Effects of dry period length on metabolic status, fertility, udder health, and colostrum production in 2 cow breeds

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ABSTRACT

Shortening the dry period improves postpartum energy balance, which has potential positive effects on metabolic health. This concept has been mainly studied in Holstein cows. The aim of this study was to assess the effect of a short dry period of 4 wk, compared with a standard dry period of 8 wk, on the metabolic status, progesterone profiles, health, and colostrum quality of dairy cows of 2 breeds, Swedish Red (SR) and Swedish Holstein (SH), not treated with antibiotics at dry off. The IgG uptake in calves was also studied to reflect the colostrum quality when shortening the dry period. Cows of both SH and SR were blocked by breed and parity and then randomly allocated to a short dry period of 4 wk (4W, $n = 43$) or a conventional dry period of 8 wk (8W, $n = 34$). Blood samples were collected wk -8 , -4 , -2 , -1 and 1 , 2 , 3 , 4 , 6 , 9 , and 12 relative to calving. Prepartum, cows with a 4-wk dry period had higher concentrations of nonesterified fatty acids and lower concentrations of insulin-like growth factor-1 and insulin than 8W cows. Postpartum, plasma concentration of nonesterified fatty acids was lower, whereas plasma insulin and insulin-like growth factor-1 tended to be higher for 4W cows than for 8W cows. Plasma concentration of β -hydroxybutyrate did not differ between dry period lengths. Swedish Holstein cows with a 4W dry period responded with a lower concentration of insulin prepartum than SR and SH on an 8W dry period. The dry period length had no effect on the proportion of disturbed progesterone profiles; disturbed progesterone profiles occurred in 30% of the 4W cows and 47% of the 8W cows. In this trial, only 48.8% of the SR cows had a normal progesterone profile, which differed from the SH where 76.5% had a normal profile. Fertility-related diseases (endometritis, pyometra, anestrus, ovarian cyst) did not differ between the 2 dry period groups: 21% in the 8W group versus 12% in the

4W group, whereas mastitis tended to be more common: 26% of the 4W cows versus 9% of the 8W cows. A short dry period resulted in less colostrum but with a higher content of protein and somatic cell count. Calves were fed colostrum from their dam, and the IgG and total protein in plasma did not differ between calves to mothers with different a dry period length. Shortening the dry period could improve metabolic status in cows of both SH and SR breed postpartum, without compromising the colostrum quality. Health and progesterone profiles were not affected by the dry period length for SH or SR in this study.

Key words: short dry period, metabolism, fertility, colostrum

INTRODUCTION

The modern dairy cow is often high yielding. The 2 major breeds in Sweden, Swedish Red (SR) and Swedish Holstein (SH), produce 9,747 and 10,452 kg of ECM per year, respectively (Växa, 2017). Most studies examining the effects of dry period (DP) length on the metabolism of dairy cows have been performed using the Holstein breed. Thus, it is not clear whether cows of dual-purpose breeds, such as the SR, respond in the same way to shortened DP. The high-yielding SH breed might be more suitable for a short DP.

The rapidly increasing milk yield after parturition in these breeds increases the metabolic load of the cow and the disease incidence also peaks immediately after parturition (Ingvarsen, 2006). High milk yield may result in a severe negative energy balance (NEB), which is known to influence fertility (Wathes et al., 2007); a long interval between parturition and first ovulation and luteal activity, and a low conception rate, are reported to be related to NEB (de Vries and Veerkamp, 2000). One way to reduce NEB, and thereby to alleviate the strain on the metabolism after calving, is to shorten or omit the DP (Gümen et al., 2005; Chen et al., 2015a). The main reason for improved energy balance (EB) on shortening the DP is decreased milk production postpartum without affecting DMI (Rastani

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et al., 2005; van Knegsel et al., 2014; Andrée O'Hara et al., 2018). The cow's metabolic state and the degree of mobilization of adipose tissue are partly reflected by plasma concentrations of nonesterified fatty acids (NEFA; Pullen et al., 1989) and are related to EB (Ingvarsen and Andersen, 2000). Most studies (Watters et al., 2008; Klusmeyer et al., 2009), but not all (Weber et al., 2015), report a reduction in plasma concentration of NEFA in response to a short DP of 32 to 34 d. Shortening the DP induces an increase in plasma concentration of insulin in early lactation (Rastani et al., 2005; Weber et al., 2015), whereas varying responses in glucose and BHB have been reported (Klusmeyer et al., 2009; Weber et al., 2015).

