived from the production data of the 16 participating dairy farms. Shortening or omitting the DP affected lactation curves (including solids content), calving intervals and the probability of culling for fertility reasons. The

probability of other culling (per lactation) was assumed to be equal across DP lengths. Other culling was more likely to occur during early than late lactation.

Culling during early lactation had a smaller impact on effective lactation yields (from 60 days before calving until culling) for cows with no DP than for cows with a standard DP, because part of the milk yield was already produced before calving. This insight could be a financial incentive to omit the DP of cows with a high culling risk, in addition to the incentive of improved metabolic status. Model results also showed that, although effective lactation yields do not change over time (Chapter 6), the introduction of no DP results in a dip in milk production of the herd in the second year the strategy is applied (Chapter 7). This is important information for a farmer who considers omitting the DP.



Figure 1: Schematic lactation of a cow for 4 calendar years. Differences in effective lactation yield between a cow with a conventional DP (solid line) and a cow with no DP (dashed line) are given below the horizontal axis. Culling early in lactation () has less impact in the case of no DP, because part of the milk is produced before calving.

On average, applying a short DP of 4 weeks for the entire herd reduced milk yield of the herd by 3.1%, and applying no DP reduced milk yield of the herd by 3.5% (Chapter 7). The 'average farm', however, does not exist, and there are large differences in absolute production level and in impact of DP length on milk yield between farms (Santschi et al., 2011a; Steeneveld et al., 2013). The sensitivity analysis showed that milk yield at the herd level with a short or no DP greatly depends on the impact of DP length on milk yield (Chapter 7). If milk production after a short or no DP is 1 kg per day higher than the assumed average, this would result in similar milk yields for herds with no, short and standard DP. Data about milk production, DP length and specific farm characteristics (e.g. ration, milking frequency, genetics) of many farms that apply a short or no DP would be required to disentangle causal factors behind this farm effect.

In conclusion, the assumed trade-off between metabolic status and milk yield was correct in its direction: overall milk yield is reduced by shortening or omitting the DP. However, milk losses per effective lactation (3% and 11% in parity 2 and 4% and 8% for older cows; Chapter 6) and total milk

losses at the herd level (3.1 and 3.5%; Chapter 7) are smaller than previously reported milk losses based on the lactation after a short or no DP (4.5% and 19.1%; Van Knegsel et al., 2013). This suggests that milk revenues in herds with a short or no DP will be affected to a lesser extent than may previously have been assumed, which has a positive impact on net partial cash flows. Moreover, a smaller reduction in annual milk production is expected to have a smaller negative impact on GHG emissions per unit milk, due to greater dilution of GHG emissions related to maintenance (Garnsworthy, 2004; Van Middelaar et al., 2014). Positive impacts of a short or no DP on cow health may therefore compensate these negative impacts on net partial cash flows and GHG emissions per unit milk. Impacts of a short and no DP on cash flows and GHG emissions will be discussed in the next section.

2.2 Impacts on cash flows and greenhouse gas emissions

The impact of DP length on cash flows and GHG emissions depends on many factors. In addition to the impact of DP length on total milk yield described in the previous section, cash flows and GHG emissions will depend on the impact of DP length on fertility, culling and disease incidence, and on a possible change in feed composition. Impacts of DP length on cash flows and GHG emissions through milk yield, fertility and culling were assessed in the herd simulation model (Chapter 7).

The reduction in total milk yield and solids in herds with short or no DP reduced revenues from milk (Chapter 7). On average over the first 5 years, net partial cash flows were reduced by \in 12 per cow per year when the DP was shortened, and by \in 16 when the DP was omitted. Moreover, GHG emissions per unit milk were increased by 0.8% (i.e. 8 kg CO₂ equivalents per t FPCM) when the DP was omitted. The reduction in net partial cash flows for this baseline scenario seems relatively small compared with the average annual family labour income of Dutch dairy farmers of \in 42,322 between 2008 and 2016 (Wageningen Economic Research, 2017). Also, the impact of DP length on GHG emissions per unit milk was smaller than the variation that was found between farms with the same DP length, and smaller than improvements in GHG emissions that can be made through, for instance, 5% reduction in replacement rate (-16 to -23 kg CO₂ equivalents per t FPCM with economic allocation) (Van Middelaar et al., 2015).

Net partial cash flows and GHG emissions per unit milk were strongly linked to total milk production and consequently to the impact of short and no DP on milk yield. One kg per day higher yields after a short or no DP increased net partial cash flows to \pounds_{35} and \pounds_{42} per cow per year

compared with a conventional DP. Similarly, the increase in GHG emissions per unit milk could be offset by a one kg per day higher yield after a short or no DP and worsened by a lower yield. Fertility affected cash flows and GHG emissions through the length of calving intervals and through the probability of culling for fertility reasons (fertility culling). Median calving intervals were shorter after a short and no DP compared with a conventional DP, although differences were not significant for cows in parity >2 after a short DP (Chapter 5, 6). Calving intervals were shortened most by omission of the DP. For the model analysis in Chapter 7, fertility culling was assumed to equal the proportion of long calving intervals (>518 days) in the commercial dataset that was used in Chapter 6. Fertility culling was reduced most for cows with no DP (from about 8% to 4%), with intermediate results for a short DP, compared with a conventional DP. Both shorter calving intervals and reduced fertility culling compensated milk losses and net partial cash flows of herds with a short and no DP, with a larger reduction in GHG emissions per unit milk was realised by reduced fertility culling, especially for herds with no DP, than by shorter calving intervals.

A reduction in general culling rate (i.e. all culling except fertility culling) increased net partial cash flows for all DP lengths, but this effect was much smaller than the effect of a reduction in milk losses. A reduction in general culling rate also reduced GHG emissions of milk production, and, unlike net partial cash flows, this effect was stronger than the effect of milk yield. This implies that reducing milk losses is economically more attractive, whereas reducing culling rate is more attractive from a GHG perspective (Chapter 7). An explanation for this finding is that the economic costs of rearing a replacement heifer are to a large extent covered by the revenues from meat from a culled cow, whereas the GHG emissions related to rearing a replacement heifer are only partly compensated by the avoided burden of meat production elsewhere. However, there is no trade-off between the two, as both a reduction in milk losses and a reduction in culling rate are estimated to improve cash flows and GHG emissions in synergy. Positive effects of increased milk yield and lifespan on GHG emissions of milk production are in accordance with previous findings (Weiske et al., 2006; Van Middelaar et al., 2014).

Shortening or omitting the DP could reduce disease costs and GHG emissions per unit milk through a reduction in disease incidence (Köpf et al., 2014; Liang et al., 2017; Mostert et al., 2018a,b). Shortening or omitting the DP may be expected to reduce the incidence of diseases that are associated with a severe NEB or high plasma FFA concentrations during early lactation, such as ketosis, metritis and displaced abomasum (Kaneene et al., 1997; Ingvartsen, 2006; Chapinal et al., 2011). Moreover, this reduction in disease incidence is expected to be larger for omitted than shortened DP, based on the greater improvement of the energy balance (Rastani et al., 2005; Van Knegsel et al., 2014b). Diseases were not explicitly included in the model, because data on disease incidence following a short or no DP is limited and inconsistent (Van Knegsel et al., 2013). Consistent results have been reported regarding the impact of no DP on ketosis, although the number of animals per treatment group was limited (13 to 49 cows) (Rastani et al., 2005; Schlamberger et al., 2010; Köpf et al., 2014). In these 3 studies, ketosis did not occur in cows with no DP, whereas its incidence was 4.8% to 19% in cows with a standard DP. Disease costs may consist of treatment and veterinary costs, revenues foregone due to a decrease in sold milk and costs related to increased days open, early culling or death. The simulated impact of DP length on milk yield and calving intervals in Chapter 7 was based on actual data and therefore implicitly included impacts of diseases on the amount of milk produced and days open. Treatment and veterinary costs were not accounted for, however, though these could amount to considerable costs, e.g. \$52 (Liang et al., 2017) and €185 per case of ketosis (Köpf et al., 2014). If the simulated herd has a ketosis incidence of 11.8% during early lactation of parity >1 cows (Vanholder et al., 2015), and omission of the DP eliminated this ketosis, this would prevent about 10 cases of ketosis per herd per year. The reduced costs of this disease alone would be \$5.20 to €18.50 per cow per year, which would compensate 26% to 116% of the reduction in net partial cash flows in herds with no DP. Moreover, ketosis also increases the risk of other diseases, including lameness, mastitis and metritis (Berge and Vertenten, 2014; Raboisson et al., 2014), the treatment of which may result in discarded milk. This additional risk resulted in discarded milk costs of €18 per case of subclinical ketosis (Mostert et al., 2018a). Discarded milk also increases the GHG emissions per unit of sold milk, because the milk is produced but does not share the burden of produced GHGs (Mostert et al., 2018b). A lower disease incidence, therefore, could improve cash flows and GHG emissions in synergy.