Some studies indicate that fertility traits are improved when the DP is shortened. For example, the interval from calving to first ovulation is reported to be shorter when the DP is reduced from 55 to 34 d (Watters et al., 2009). Another study found no improvement in first service conception rate, services per conception rate, or conception rate on shortening the DP from 49 to 28 d (Pezeshki et al., 2008). Cows in NEB with a prolonged interval to first ovulation have been found to have increased levels of NEFA and reduced levels of IGF-1, which can lead to impaired follicular growth, reduced estradiol synthesis, and delayed ovulation (Wathes et al., 2007).

It is unclear how shortening the DP affects udder health. For example, in a retrospective study, Kuhn et al. (2006) found an increase in postpartum SCC in milk, whereas Gulay et al. (2003) observed a tendency for lower SCC and van Knegsel et al. (2014) found no differences in SCC between short and conventional DP length.

The influence of DP length on mastitis incidence has been examined in only a few studies, which show that short DP length generally does not influence mastitis incidence (as reviewed by van Knegsel et al., 2013). However, the effect of short DP on SCC and mastitis incidence has been investigated in even fewer studies where cows were not treated with preventive dry cow therapy (Van Hoeij et al., 2018).

Calves are born agammaglobulinemic because of the physiology of the bovine placenta and are instead provided with immunoglobulins from colostrum. Uptake of immunoglobulins is influenced by the time of feeding after birth, amount, and how it is administered to the calf, and also by the immunoglobulin content in the colostrum (Weaver et al., 2000). So far, most studies on the effect of DP on colostrum quality have shown no or a limited reduction in colostrum volume and IgG content (Rastani et al., 2005; Klusmeyer et al., 2009; Shoshani et al., 2014). In one study on uptake of immunoglobulins in the calf, Mayasari et al. (2015) found

no differences in immunoglobulin uptake in calves born to mothers with short or conventional DP length.

The objective of the present study was to evaluate how metabolic status, fertility (in this case progesterone profiles), udder health, colostrum, and IgG uptake in the calf were affected by shortening the DP in dairy cows of 2 breeds. We hypothesized that the SR cows would be less suited for a short DP than SH cows and that the metabolic status would be improved in the 4W cows.

MATERIALS AND METHODS

The work was carried out during the winter feeding period in 2 consecutive years (2012–2014) at the Swedish Livestock Research Centre, Swedish University of Agricultural Sciences, Lövsta, Uppsala, Sweden. Mean herd annual milk production per cow was 10,514 kg of ECM for the SH cows and 9,263 kg of ECM for the SR cows during 2012–2013 and 11,143 kg of ECM for the SH cows and 9,734 kg of ECM for the SR during 2013–2014. The experimental design and all handling of animals were approved by Uppsala Ethics Committee, Sweden (C217812/13).

Animals and Management

Multiparous cows of SR ($n = 43$) and SH ($n = 34$) were blocked by breed and parity and then randomly allocated to 2 different treatments: a conventional DP of 8 wk (8W) or a short DP of 4 wk (4W). The cows were allocated to the 2 treatments, respectively, well in advance of entering the trial. Several of them were excluded because they did not fulfil the inclusion criteria as defined below. This resulted in uneven treatment groups. Actual mean DP $\pm$ standard error length was 58.8 ± 0.7 d for the 8W group and 30.7 ± 0.7 d for the 4W group. The cows were studied from 10 wk before expected calving to 12 wk postpartum. All cows included had an udder disease score <5 and yielded at least 15 kg of milk/d at 10 wk prepartum. Neither dry cow antibiotic therapy nor teat sealants were administered at drying-off or during the DP. The information about udder disease score was obtained from the Swedish Official Milk Recording Scheme and is based on individual SCC from 3 consecutive monthly test milkings, corrected for milk yield, breed, parity, and DIM. The scale runs from 0 to 9 and expresses the probability of IMI, where each class increase the risk by 10% so >5 indicates $>50\%$ probability (Brolund, 1990).