In Chapter 7, the baseline ration was based on the average Dutch ration for dairy cows (CBS, 2014). In a second ration, the proportion of concentrate was reduced to mimic a reduction in concentrate intake to match the lower milk yield of cows with a short or no DP. A similar reduction in concentrate intake was not detrimental for milk production after omission of the DP in an animal experiment (Van Hoeij et al., 2017). Compared with the baseline ration, this second ration only reduced feed costs by \pounds 1 per cow per year and GHG emissions by 4 kg CO₂ equivalents per t FPCM (0.4%). A larger reduction in concentrates, or a shift towards ingredients with a lower price or environmental impact, may have a bigger impact on cash flows and GHG emissions (Van Middelaar et al., 2015), but could also further reduce milk production (Reist et al., 2003).

In conclusion, shortening and omitting the DP reduced net partial cash flows and increased GHG emissions per unit milk in the baseline model. However, negative impacts of short and no DP on cash flows and GHG emissions are relatively small, and could be offset by a reduction in disease incidence and culling. Such an improvement in health may be expected, given that the main motive to shorten or omit the DP is to improve the energy balance and metabolic status during early lactation.

3 Trade-offs between aspects of welfare?

Shortening and omitting the DP are expected to have a small negative impact on cash flows and GHG emissions, which may be offset by improved health (Chapter 7; section 2.2). The main motive to shorten or omit the DP, therefore, remains to improve energy balance and metabolic status during early lactation (Chapter 1 section 1.2.1). However, the NEB is not the only challenge for cow welfare. Good welfare includes good health and the absence of disease, feeling well and being able to express natural behaviour (Fraser et al., 1997). Diseases and behaviour can be assessed relatively easily, but animal feelings cannot be assessed directly (Dawkins, 1990). Generally, behavioural or physiological parameters are used as indirect measures of how an animal feels (Broom, 1996).

Aside from improved energy balance and metabolic status, the idea to shorten or omit the DP raised at least three welfare concerns. First, the feeling aspect of welfare may be negatively affected when the cow has no DP, and consequently no non-productive period to rest. Second, udder health may be negatively affected by the absence of the non-productive period to recover. Third, the health implications of short and no DP for the unborn and new-born calf were unknown. In other words, trade-offs might exist between different welfare consequences of shortening or omitting the DP. These trade-offs will be addressed in the following sections. To address the first concern, behaviour of cows with and without a DP will be discussed in relation to possible consequences for feeling during early and late lactation. Next, literature on the effects of short and no DP on udder health and calf health will be discussed.

3.1 Impact of having or not having a dry period on cow behaviour

Dairy cows adapt their behaviour to their state (lactation stage, production level, disease) and to external factors (housing, feed, management) (Krohn et al., 1992; Huzzey et al., 2006; Fregonesi et al., 2007; Gomez and Cook, 2010; Norring et al., 2012; Maselyne et al., 2017). Differences in the

resulting time budgets may simply reflect adaptation to specific internal and external situations, but a severe constraint on behaviours, such as lying and feeding, may have negative consequences for welfare (Munksgaard et al., 2005; Korte et al., 2007). Put another way, the extent to which strongly preferred behaviours can be shown is a measure of good welfare (Broom, 1996). The strong motivation of dairy cows to lie down has been demonstrated with different experimental designs, e.g. deprivation, time constraint and operant experiments (i.e. work to gain access to lying space) (Metz, 1985; Jensen et al., 2005; Munksgaard et al., 2005; Tucker et al., 2018). For example, cows that had access to feed, social contact and a place to lie down for only 12 hours per day for 2 weeks, prioritised lying during this period, which reduced feed intake and caused weight loss (Munksgaard et al., 2005). Feed intake of these cows was partly compensated by a faster feeding rate. If the experiment had lasted longer, more severe weight loss could have increased the motivation to feed, and consequently could have increased feeding at the expense of lying time.

Lying time differs between different stages in lactation (Bewley et al., 2010; Maselyne et al., 2017). Throughout lactation, daily lying time (of Holstein cows in loose-housing systems) first decreased from 11.1 hours in week 1 to 10.4 hours in week 4 after calving, and subsequently increased until about 200 days in milk, reaching a plateau at 12.5 hours of lying per day (Maselyne et al., 2017). This plateau value is similar to the relatively inelastic demand for rest of 12-13 hours by 2-month pregnant heifers (Jensen et al., 2005), and of 13 hours by cows at 198 (SD:12) days in milk (Tucker et al., 2018). Also, it is close to the 12 hours per day that non-lame cows at 167 (SD: 95) days in milk spent lying in loose-housing systems, independent of bedding type (mattress or sand) (Gomez and Cook, 2010). The low lying time during early lactation (Maselyne et al., 2017) may be indicative of time constraints, discomfort due to the size of the udder, or volume of milk or processes in the udder, or the NEB (Haley et al., 2000; Munksgaard et al., 2005; Norring et al., 2012; Løvendahl and Munksgaard, 2016). A low lying time in itself may reflect reduced welfare of cows during early lactation, if cows are strongly motivated to lie down for 12-13 hours per day but express this behaviour to a limited extent (Broom, 1996). Moreover, excessive standing time (i.e. a shorter lying time) on hard floors is a risk factor for lameness (Knott et al., 2007; Cook and Nordlund, 2009). This risk may be especially high around parturition and during early lactation, when the physiological changes may increase the risk for claw horn disruption (Knott et al., 2007) and lying time is shortest (Maselyne et al., 2017).

Although the DP is often considered a rest period, daily lying time during the DP was previously reported to be 12 hours per day (Huzzey et al., 2005; Schirmann et al., 2011), similar to the abovementioned results from mid-lactation onwards. This suggests that having a DP does not increase lying time, and that the milking process poses no constraint to lying time. In our experiment, however, dry cows (with a short DP) had longer lying times than lactating cows (with no DP) at 4 weeks before calving (13.7 vs. 12.6 hours per day; Chapter 3). The lying time of cows with no DP was above 12 hours per day and feeding rate remained stable over the 6 weeks before calving (i.e. they did not eat faster). This suggests that the shorter lying time for cows with no DP than for cows with a DP before calving may not be experienced as a time constraint. Instead, cows with a DP may have increased their lying time because of the limited options to perform other activities and the limited size of the dry cow pen in the experiment (same density but fewer cows than the lactating herd; Chapter 3). Cows with a DP also had a 41% lower step count (as a measure for walking) than that of cows with no DP, which was a direct consequence of not going through the milking parlour (Chapter 3). One could wonder if such a 'rest period' might have negative consequences for cow health due to reduced physical fitness (Gustafson, 1993; Davidson and Beede, 2009). The lying time and walking activity of cows with a DP may depend on the farm size and housing facilities of dry cows (Telezhenko et al., 2012). Similarly, no and short DP result in fewer days dry per cow per year, and consequently could result in increased stocking density and competition in the lactating herd (Santschi et al., 2011a).

Having or not having a (short) DP also altered the time budgets of cows during early lactation (Chapter 3). At 4 weeks after calving, cows with no DP had a greater lying time than cows with a DP (11.6 vs 10.7 hours per day) (Chapter 3). Considering that lying time was lowest at this moment in lactation (Maselyne et al., 2017), and that cows without a DP lie down 1 hour longer than cows with a DP (Chapter 3), cows with a DP may also be motivated to lie down longer. If cows do not lie down longer despite the motivation to do so, this would imply that cows are not willing or able to do so for other reasons, possibly due to a greater motivation to be milked or to feed. Cows with a (short) DP did not seem to lie down less due to feeding time constraints, because feeding time during early lactation was not different (Chapter 3). In contrast, a negative association was found between milk yield and lying time, similar to the findings of Norring et al. (2012) at 8 weeks in lactation, which could support the hypothesis that cows lie down less due to discomfort related to the size of the udder, volume of milk or processes in the udder. A prolonged state of NEB may also cause discomfort (Webster, 2000; Roche et al., 2009). The discovered correlations of lying time with milk yield (-0.22) and energy balance (0.28) were weak (Chapter 3), but moderate with plasma FFA concentration (-0.43) (Chapter 4). The greater lying time and feed intake of cows with no DP (Chapter 3), in combination with their improved energy balance (Van Hoeij et al., 2017) and better metabolic status (Chapter 4), could imply that these cows had better welfare than cows with a short DP.

3.2 Udder health

Udder health may be positively and negatively affected by shortening or omitting the DP. A short DP can result in a lower milk production at dry-off, which reduces udder pressure and the risk of new intramammary infections at dry-off (Rajala-Schultz et al., 2005; Bertulat et al., 2013). However, the DP is traditionally used by the farmer to treat subclinical intramammary infections (Santman-Berends et al., 2016). This treatment may not be possible when the DP is shorter than the treatment duration. Shortening the DP did not affect SCC after calving compared with a conventional DP when cows were treated with dry cow antibiotics (Rastani et al., 2005; Van Knegsel et al., 2014b). Omitting the DP increased SCC after calving in some (Klusmeyer et al., 2009; Van Knegsel et al., 2013, 2014b; Van Hoeij et al., 2016) but not all studies (Rastani et al., 2005; Köpf et al., 2014). The higher SCC after no DP than after a conventional DP was not associated with a greater incidence of clinical mastitis (Van Hoeij et al., 2016). However, the incidence of clinical mastitis after no DP was numerically higher than after a short DP in a recent experiment (Van Hoeij et al., 2018). The limited animal numbers do not allow for definitive conclusions on the impact of no DP on udder health. Aside from a possible increase in mastitis, a higher SCC after no DP might be explained by three other factors. First, no dry cow antibiotics are used before calving when the DP is omitted. Second, milk yield after calving is lower when the DP is omitted, which reduces the dilution and consequently increases the concentration of the absolute number of cells (Steeneveld et al., 2013). Third, renewal of udder cells during lactation increases when the DP is omitted (Annen et al., 2008), whereas renewal of udder cells would normally occur at a high rate during the DP (Capuco et al., 2001).

3.3 Calf health

Calf health could be affected by shortening or omitting the DP due to differences in late gestation or in colostrum composition during early lactation. Omission of the DP reduced gestation length by 3 days, and the birth weight of calves by 1.5 kg, compared with a conventional DP (Mayasari et al., 2015). A short DP did not affect gestation length or birth weight. Combined with the greater walking activity during late gestation for cows with no DP than cows with a DP (Chapter 3), this might explain the ease of calving reported by farmers that applied no DP. After calving, no DP results in a lower concentration of antibodies (specifically IgG and IgM) in colostrum compared with a short or conventional DP (Rastani et al., 2005; Mayasari et al., 2015). The lower concentration of antibodies can be explained by antibodies being secreted in milk, instead of accumulating, in the days before calving (Baumrucker et al., 2014). The quantity of colostrum in case of a short DP in most studies was sufficient to fulfil the needs of the calf (Rastani et al., 2005; Watters et al., 2008; Shoshani et al., 2014; Mayasari et al., 2015). The high variability in colostrum quality when the DP is omitted (Baumrucker et al., 2014), however, could call for a larger quantity of colostrum being fed to calves to ensure an adequate intake of antibodies. Growth of calves in the first 12 weeks of life was not affected by a short or no DP (Mayasari et al., 2015). Some farmers that omitted the DP did experience problems with calf health. These farmers either switched to a short DP or fed all new-born calves colostrum produced by heifers to solve this issue.

In summary, omitting the DP seems to improve the energy balance and metabolic status of dairy cows without compromising their time for resting during late gestation. Moreover, lying and feeding time during early lactation improved in synergy with energy balance and metabolic status. A trade-off may exist with udder health should omission of the DP increase the risk of mastitis. There is no trade-off with calf health in the case of a short DP, whereas there is a risk of a trade-off with calf health in the case of no DP, due to a more variable and lower colostrum quality.

4 Short and no dry periods in practice

4.1 Experience of farmers

The effect of DP length on milk production was analysed in collaboration with 16 farmers that applied a short or no DP in practice, mostly since 2010/2011. Reasons to apply a short or no DP varied, but famers generally mentioned ease of management and robust or healthy cows (Steeneveld et al., 2013). Two farmers still apply no DP for the whole herd (farms B and E in Chapter 5). These farms are not alike: farm B has an above-average production level, whereas farm E has a below-average production level and an exceptionally low replacement rate (<10%). Both farms have shorter than average calving intervals (Chapter 5). Farmers that stopped omitting the DP mentioned that they were unsatisfied with the total milk production (and mainly quit after the first omission of the DP), or had problems with calf health (see section 3.3). In future, farmers that are considering omitting the DP could be informed in advance about the expected reduction in milk production per effective lactation and at the herd level. Informing farmers about the timing of milk production is especially important, because milk yield is lowest after the first omission of the DP, and a dip in total milk production is expected in the second year the strategy is applied. In some cases, cows dried off spontaneously and fattened before the next calving. In an experimental setup, lactation persistency and short calving intervals were essential for successful omission of the DP

over multiple lactations (Chen, 2016). All other farmers appear to have switched to a DP from 3 to 6 weeks, which generally consists of a DP of 3-4 weeks for cows that are not treated, and 6 weeks for cows that are treated for subclinical mastitis. Several farmers mentioned that they do not, or rarely, use antibiotics at dry-off, and only in some cases of clinical mastitis, because they perceive that most cows are robust and recover spontaneously.

The amount of labour on farms may increase or decrease when the DP is shortened or omitted. Both shortening and omitting result in extra milking days. This increases the amount of labour in case of conventional milking and may require extra capacity in case of automated milking. Moreover, more labour may be required for youngstock management to ensure calf health when the DP is omitted. Less labour may be required for preparation of the ration when only one ration is used (Heeren et al., 2014). Also, less labour may be required for regrouping and drying off. For example, one farmer applies a DP of 3 weeks for all cows, without regrouping dry cows. Selection gates of the automated milking system simply keep cows from milking, and dry cows eat the same ration as lactating ones, except for the concentrate allowance. Automated milking, feeding one ration and not regrouping the cows greatly reduce labour for the farmer. In other cases, the qualitative aspect of labour may outweigh a possible quantitative increase in working hours when shortening or omitting the DP. Farmers that applied a short or no DP with a conventional milking system preferred additional daily labour for milking over incidental tasks, such as regrouping and drying off, and over unpredictable labour in case of illness.

4.2 Recommendations to farmers

A tailored DP length for each cow has been proposed to treat subclinical mastitis in cows with a high SCC, and to shorten or omit the DP of healthy cows depending on their history regarding metabolic disorders and fertility, and their current yield and persistency (Van Hoeij, 2017). Such a cow-specific approach likely requires more planning and monitoring than no DP or one DP length for all cows. A tailored DP length may, therefore, not be compatible with the attitude of the farmers participating in the current project, who valued ease of labour. Moreover, variation within the herd may increase when no, short and conventional DP are applied. Therefore, the success of tailored DP will depend on the attitude of the farmer and the possibilities to, for example, automatically adjust the feeding and milking regimes of individual cows.

If I were asked to recommend a DP strategy to a farmer, I would respond that it depends on the farm-specific circumstances and the attitude of the farmer. No DP would be my preferred DP length

regarding cow welfare, because of the clear and consistent improvement in energy balance during early lactation, in combination with fewer changes in routine and potential benefits for health and lifespan of the dairy cow. The trade-off with net partial cash flows is small and may be a synergy if omitting the DP reduces veterinary costs. Similarly, the trade-off with GHG emissions per unit milk is small and may be a synergy if lifespan is increased. However, omitting the DP may not be suitable for a farmer who values high milk production, or on a farm with insufficient labour, barn space or milking capacity to accommodate the increase in lactating cows. Moreover, omitting the DP may not be beneficial to welfare when cows have a high SCC, when calf health is compromised and when cows spontaneously dry off due to low persistency or long calving intervals and consequently fatten before calving. Under such circumstances, improved hygiene, feed and fertility management would be desirable, and cows and calves should not be put at risk by omitting the DP. Under such circumstances, therefore, I would recommend giving cows a DP. A conventional DP of 2 months may not be beneficial for the cow, however, because of the challenge at dry-off, many changes in routine and an expected NEB of about 3 months after calving. Therefore, a short DP of 4 weeks would in my opinion be preferred over a conventional DP, to reduce the number of transitions and improve the NEB after calving. In the case of a high SCC during late lactation, a DP of 6 weeks may be desirable to allow treatment of subclinical mastitis.