Cows subjected to the 4W treatment were milked until 4 wk before the expected calving date, and cows in the 8W group were milked until 8 wk before expected calving date. Milking was carried out in an automatic

rotary system (DeLaval AMR, Tumba, Sweden) twice a day, at 0530 and 1530 h. Due to farm practicalities, 13 of the cows were milked in a voluntary milking system (DeLaval) for the last 6 to 12 wk of the study. All cows were kept indoors in a loose-housing system, with free access to cubicles with rubber mats and wood shavings. At drying off, cows were moved to a drying-off pen after morning milking (d 1), milked again once on d 3 and on d 6, and if milk yield >25 kg/d in the week before drying off, again on d 8. Artificial insemination was performed by trained personnel approximately 12 h after detected estrus. The concentrate allowance was gradually reduced, over the course of 1 wk, to zero 2 d before drying off. Feeding and feed composition has been published earlier as well as milk yield, milk composition, DMI, BCS, and EB (Andrée O'Hara et al., 2018).

Blood Sampling and Analysis

Blood samples were taken 8, 4, 2, and 1 wk prepartum and 1, 2, 3, 4, 6, 9, and 12 wk postpartum. Blood was harvested from the coccygeal artery or vein into heparinized 10-mL vacuum tubes with lithium heparin as anticoagulant (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ) at approximately 1000 h each time. The samples were centrifuged at +4°C within 1 h of sampling and plasma samples were stored at -20°C until further analysis. After thawing, plasma samples were analyzed for glucose using enzymatic colorimetric tests (Glucose LiquiColor, Human, Wiesbaden, Germany) and spectrophotometry (Ultrospec K, Boule Nordic, Huddinge, Sweden). The concentration of insulin was analyzed using a commercial enzyme immunoassay method adapted for bovines (Mercodia Ultrasensitive Bovine Insulin ELISA, Mercodia AB, Uppsala, Sweden). The concentration of NEFA was analyzed using an enzymatic colorimetric test (NEFA C, Wako Chemicals GmbH, Neuss, Germany). The concentration of IGF-1 was determined using a commercial enzyme immunoassay (Bovine Insulin-like Growth Factor 1 ELISA catalog no. KT-18278, Kamaya Medical Company, Seattle, WA). The concentration of BHB in plasma was analyzed with a kinetic enzymatic method (RANBUT, Randox Laboratories Limited, Crumlin, UK) and was only analyzed for the 45 cows sampled during yr 2.

Milk Progesterone Analysis and Progesterone Profiles

The concentration of progesterone was determined in milk samples collected twice weekly from 2 to 12 wk postpartum using a commercial ELISA (Ridgeway Sci-

ence Ltd., Alvington, UK). Progesterone was then plotted for each cow and classified into 4 different profiles according to Petersson et al. (2006): (A) normal cyclicity, first rise in progesterone before d 56 postpartum, followed by regular cyclicity; (B) delayed cyclicity with low progesterone for at least 56 d after calving; (C) cessation of cyclicity, with a normal start but cyclicity interrupted with low progesterone for at least 14 d; and (D) prolonged luteal phase, with a normal start but with extended periods of high progesterone of at least 20 d. Milk samples for progesterone were missing for part or all of the period for 2 cows, resulting in no progesterone profiles for these cows. Commencement of luteal activity was defined as a rise in progesterone above 5 ng/mL.

Visual estrus detection was performed 3 times daily.

Health

The cows were checked daily for disease detection and received veterinary treatment when needed. All veterinary treatments were recorded for each cow. Dinolytic vet (Orion Pharma AB Animal Health, Danderyd, Sweden) was used to treat anestrus cows and cows diagnosed with endometritis and pyometra. Clinical mastitis cases received selective antibiotic treatment based on laboratory bacterial culture results, and if necessary, nonsteroidal anti-inflammatory drugs. Mastitis cases were defined as cows diagnosed with and treated for mastitis, based on the following clinical signs: fever, swollen udder, pain, and changed color of milk. Treatment decisions were based on these clinical mastitis signs in combination with a raise in udder health class of 1 to 2 scores and a SCC >800,000 cells/mL of milk.

Milk samples (25 mL) were collected once a month from an afternoon milking and the following morning once every 4 wk into tubes prepared with bronopol and stored at 4°C. Milk SCC was analyzed using fluorescence-based cell counting (Fossomatic 5000, Foss, Hillerød, Denmark). Somatic cell count on the regular milk testing closest to 8 wk prepartum was on average 81,500 cells/mL of milk for the 4W cows and 65,400 cells/mL of milk for the 8W cows.

Colostrum and Calves

Colostrum was weighed manually for the first 5 milkings, and a milk sample of 25 mL was collected during the first and fifth milkings. Colostrum was analyzed for composition of fat and protein by infrared Fourier transform spectroscopy (MilkScan FT120 Foss) and SCC as described above.

All calves were fed 2.5 to 3 L of colostrum with a nipple bucket within 12 h of birth, and all calves except

one were fed within 6 h. Calves were weighed after their first meal. Blood plasma was harvested once from the jugular vein during d 2 to 9 and once during d 56 to 64, at approximately 1100 h. The samples were centrifuged at +4°C within 1 h of sampling and the plasma samples were stored at −20°C until further analysis. After thawing, the concentration of IgG in plasma from calves was determined using a 2-sited ELISA (Bovine IgG ELISA, Kamiya Biomedical Company) on a combined shaker and plate reader (Multiskan FC, Thermo Fisher Scientific, Waltham, MA). Blood plasma samples from calves were analyzed for total protein in plasma immediately after centrifuging, using an optical refractometer (Master Refractometer, Atago, Tokyo, Japan). One calf from a SR cow in the 4W treatment died at birth. Of the remaining 44 calves, 15 had to be excluded from the data set regarding total protein content in plasma, due to missing information about the amount of colostrum fed or to the calf being fed colostrum from a cow other than the mother.

Statistical Analysis

Data were analyzed with the statistical software SAS 9.3 (SAS Institute Inc., Cary, NC). The PROC MIXED model with repeated measurements was used to study plasma metabolites in prepartum lactation wk −8, −4, −2, and −1, and then postpartum lactation wk 1, 2, 3, 4, 6, 9, and 12, in 2 separate data sets. In the plasma figures, however, all measured weeks were included. The following model was used:

$$Y = \text{treatment} + \text{breed} + \text{parity class} \\ + \text{lactation week} + \text{cow} + \text{year},$$

where cow and year are random effects and Y is the dependent variable. Multiparous cows (parity 3 or older after parturition) formed one parity class (parity ≥ 3) and all cows approaching their second parturition another (parity 2). Interactions between treatment and breed, treatment and parity class, and treatment and lactation week were included in all models, but taken out of the model when not significant ($P > 0.05$). Because observations were made several times on the same animal, the model also estimated the correlation in time using a spatial power correlation structure. Least squares means were calculated using the SAS statement LSMEANS/PDIFF. The data on SCC, insulin, and plasma metabolites NEFA and IGF-1 were $\log_{10}$ -transformed before statistical analyses to achieve normally distributed residuals. The 3 milk sample analyses per cow were statistically evaluated with the same mixed model as presented above and the cell

count had to be $\log_{10}$ -transformed to achieve normally distributed residuals. Interactions between treatment and breed, treatment and parity class, and treatment and lactation week were included in the model, but were all taken out of the model because they were not significant ($P > 0.05$).

Progesterone profiles were divided into 2 groups, normal (A) or disturbed (B–D), and treatment effects were then evaluated by a χ^2 test provided by OpenEpi (www.OpenEpi.com). The same program was used to evaluate proportion of animals suffering from mastitis or fertility-related diseases.

Colostrum parameters and days to first rise in progesterone were statistically evaluated with the same mixed model as presented above, only without lactation week, cow, and the correlation structure for time. For calves, total protein in plasma and IgG uptake were statistically evaluated by comparing only treatments with the Wilcoxon rank sum test in SAS 9.3 because the residuals were not normally distributed and transformation did not improve their distribution.

RESULTS

Plasma Metabolites

Treatment, breed, and parity had no effect on concentrations of plasma glucose or BHB. The concentration of IGF-1 was higher prepartum, and lower in the first week postpartum, in 8W cows than in 4W cows (Figure 1). Swedish Red cows had higher concentrations of IGF-1 than SH cows (Table 1). Younger cows had higher concentrations of IGF-1 postpartum than multiparous cows (parity 2: 84.3 ng/mL; parity ≥ 3 : 73.8 ng/mL; $P < 0.01$).

The concentration of insulin in plasma varied over weeks (Figure 1) and an interaction was observed between treatment and week (<0.05) in 8W cows, whereby insulin was higher 4 wk prepartum and lower 6 wk postpartum. Insulin differed between breeds postpartum, with SR cows having higher concentrations of insulin in plasma than SH cows (Table 1).

The plasma concentration of NEFA was affected by week (Tables 1 and 2) and an interaction was observed between treatment and week ($P < 0.02$), with NEFA being numerically highest for 8W SH cows during the second week after calving (Figure 1).