5 Improving sustainability

Shortening or omitting the DP can improve cow welfare with limited impact on net partial cash flows and GHG emissions. Therefore, both strategies have the potential to improve these aspects of the sustainability of dairy farming. However, shortening or omitting the DP do not seem very promising strategies to improve net partial cash flows and GHG emissions in dairy farming. If a farmer aims for an increase in net partial cash flow, or the dairy sector aims for a reduction of total GHG emissions (Reijs et al., 2016), other strategies may be more suitable.

Chapter 8 evaluated different lactation length strategies, in combination with improved production level and persistency. A higher production level and persistency, ideally in combination with an increased lifespan of dairy cows, greatly increased net partial cash flows and reduced GHG emissions per unit milk (Chapter 8). The results regarding increased production level and persistency in Chapter 8 were estimated with milk production far above the Dutch average, whereas other factors remained unchanged. An optimisation model, accounting for e.g. feed intake constraints and changes in feed production on-farm, could provide more realistic results. For example, an increase in milk yield was shown to increase labour income and reduce GHG emissions per unit milk in a Dutch farm situation under conditions optimised for labour income or GHG emissions (Van Middelaar et al., 2015). Conclusions may be different now, however, because the milk quota has been replaced by a phosphate excretion quota (Klootwijk et al., 2016). Each Dutch dairy farm now has a phosphate excretion quota based on its herd size in July 2015 and standard excretion factors (RVO, 2018). The standard excretion factor of phosphate per cow per year depends on milk production and would be 42.7 kg for cows with a conventional DP and 42.0 kg for cows with a short or no DP in the baseline simulation (Chapter 7). Shortening and omitting the DP, therefore, seems possible with a maintained or slightly increased herd size when the farm is within its phosphate quota. An increase in milk yield, however, is not possible within the phosphate quota unless phosphate efficiency is increased or the number of cows or youngstock is reduced. A reduction in youngstock reduces animal phosphate excretion by 9.6 kg/ head (<1 year of age) and 21.9 kg/head (>1 year of age). The number of youngstock may be reduced without consequences for net partial cash flows and GHG emissions by extending heifer lactations if this increases lifespan and thereby reduces replacement rate (Chapter 8).

A trade-off with welfare may be expected when production levels are increased, however, because this is expected to simultaneously increase the NEB during early lactation (Veerkamp, 1998). Moreover, although extending lactations would reduce milk production at dry-off and the frequency of the NEB during early lactation, the severity of the NEB will remain the same. Considering net partial cash flows, GHG emissions per unit milk and two aspects of cow welfare, increasing lifespan and lactation persistency, but not peak yield of cows, could contribute to sustainable milk production (Table 1). Lactation persistency and lifespan could be increased in combination with short or no DP. Extended lactations, moreover, may be used to facilitate an increase in lifespan of dairy cows.

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	Net partial	GHG emissions	NEB during	Transitions
	cash flow	per unit milk	early lactation	for dairy cow
î lactation persistency	++	+	0	0/-
û peak yield	++	+	-	-
û lifespan	+	++	0	0
no dry period	+/-*	+/-*	++	++
short dry period	+/-*	+/-*	+	+
extended lactation heifers	+/-*	+/-*	0	+

Table 1: Qualitative sustainability impacts of an increase in lactation persistency, peak yield or lifespan, of omitting or shortening the dry period, and of extending lactation length of heifers.

*) Result depends on assumptions regarding lifespan, disease incidence and milk yield.

With the evaluated Dutch lactation curves, heifers had a persistent milk production that may make them suitable for extended lactations (Chapter 8), and heifers had a higher effective lactation yield than second parity cows when the DP was omitted (Chapter 7). Therefore, we might try extending heifer lactations in combination with omission of the DP in order to improve cash flows, GHG emissions and cow welfare in synergy. Extending heifer lactations by 2 or 4 months, in combination with no DP, was evaluated using the combined models of Chapter 7 and 8, to forecast expected consequences with current production levels. The combined strategy reduced overall milk yield and net partial cash flows and increased GHG emissions per unit milk, compared with omission of the DP alone (Appendix, Table 9.a). Extending lactations of heifers reduced effective lactation yields of heifers as well as second parity cows, because less extra milk was produced in the weeks before calving. This suggests that omission of the DP is most successful with short lactations.

Dutch goals regarding production and GHG emissions of the dairy sector (Reijs et al., 2016) may not be reached with a short or no DP (Chapter 7), even when combined with a reduction in disease incidence (Mostert et al., 2018b; Özkan Gülzari et al., 2018), an increase in lactation persistency (Chapter 8) and changes in e.g. manure management and feed components (Chadwick et al., 2011; Van Middelaar et al., 2015). If we want to meet the relatively stable European or the increasing global demand for dairy products (Alexandratos and Bruinsma, 2012) while reducing total GHG emissions in the dairy sector, this might require an increase in cow production levels. This would increase the NEB of dairy cows during early lactation (Veerkamp, 1998) and could increase discomfort related to the udder. Moreover, an increase in milking frequency and higher energy requirements could increase milking and feeding time and consequently constrain the time budget of dairy cows. Especially if society does not accept a further increase in milk yield and NEB of the dairy cow, increasing peak yield may not be a sustainable solution (Van Calker et al., 2005; Oltenacu and Broom, 2010). A sustainable solution to reducing total GHG emissions in the dairy sector may, therefore, be found in reduced consumption of dairy products. Alternatives to dairy products with a lower carbon footprint could be considered to limit dairy production and consumption at the EU level (Bryngelsson et al., 2016).

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6 Conclusions

This thesis is the first to make an integrated assessment of consequences of short and no DP for economic, environmental and social sustainability impacts of milk production. Novelties in this thesis include the integrated assessment of consequences of short and no DP 1) for overall milk production, using the effective lactation yield and annual production at the herd level, 2) for net partial cash flows, 3) for GHG emissions per unit milk and 4) for behaviour of dairy cows in the months around calving. The large dataset from commercial farms that applied no and short DP over multiple lactations enabled me to analyse consequences for milk production in practice. The animal experiment enabled me to study lying and feeding behaviour in relation to individual milk production, energy balance and metabolic status.

The effective lactation yield and herd simulation model provided insights into the consequences of short and no DP. The highest milk production is obtained for a conventional DP of 56 days. However, reductions in effective lactation yields due to short or no DP are smaller than was previously assumed based on the reduction in milk yield after calving. Reductions in milk yield in case of no DP differed over time and were largest in the second year that no DP was applied.

The impact of short or no DP on net partial cash flow and GHG emissions per unit milk was small but negative when no reduction in disease incidence and culling rate were assumed. A potential reduction in disease costs and a reduction in culling rate, however, may compensate for these impacts.

The impact of short and no DP will be different across farms due to farm-specific effects of DP length on factors such as milk yield, fertility, calf and cow health and lifespan. More research is needed in which multiple farms are monitored for multiple years in order to identify factors that explain variations between farms.

Behaviour of dairy cows with no or a (short) DP was observed at 4 weeks before calving and 4 weeks after calving. Before calving, cows with no DP had a 1 hour shorter lying time than cows with a DP, but their time for resting did not seem compromised. After calving, lying time was shorter than before calving, and cows with no DP had a 1 hour longer lying time than cows with a short DP. Cows with no DP also had a greater feed intake and therefore seemed better adapted to the next lactation.

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Appendices

Chapter 5

- Appendix 5.a Parameter values for the Wilmink curves used as standard lactation curves
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Chapter 9

Appendix 9.a Milk production, net partial cash flow and greenhouse gas emissions of dairy herds with dry periods of 56, 28 or 0 days and 0, 2 or 4 months extended lactations for heifers **Table 5.a** parameter values for the Wilmink curves used as standard lactation curves for cows in parity 1 and additional effects (on a and b) for cows with no (n=234), short (n=323) or conventional (conv., n=260) dry period (DP) lengths in parity 2. All fixed effects were highly significant (Wald test, 1631 degrees of freedom, P < 0.001).

			No DP, parity 2		Short DP ¹ , parity 2		Conv. DP ¹ ,	parity 2
	estimate	SE	estimate	SE	estimate	SE	estimate	SE
а	33.69	0.36	3.03	0.48	7.83	0.42	10.09	0.45
b	-0.046	0.0012	-0.0261	0.0016	-0.0291	0.0014	-0.0315	0.0015
с	-9.51	0.29						
k	0.0191	0.0012						
S^2 a	56.17	3.12						
s ² b	0.00065	0.00004						
S ² c	75.52	4.85						
COVab	-0.153	0.011						
$\rm COV_{bc}$	0.138	0.013						
COVac	-37.36	3.72						
S ² e	6.75	0.09						

¹Short dry period: 20 d to 40 d; conventional dry period: 49 d to 90 d

Table 5.b Estimates¹ for 305-d, 365-d and effective lactation (eff.) yield² and median days open (DO) of second-parity cows per dry period (DP) category per farm.

	No DP				Short DP ³				Conventional DP ³			
farm	305-d	365-d	eff.	DO	305-d	365-d	eff.	DO	305-d	365-d	eff.	DO
А	24.6	23.0	22.6	90	28.6	25.1	24.7	92	31.7	26.6	25.6	112
В	27.5	26.2	26.4	77	31.3	27.8	28.1	85	32.9	27.6	27.1	114
С	25.3	23.8	24.1	70	-	-	-	-	-	-	-	-
D	23.0	22.1	21.8	86	26.6	23.6	23.3	90	29.8	25.3	24.9	105
Е	23.1	21.4	21.4	77	26.8	23.4	23.1	90	29.1	24.5	23.9	114
F	21.7	20.8	20.2	93	26.5	23.4	23.2	87	29.3	24.8	24.6	93
G	20.8	20.4	20.0	85	27.1	24.2	23.6	94	29.6	25.1	24.4	111
Н	22.6	21.6	21.5	82	27.9	24.8	24.6	89	30.6	25.9	25.4	98
Ι	24.3	22.9	23.1	75	28.7	25.2	25.4	76	31.9	26.8	26.5	96
J	24.9	23.9	24.0	78	30.7	27.2	27.3	81	-	-	-	-
К	-	-	-	-	28.4	24.9	24.5	101	30.5	25.5	24.5	129
L	-	-	-	-	30.2	26.7	26.6	94	32.7	27.6	27.4	106
М	-	-	-	-	25.6	22.7	22.3	91	26.9	22.7	22.1	105
Ν	-	-	-	-	29.2	25.7	25.0	101	30.0	25.3	24.5	115
0	-	-	-	-	29.4	25.8	25.0	103	32.6	27.5	26.4	122

¹Using SAS statements LSMEANS for fixed and ESTIMATE for random effects

²Kg FPCM per cow per day

³Short dry period: 20 d to 40 d; conventional dry period: 49 d to 90 d

Parameter	Component	Estimate	SE	df	Р
а	Intercept	20.9226	0.3643	6654	<.0001
	Par 1	-8.9916	0.2672	7922	<.0001
	Par 2 – standard DP	-0.00119	0.2797	7843	0.9966
	Par 2 – short DP	-2.9724	0.3071	7654	<.0001
	Par 2 – no DP	-6.7875	0.3195	7444	<.0001
	Par >2 – standard DP	3.5600	0.2393	5856	<.0001
	Par >2 – short DP	0.7425	0.2807	5957	0.0082
	Par >2 – no DP 1 st time	-2.5319	0.3433	5840	<.0001
	Par >2 – no DP 2 nd time	0			
	Covariate ^a	0.002543	0.000037	5672	<.0001
b	Intercept	-0.08350	0.000540	6840	<.0001
	Par 1	0.03878	0.000755	5688	<.0001
	Par 2	0.01274	0.000756	5697	<.0001
	Par >2	0			
С		-16.0916	0.1971	3550	<.0001

Table 7.a Standard errors and P-values for fitted parameters a, b, and c of the lactation curves.

^aFirst parity 305 yield in kg fat-and-protein-corrected milk.

Table 7.b Emission factors for N₂O and CH₄ emissions from manure, on pasture and in stables.

Emission factors	Pasture	Stable
N ₂ O-N direct	0.033 kg/ kg N ^a	0.0015 kg/ kg TAN ^{e,1}
NH ₃ -N	0.053 kg/ kg TAN ^{a,1,2}	0.1 kg/ kg TAN ^e
NO _x -N	0.012 kg/ kg Nª	0.0015 kg/ kg TAN ^e
NO ₃ -N leaching	0.12 kg/ kg N ^b	
CH ₄	0.11 kg/ m ³ manure ^c	0.746 kg/ t manure ^c
N_2O-N via NH_3	0.01 kg/ kg NH ₃ -N ^d	0.01 kg/ kg NH ₃ -N ^d
N ₂ O-N via NO _x	0.01 kg/ kg NO _x -N ^d	0.01 kg/ kg NO _x -N ^d
N ₂ O-N via NO ₃ -	0.0075 kg/ kg NO₃⁻-N ^d	

References: a: (Vonk et al., 2016); b: (Velthof and Mosquera, 2011); c (De Mol and Hilhorst, 2003); d (Dong et al., 2006); e: (Vries et al., 2011)

¹TAN = Total Ammoniacal Nitrogen

 2 calculated value: 1.98 \times 10 $^{-5}$ \times (N-content ration)^{3.664}; N-content of the ration in summer (i.e. when on pasture) is 30.31 g/ kg DM

Table 9.a Average annual milk production and net partial cash flow, and greenhouse gas emissions per
unit fat-and-protein-corrected milk (FPCM) of herds with dry period lengths of 56, 28, or 0 days, and
baseline lactation lengths (BL), or 2 or 4 months extended lactations for heifers. Averages and SD of 100
herds with 100 cows, over the first 5 years the strategy is applied.

		Dry period length						
		56 days		28 days		0 days		
		Avg SD		Avg	SD	Avg	SD	
Milk	BL	871	12	851	15	840	20	
(t herd ⁻¹ y ⁻¹)	H+2	863	13	842	18	830	23	
	H+4	851	16	830	24	818	28	
NPCF ^a	BL	170	4	168	4	167	5	
(k€ herd ⁻¹ y ⁻¹)	H+2	168	4	166	4	165	5	
	H+4	166	4	164	5	162	6	
GHG emissions	BL	938	18	945	17	942	18	
(Kg CO ₂ -eq per t FPCM)	H+2	937	18	946	17	944	19	
	H+4	938	18	946	18	946	19	

^aCosts for calving and artificial insemination were included in the analysis of NPCF.
Summary

A high-producing dairy cow experiences a negative energy balance (NEB) for about 3 months after calving, which is associated with impaired health and fertility. Moreover, the cessation of milking at the start of the dry period (DP) has become a challenge due to the high milk yield at dry-off. Shortening and omitting the DP are strategies to improve cow health and ease the transition period around calving. Both strategies partly shift milk production from early lactation to the weeks before calving, and improve the energy balance, metabolic status and fertility in early lactation. Consequences of shortening or omitting the DP for other aspects of cow welfare besides health, for the farmer and for global warming, however, are currently not well known. The aim of this thesis is to evaluate and integrate sustainability impacts of short or no DP in dairy cows, with a focus on cow welfare, cash flows and greenhouse gas (GHG) emissions.

Good welfare consists of good health, feeling well and being able to express natural behaviour. The feelings aspect of welfare can be indirectly addressed by monitoring behaviour. Changes in behaviour, such as an increase in feeding rate or a reduction in lying time, could be indicative of time constraints and reduced welfare of dairy cows.

To address the impact of a DP on the feelings aspect of welfare, behaviour of dairy cows with and without a DP was monitored. First, however, the sensor measuring lying behaviour was validated (**Chapter 2**). To validate lying records, data were compared between simultaneously recording sensors on the left and the right hind legs of cows (N=28). Results indicated that short records of lying (<33 s) were mostly false. Therefore, these records were discarded in the next experiment.

Lying behaviour, feeding behaviour and steps of dairy cows were measured in late gestation and early lactation in cows with a short (30 d) or no DP (**Chapter 3**). In late gestation, cows with a short DP were fed a DP ration and housed in a dry cow group, and cows with no DP were fed a lactation ration and housed in the lactating herd. In early lactation all cows were fed the same lactation ration in the lactating herd. In late gestation (week 4 before calving), no DP reduced daily lying time (-1 h) and increased the number of steps (+70%) compared with a short DP. Differences in lying time and number of steps in late gestation were associated with going through the milking parlour of cows with no DP, compared with cows with a short DP. In early lactation (week 4 after calving), no DP resulted in a higher feed intake and longer lying time (+1 h) in early lactation compared with a short DP. The absolute daily lying time (12.6 h) and relatively constant feeding rate suggest that feelings of cows with no DP were not impaired by milking in late gestation.