Fertility

In terms of progesterone profile (Table 3), cows in the 4W group had a 36% lower risk of a disturbed progesterone profile than cows in the 8W group but no difference was observed between 4W and 8W in the

Figure 1. Least squares means ($\pm$ SE) of blood plasma concentrations of BHB (A), glucose (B), IGF-1 (C), insulin (D), nonesterified fatty acids (NEFA; E) for the treatments with a 4-wk (4W) and 8-wk (8W) dry period in 2 cow breeds: Swedish Red (SR) and Swedish Holstein (SH). Sampling started 8 wk before expected calving and continued to 12 wk postpartum [4W SH, $n = 20$; 4W SR, $n = 23$; 8W SH, $n = 15$; 8W SR, $n = 19$ for BHB (A); 4W SH, $n = 13$; 4W SR, $n = 13$; 8W SH, $n = 8$; 8W SR, $n = 11$ (B, C, D, and E)].

proportion of cows with normal progesterone profiles [15/32 (47%) of 4W cows vs. 13/43 (30%) of 8W cows].

Among the disturbed profiles, profile D (i.e., prolonged luteal phase) was the most common for cows in both the 4W and 8W groups. Three cows in the 8W group and 11 cows in the 4W group became pregnant within d 62 to 84 of lactation (within the trial period), but no cow had a prolonged luteal phase because of pregnancy. One cow in the 8W group did not start cycling within the 12 wk and thus fell into progesterone profile B. The SH cows had a larger proportion of normal progesterone profiles than did the SR cows

(SH 77% vs. SR 49%; $P < 0.05$). No differences were observed between treatment groups and breeds when studying commencement of luteal activity ($P > 0.05$).

Udder Health

Somatic cell count did not differ ($P < 0.1$) between 8W cows and 4W cows (back-transformed LSM 81,000 and 120,000 cells/mL, respectively) and was not affected by breed or parity. However, cows in the 4W group had higher SCC at the first and fifth milking. Mastitis tended to be more common among 4W cows,

Table 1. Associations (LSM) between dry period (DP) length and postpartum concentrations of plasma metabolites, IGF-1, and insulin in 2 different cow breeds (SH = Swedish Holstein; SR = Swedish Red) with 2 different DP lengths [4 wk (4W) and 8 wk (8W)]

Item	DP length						DP length and breed						Breed						P-value ²			
	4W	8W	SEM ¹	4W SH	4W SR	8W SR	8W SH	8W SR	8W SH	SEM ¹	SH	SR	SEM ¹	DP	B	p ³	W ⁴	DP × p				
Cows, no.	43	34		20	23	15	15	19			35	42										
BHB, ^{5,6} mmol/L	0.59	0.61	0.03	0.65	0.53	0.61	0.61	0.60		0.04	0.64	0.57	0.03	0.65	0.11	0.43	<0.01	—				
Glucose, ⁵ mmol/L	3.37	3.36	0.21	3.34	3.40	3.34	3.34	3.37		0.22	3.34	3.39	0.21	0.83	0.47	0.14	<0.01	—				
IGF-1, ^{5,7} ng/mL	77.3	70.4		68.0	87.4	66.3	66.3	75.3			66.8	81.4		0.06	<0.01	<0.01	<0.01	—				
Insulin, ^{5,8} µg/L	0.41	0.32		0.34	0.48	0.31	0.31	0.34			0.32	0.41		0.06	0.04	0.12	<0.01	—				
NEFA, ^{5,9} mmol/L	0.15	0.20		0.14	0.16	0.19	0.19	0.20			0.17	0.18		<0.01	0.32	0.13	<0.01	<0.01				

¹Pooled standard error of LSM.
²DP = dry period length; B = breed; p = parity; W = week.
³Parity was divided into 2 parity classes: p2 = parity 2, p ≥ 3 = parity 3 or older.
⁴Samples were collected during wk 1, 2, 3, 4, 6, 9, and 12 postpartum.
⁵LSM per sample week is presented in Figure 1. NEFA = nonesterified fatty acids.
⁶BHB analysis was performed only during yr 2; 4W SH, n = 13; 4W SR, n = 13; 8W SH, n = 8; 8W SR, n = 11.
⁷Statistical analysis was based on natural log-transformed data, but means are back-transformed to the original scale. The 95% CI were generally 17.4 units lower and 22.8 units higher than the mean.
⁸Statistical analysis was based on natural log-transformed data, but means are back-transformed to the original scale. The 95% CI were generally 0.15 units lower and 0.26 units higher than the mean.
⁹Statistical analysis was based on natural log-transformed data, but means are back-transformed to the original scale. The 95% CI were generally 0.02 units lower and 0.03 units higher than the mean.