Moreover, the greater feed intake and increased lying time in early lactation suggested that cows with no DP were better adapted to the start of the next lactation than cows with a short DP.

Subsequently, cow behaviour in week 4 after calving was associated with metabolic status to study the relation between behaviour and physiology in early lactation (**Chapter 4**). Physiological indicators of high metabolic load (high plasma FFA concentration, low plasma IGF-1 concentration) were negatively associated with number of meals, feed intake and daily lying time. These results suggest that a compromised metabolic status in early lactation is reflected in altered cow behaviour during this period.

An accurate estimate of the impact of DP length on milk production is essential for an accurate estimate of the impact of DP length on cash flows and GHG emissions. Therefore, a novel measure for milk yield was developed to compare yields of cows with different DP length, and consequences of DP length for milk yield were assessed over two consecutive lactations.

To compare milk yield between cows with different DP lengths, accounting for extra milk before calving and possible changes in calving interval, the 'effective lactation yield' measure was developed (**Chapter 5**). The 'effective lactation yield' was defined as the daily yield from 60 days before calving to 60 days before the next calving, to account for additional milk yield before calving and for differences in calving interval. Accounting for additional milk yield before calving had a major impact on yield comparisons of cows with different DP lengths. For example, omission of the DP reduced the 305-d yield of second parity cows by 7.0 kg per day, and the effective lactation yield by only 3.1 kg per day, compared with a conventional DP. Correcting for calving interval will especially affect milk yield comparisons when calving interval is affected by DP length.

The impact of DP length on effective lactation yields of second and greater parity cows was assessed over multiple lactations (**Chapter 6**). In line with earlier studies, the reduction in milk yield compared with a standard DP was larger for no DP than for a short DP. A second omission of the DP resulted in a higher milk yield after calving than a first omission of the DP. This higher milk yield after calving, however, did not result in a higher effective lactation yield, because these cows also produced a smaller amount of extra milk in the weeks before calving. Therefore, the effective lactation yield did not differ between the first and a subsequent shortening or omission of the DP.

The impact of DP length on milk production and cash flows at herd level and GHG emissions per unit of milk were evaluated using a dynamic stochastic simulation model (**Chapter 7**). Modelled impacts of DP length were derived from production data of dairy farms that voluntarily managed cows for a short or no DP. Introduction of no DP resulted in a dip in milk production of the herd in

the second year the strategy was applied. On average, applying a short DP reduced milk yield of the herd by 3.1%, and applying no DP reduced milk yield of the herd by 3.5%. Partial cash flows were computed from revenues from sold milk, calves and culled cows, and costs from feed and rearing youngstock, and GHG emissions were computed using a life cycle approach. On average, short and no DP reduced partial cash flows by €12 and €16 per cow per year, and increased GHG emissions by 0.8% and 0.5%, respectively. These relatively small negative impacts of short and no DP on cash flows and GHG emissions may be offset by improved cow health and lifespan.

Instead of shortening or omitting the DP, lactations of dairy cows can be extended to reduce the frequency of critical transition periods and thereby improve cow health. The impact of extending lactations on milk yield, cash flows and GHG emissions was explored (**Chapter 8**) using empirical data of cows managed for conventional and extended lactation lengths. Extending lactations by 2 or 4 months reduced milk yield of the herd, reduced the net partial cash flow per herd per year and increased GHG emissions per unit milk compared with the conventional lactation length. The sensitivity analysis showed that the negative impact of extending lactations on cash flows could be compensated by an increase in lactation persistency, whereas the negative impact on GHG emissions could be compensated by an increase in lifespan of cows.

In the discussion, sustainability impacts of short and no DP were integrated (**Chapter 9**). The integrated consequences of DP length for cow welfare, cash flows and GHG emissions can facilitate informed decision-making by dairy farmers. Consequences for udder and calf health and farmers' experience were also discussed. The existing literature appears too limited to allow for definitive conclusions on the impact of no DP on udder health, whereas shortening the DP did not seem to affect udder health. Colostrum quality after no DP, but not after a short DP, is lower than after a conventional DP, and this could call for a larger quantity of colostrum being fed to calves to ensure an adequate intake of maternal antibodies. Farmers that voluntarily managed cows for a short or no DP valued the ease of management and perceived cows to be healthier or more robust.

To conclude, this thesis assesses and integrates consequences of short and no DP for behaviour of dairy cows, for overall milk production (using the effective lactation yield), for net partial cash flows and for GHG emissions per unit milk. Cows with no DP seemed better adapted to the next lactation than cows with a DP. The estimated impacts of short and no DP on cash flows and GHG emissions were small but negative compared with a conventional DP. Improved cow health and lifespan after a short or no DP could compensate these impacts.

Samenvatting

Een hoogproductieve melkkoe heeft in de maanden na afkalven een negatieve energiebalans, die de kans op ziekte en vruchtbaarheidsproblemen vergroot. Daarnaast is het risicovol om een koe met een hoge melkproductie droog te zetten. Het verkorten of weglaten van de droogstand vergemakkelijkt de transitieperiode rondom afkalven, zorgt voor extra melkproductie in de weken voor afkalven en verlaagt de melkproductie na afkalven. De lagere melkproductie verbetert de energiebalans, metabole status en vruchtbaarheid in vroege lactatie. Gevolgen van het verkorten of weglaten van de droogstand voor de koe (buiten metabole status), de veehouder en het klimaat zijn nog onduidelijk. In dit proefschrift zijn gevolgen van het verkorten of weglaten van de droogstand voor het welzijn van de koe, het inkomen van de veehouder en broeikasgasemissies onderzocht.

Onder goed welzijn wordt verstaan dat een dier gezond is, zich goed voelt, en natuurlijk gedrag kan vertonen. Omdat niet direct gemeten kan worden hoe een dier zich voelt, wordt gedrag gebruikt als indirecte maat. Veranderingen in gedrag, zoals een snellere voeropname of een afname in ligduur, kunnen bij melkvee wijzen op verminderd welzijn.

Om te bepalen of (het niet hebben van) een droogstand beïnvloedt hoe een koe zich voelt, is het gedrag van koeien met en zonder droogstand gemonitord met sensoren. Eerst is de sensor om liggedrag mee te meten gevalideerd (**Hoofdstuk 2**). Hiervoor zijn de geregistreerde ligperiodes vergeleken tussen 2 sensoren die gelijktijdig liggedrag registreerden aan de linker en rechter achterpoot van 28 koeien. Korte geregistreerde ligperiodes (<33 seconden) bleken vaak onjuist, en werden daarom niet meegenomen in de analyse bij de volgende studies.

Liggedrag, voeropnamegedrag en stappen van koeien met een korte (30 dagen) of geen droogstand zijn gemeten in week -4 en 4 ten opzichte van afkalven (**Hoofdstuk 3**). In week -4 waren koeien met een korte droogstand gehuisvest met droge koeien en kregen zij een droogstandsrantsoen, terwijl koeien zonder droogstand in de lacterende groep bleven en een lactatierantsoen kregen. In week 4 kregen alle koeien hetzelfde lactatierantsoen in de lacterende groep. Koeien zonder droogstand hadden in week -4 een kortere ligduur (-1 uur/ dag), en zetten meer stappen (+70%) dan koeien met een korte droogstand. Deze verschillen werden veroorzaakt door het twee keer per dag melken van koeien zonder droogstand. In week 4 hadden koeien zonder droogstand een langere ligduur (+1 uur/ dag) en een hogere voeropname dan koeien met een korte droogstand. De relatief hoge absolute ligduur in week -4 (12.6 uur/ dag) en de stabiele vreetsnelheid over de weken voor afkalven suggereren dat het welzijn van koeien zonder droogstand niet verminderd was door het tweemaal daags melken. De langere ligduur en hogere voeropname in vroege lactatie suggereren

dat koeien zonder droogstand zich makkelijker aanpasten aan de start van de volgende lactatie dan koeien met een korte droogstand.

Vervolgens zijn de relaties bestudeerd tussen gedrag en metabole status in week 4 na afkalven (**Hoofdstuk 4**). Bloedwaarden die gerelateerd zijn aan een hogere metabole belasting (hoge concentratie vrije vetzuren, lage concentratie IGF-1) waren gecorreleerd met minder maaltijden, een lagere voeropname en een kortere ligduur. Deze resultaten geven aan dat de metabole status van gezonde koeien in vroege lactatie wordt gereflecteerd in gedrag.

Een nauwkeurige schatting van het effect van droogstandslengte op melkproductie is essentieel voor een nauwkeurige schatting van bijbehorende gevolgen voor inkomen en broeikasgasemissies. Daarom is eerst een nieuwe maat voor melkproductie ontwikkeld, waarmee de totale melkproductie van koeien met verschillende droogstandslengtes kon worden vergeleken.

De maat 'effectieve lactatie' is gebaseerd op de melkproductie tussen twee droogstandsbeslissingen, en wordt berekend als de gemiddelde melkproductie van 60 dagen voor afkalven tot 60 dagen voor het volgende afkalfmoment (**Hoofdstuk 5**). De extra melk die geproduceerd wordt vóór afkalven wanneer de droogstand wordt verkort, wordt daardoor toegeschreven aan de effectieve lactatie. Ook wordt er gecorrigeerd voor verschillen in tussenkalftijd. Met name het toeschrijven van de extra melk vóór afkalven aan de effectieve lactatieproductie had een groot effect op de vergelijking van melkproductie tussen koeien met en zonder droogstand. Zo was de 305-dagen productie van tweedekalfs koeien 7.0 kg per dag lager wanneer koeien geen droogstand hadden, terwijl de effectieve lactatieproductie slechts 3.1 kg per dag lager was.

Vervolgens zijn de gevolgen van het verkorten of weglaten van de droogstand voor twee opeenvolgende lactaties op melkproductie geanalyseerd (**Hoofdstuk 6**). In overeenstemming met eerder onderzoek resulteerde de conventionele droogstand in de hoogste melkproductie, en werd de melkproductie sterker verlaagd wanneer de droogstand werd weggelaten dan wanneer deze werd verkort. De effectieve lactatieproductie was niet verschillend tussen de eerste en tweede keer dat de droogstand werd verkort of weggelaten. Er waren wel verschillen in wanneer de melk werd geproduceerd: de tweede keer dat de droogstand werd weggelaten was de melkproductie vóór afkalven lager en na afkalven hoger dan de eerste keer dat de droogstand werd weggelaten.

Gevolgen van het verkorten of weglaten van de droogstand voor netto opbrengsten en broeikasgasemissies per eenheid melk zijn bepaald met een model (**Hoofdstuk** 7). Het model werd ontwikkeld om lactaties van koeien met een conventionele, korte of geen droogstand na te bootsen voor bedrijven met 100 koeien, en is gebaseerd op de melkproductieregistratie van

praktijkbedrijven die de droogstand al enkele jaren bewust verkorten of weglaten. Het weglaten van de droogstand verlaagde de melkproductie van de koppel over de eerste 5 jaar gemiddeld met 3.5%, met het grootste melkverlies in het tweede jaar dat de strategie werd toegepast. Een verkorte droogstand verlaagde de melkproductie gemiddeld met 3.1% vergeleken met een conventionele droogstand. Voor een schatting van de economische effecten van droogstandslengte zijn de opbrengsten van melk, afgevoerde koeien, en verkochte kalveren, en de kosten van voer en jongvee opfok per kudde per jaar bepaald. De broeikasgasemissies zijn bepaald met een levenscyclusanalyse. Gemiddeld over 5 jaar, verminderden een verkorte en geen droogstand de netto opbrengsten met €12 en €16 per koe per jaar, en werden broeikasgasemissies per eenheid melk met 0.8% en 0.5% verhoogd. Een verlaging in ziektekosten per koe en een verlengde levensduur van koeien, die op basis van de verbeterde energiebalans in vroege lactatie verwacht zou kunnen worden, kan deze effecten compenseren. Deze mogelijke verbeteringen in gezondheid zijn niet in de modelvergelijking meegenomen.

In plaats van het verkorten of weglaten van de droogstand om de transities rondom afkalven milder te maken, kan de lactatie van koeien worden verlengd zodat de transitieperiode minder frequent wordt doorgemaakt. Gevolgen van 2 of 4 maanden verlengde lactaties voor melkproductie, inkomen en broeikasgasemissies werden daarom geanalyseerd (**Hoofdstuk 8**). Het verlengen van lactaties verlaagde de melkproductie van de koppel en de netto opbrengsten, en verhoogde broeikasgasemissies per eenheid melk. Een gevoeligheidsanalyse wees uit dat de negatieve gevolgen konden worden gecompenseerd door een verhoogde persistentie van melkproductie of een verlengde levensduur van koeien.

In de discussie zijn gevolgen van het verkorten of weglaten van de droogstand voor de koe, de veehouder, en broeikasgasemissies op een rij gezet (**Hoofdstuk 9**). Dit overzicht kan bijdragen aan een bewuste keuze voor een bepaalde droogstandslengte door melkveehouders. Gevolgen voor uiergezondheid en kalvergezondheid en ervaringen van veehouders werden ook besproken. De beschikbare literatuur over het effect van droogstandslengte op uiergezondheid laat zien dat het verkorten van de droogstand geen invloed heeft op uiergezondheid, maar is te beperkt om definitieve conclusies te trekken over het effect van het weglaten van de droogstand. De biestkwaliteit is gelijk na een verkorte en een standaard droogstand, maar vermindert wanneer de droogstand wordt weggelaten. Vanwege de verminderde biestkwaliteit kan een grotere hoeveelheid biest nodig zijn om voldoende opname van maternale antistoffen door het kalf te garanderen. Veehouders die de droogstand bewust verkorten of weglaten noemen als redenen het arbeidsgemak en dat de koeien gezonder of robuuster zijn.

In dit proefschrift zijn effecten bestudeerd van het verkorten of weglaten van de droogstand op het gedrag van koeien, op melkproductie (met de maat 'effectieve lactatie'), op inkomen en op broeikasgasemissies per eenheid melk. Koeien zonder droogstand leken beter aangepast aan de volgende lactatie dan koeien met een droogstand. Gevolgen van het verkorten of weglaten van de droogstand voor netto opbrengsten en broeikasgasemissies waren klein maar negatief. Een verbeterde gezondheid en een verlengde levensduur van koeien kunnen deze gevolgen compenseren.

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Akke Kok was born in Ede, the Netherlands, in 1990. She obtained her BSc degree in Biology (2011) and her MSc degree in Environmental Biology (2013) from Utrecht University (both cum laude). During her BSc, Akke studied third-party interventions in wild common ravens (Konrad Lorenz Research Station, Austria). During her MSc, she assessed the impact of alternative weaning and separation methods on weaning distress after prolonged suckling in dairy calves (Louis Bolk Institute, the Netherlands),

and she investigated the regulation of elongation in *Arabidopsis thaliana* seedlings (Plant Ecophysiology, Utrecht University, the Netherlands).

After graduation, Akke started as a PhD student in the Animal Production Systems group of Wageningen University & Research. In collaboration with the Adaptation Physiology group and the Business Economics group, she investigated impacts of short or no dry periods in dairy cows on cow behaviour, milk yield, cash flows, and greenhouse gas emissions. Her PhD research was part of an interdisciplinary project called 'Customised dry period', which was financed by DairyNL (Zuivel NL) and the Dutch Ministry of Economic Affairs (EZ), as part of the 'Sustainable Dairy Chain initiative' (Duurzame Zuivelketen). Akke was granted a 'Short Term Scientific Mission' by DairyCare (COST Action FA1308) to go to Scotland for collaborative work with Bert Tolkamp and Marie Haskell at Scotland's Rural College. She was awarded the Novus travel grant for her presentation entitled 'Comparing cows – including dry period and lactation length in a yield measure' at the EAAP (European Association for Animal Production) conference in 2015.

Currently, Akke is working as a postdoctoral fellow with the Animal Production Systems group and the Adaptation Physiology group of Wageningen University & Research.

Publications

Refereed scientific journals

- Kok, A., A.T.M. van Knegsel, C.E. van Middelaar, H. Hogeveen, B. Kemp, and I.J.M. de Boer. 2015.
 Technical note: Validation of sensor-recorded lying bouts in lactating dairy cows using a 2-sensor approach. J. Dairy Sci. 98:7911–7916.
- Kok, A., C.E. van Middelaar, B. Engel, A.T.M. van Knegsel, H. Hogeveen, B. Kemp, and I.J.M. de Boer. 2016. Effective lactation yield: A measure to compare milk yield between cows with different dry period lengths. J. Dairy Sci. 99:2956–2966.
- Chen, J., A. Kok, G.J. Remmelink, J.J. Gross, R.M. Bruckmaier, B. Kemp, and A.T.M. van Knegsel.
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- Kok, A., R.J. van Hoeij, B.J. Tolkamp, M.J. Haskell, A.T.M. van Knegsel, I.J.M. de Boer, and E.A.M. Bokkers. 2017a. Behavioural adaptation to a short or no dry period with associated management in dairy cows. Appl. Anim. Behav. Sci. 186:7–15.
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- Kok, A., C.E. van Middelaar, P.F. Mostert, A.T.M. van Knegsel, B. Kemp, I.J.M. de Boer, and H. Hogeveen. 2017c. Effects of dry period length on production, cash flows and greenhouse gas emissions of the dairy herd: A dynamic stochastic simulation model. PLoS One 12:e0187101.
- Van Hoeij, R.J., A. Kok, R.M. Bruckmaier, M.J. Haskell, B. Kemp, and A.T.M. van Knegsel. Relationship between metabolic status and behavior in dairy cows in week 4 of lactation. *Submitted*
- Kok, A., J.O. Lehmann, B. Kemp, H. Hogeveen, C.E. van Middelaar, I.J.M. de Boer, and A.T.M. van Knegsel. Production, cash flows and greenhouse gas emissions of simulated dairy herds with extended lactations. *Submitted*

Abstracts in conference proceedings

Kok, A., A.T.M. van Knegsel, C.E. van Middelaar, H. Hogeveen, B. Kemp, I.J.M. de Boer. 2015. Is she lying? Validation of IceQube-recorded lying in dairy cows. 2015. In: Proceedings of the second dairy care conference, 3-4 March, Cordoba, Spain – p. 43.

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 Comparing cows including dry period and lactation length in a yield measure. In: Book of Abstracts of the 66th Annual Meeting of the European Federation of Animal Science, 31 August -4 September, Warsaw, Poland p. 140.
- Kok, A., R.J. van Hoeij, B.J. Tolkamp, M.J. Haskell, A.T.M. van Knegsel, I.J.M. de Boer, E.A.M. Bokkers. 2016. Effects of short and no dry period on milk yield and behaviour of dairy cows. In: Proceedings of the 50th Congress of the International Society for Applied Ethology, 12-15 July, Edinburg, UK p. 91.
- Kok, A., R.J. van Hoeij, B.J. Tolkamp, M.J. Haskell, A.T.M. van Knegsel, I.J.M. de Boer, E.A.M. Bokkers. 2016. Effects of omission of the dry period on behaviour of dairy cows. In: Proceedings of the Fourth DairyCare Conference 2016, 13-14 October, Lisbon, Portugal – p. 14.
- Kok, A., C.E. van Middelaar, A.T.M. van Knegsel, B. Engel, H. Hogeveen, B. Kemp, I.J.M. de Boer.
 2016. ADSA-EAAP PhD Student Travel Award: Comparing milk yield between cows with different dry period lengths over multiple lactations. In: Joint Annual Meeting, American Dairy Science Association, J. of Dairy Sci. (99), E-Suppl. 1, 19-23 July, Salt Lake City, USA p 589.
- Chen, J., Kok, A., G.J. Remmelink, B. Kemp, A.T.M. van Knegsel. 2016. Effects of dry period length on lactation curve characteristics over 2 consecutive years. In: 16th International Conference on Production Diseases in Farm Animals Book of abstracts, 20-23 June, Wageningen, the Netherlands – p. 105.
- Van Knegsel, A.T.M., N. Mayasari, J. Chen, R.J. van Hoeij, A. Kok., B. Kemp 2016. Customising dry period length to improve adaptation to lactation in dairy cows. In: 16th International Conference on Production Diseases in Farm Animals Book of abstracts, 20-23 June, Wageningen, the Netherlands – p. 105.
- Kok, A., C.E. van Middelaar, P.F. Mostert, A.T.M. van Knegsel, B. Kemp, I.J.M. de Boer, H. Hogeveen 2017. Economic and environmental impact of dry period length in dairy cows. In: Proceedings of the 7th International Conference on the Assessment of Animal Welfare at Farm and Group level, 5-8 September, Ede, the Netherlands p. 54.

Other publications related to this thesis

Kok, A., A.T.M. van Knegsel, C.E. van Middelaar. 2016. Nieuwe maat voor melkproductie: vergelijking melkgift koeien met verschillende droogstandslengte mogelijk met effectieve lactatie. Veeteelt 33 (7) 44–45.

Van Knegsel, A.T.M, J. Chen, Kok, A. 2017. Vaker weglaten droogstand kan. Veeteelt 34 (1) 52-53.

Pellikaan, F. 2018. Korte tussenkalftijd verliest heel langzaam fans. Veeteelt 35 (1) 36-41.

Education certificate

Completed training and supervision plan¹

The Basic Package (3.0 ECTS)

- WIAS Introduction Course (2014)
- WGS Course 'Ethics and Philosophy in Life Sciences' (2015)

International conferences (7.2 ECTS)

- DairyCare Conference, Cordoba, Spain (2015)
- EAAP, Warsaw, Poland (2015)
- BSAS, Chester, UK (2016)
- ISAE, Edinburgh, UK (2016)
- ADSA, Salt Lake City, USA (2016)
- DairyCare Conference, Lisbon, Portugal (2016)
- WAFL, Ede, the Netherlands (2017)

Seminars and workshops (2.9 ECTS)

- WIAS Science Day, Wageningen (2014, 2015, 2017, 2018)
- Symposium 'Solutions for climate change from animal production', Wageningen (2014)
- Symposium 'Strategies Towards a Quota-Free Dairy Production', Wageningen (2014)
- WGS PhD Workshop Carousel, Wageningen (2014)
- ISAE Benelux conference, Eersel (2014)
- ANR forum, Ghent (2015)
- Symposium 'New perspectives on transition cow management', Wageningen (2017)

Presentations (8.0 ECTS)

- WIAS Science Day, Wageningen, poster (2015, 2017)
- DairyCare Conference, Cordoba, Spain, poster (2015)
- EAAP, Warsaw, Poland, oral (awarded with Novus Travel Grant) (2015)
- ISAE, Edinburgh, UK, oral (2016)
- ADSA, Salt Lake City, USA, oral (2016)
- DairyCare Conference, Lisbon, Portugal, oral (2016)
- WAFL, Ede, the Netherlands, poster (2017)
- WIAS Science Day, Wageningen, oral (2018)

In-Depth Courses (10.2 ECTS)

- 'Applied economic modelling for the veterinary sciences', Utrecht University (2014)
- 'Advanced LCA', Aalborg University (2014)
- 'Environmental impact assessment of livestock systems', Wageningen University (2015)
- 'The application of sensors for lameness and behaviour research in ruminants', ILVO (2016)

Professional Skills Support Courses (3.0 ECTS)

- Techniques for Writing and Presenting a Scientific Paper (2015)
- Supervising MSc thesis work (2015)
- Presenting with Impact (2016)
- Reviewing a Scientific Paper (2017)

Research Skills Training (8.0 ECTS)

- Preparing own PhD research proposal (2014)
- External training period SRUC, Edinburgh, UK (2016)

Didactic Skills Training (13.5 ECTS)

- Supervision practicals 'Introduction to Animal Sciences' (2014, 2015, 2016)
- Coaching student project 'Integrated course on ruminants' (2015)
- Supervision practicals 'Sustainability Assessment of Animal Systems' (2015, 2017)
- Supervision practicals 'Systems Approach in Animal Sciences' (2017, 2018)
- Thesis supervision for 4 BSc and 3 MSc students (2014-2018)

Organisation of seminars (1.5 ECTS)

- Meetings with Farmers of WHYDRY/ Customised Dry Period Network (2015, 2016)
- Lunch meetings Animal Production Systems group (2015-2018)

Total: 57.3 ECTS

¹With the activities listed the PhD candidate has complied with the educational requirements set by the Graduate School of Wageningen Institute of Animal Sciences (WIAS). One ECTS equals a study load of 28 hours.



Colophon

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