whereas fertility-related diseases were numerically more common among 8W cows (Table 4). Other wounds and diseases were in the 4W group; one cow was diagnosed with bloat, one was lame, one had an udder wound, and one had another smaller wound. In the 8W group, one cow had a calving-related problem, one had a retained placenta, one cow had an udder wound, and one had another smaller wound.

Calves and Colostrum Quality

Cows with a 4-wk DP produced less colostrum but had a higher protein content (Table 5). Immunoglobulin G and total protein in plasma did not differ significantly between calves born to mothers with a 4- or 8-wk DP (*P* > 0.4; Table 6).

DISCUSSION

Metabolites

The plasma concentration of NEFA was higher in 8W cows than in 4W cows in early lactation. Many previous studies also report reduced concentrations of NEFA in plasma in cows after a short DP (e.g., Pezeshki et al., 2007; Watters et al., 2008; Klusmeyer et al., 2009). It is reasonable to assume that the lower concentration of NEFA in cows with a short DP was related to less severe NEB, although the relationship between EB and plasma NEFA appears to be rather weak (Reist et al., 2002; Wylie et al., 2008). The 4W cows in the present study showed a less severe dip in postpartum NEB and they returned to positive EB 9 wk before the 8W cows (Andrée O'Hara et al., 2018). It is well documented that high concentrations of NEFA in blood plasma in early lactating cows are related to increased risk of metabolic diseases (LeBlanc et al., 2005; Ospina et al., 2010). A suggested cut-off value for increased risk has been set to 0.7 mmol/L in early lactation (Ospina et al., 2010; Chapinal et al., 2011). In the present study, only 3 cows from each DP treatment had concentrations of NEFA above 0.7 mmol/L (data not shown). Thus it is not evident from the present study that a short DP reduces the risk of metabolic diseases, bearing in mind the low number of animals. Before parturition, insulin concentration was lower in 4W cows than in cows with a conventional 8-wk DP. It is reasonable to assume that the shorter DP, and thereby the prolonged lactation prepartum, prevented the rise in insulin observed in the 4W group after parturition. Lipolysis in adipose tissues of those cows was presumably reduced, which in turn contributed to the observed lower concentration of NEFA in plasma. Insulin prepartum was lower in

Table 2. Associations (LSM) between dry period (DP) length and prepartum concentrations of plasma metabolites, IGF-1, and insulin in 2 different cow breeds (SH = Swedish Holstein, SR = Swedish Red) with 2 different DP lengths [4 wk (4W) and 8 wk (8W)]

Item	DP length			DP length and breed						Breed			P-value ²				
	4W	8W	SEM ¹	4W SH	4W SR	8W SH	8W SR	SEM ¹	SH	SR	SEM ¹	DP	B	p ³	W ⁴	DP × B	DP × W
Cows, n	43	34		20	23	15	19		35	42							
BHB, ^{5,6} mmol/L	0.53	0.54	0.04	0.54	0.52	0.60	0.50	0.06	0.57	0.51	0.04	0.84	0.33	0.78	0.91	—	—
Glucose, ⁵ mmol/L	4.05	3.92	0.06	4.06	4.05	3.86	3.96	0.08	3.96	4.01	0.06	0.08	0.55	0.38	0.02	—	—
IGF-1, ^{5,7} ng/mL	104.0	124.6		99.8	108.9	119.3	129.3		109.0	118.9		<0.01	0.11	<0.01	0.07	—	0.04
Insulin, ^{5,8} µg/L	0.54	0.68		0.43 ^a	0.67 ^b	0.71 ^b	0.66 ^b		0.55	0.66		0.05	0.13	0.10	0.04	0.03	—
NEFA, ^{5,9} mmol/L	0.09	0.07		0.09	0.09	0.07	0.06		0.08	0.07		0.01	0.45	0.32	0.02	—	0.03

^{a,b}Values within rows with different superscripts differ significantly ($P < 0.05$).
¹Pooled standard error of LSM.
²DP = dry period length; B = breed; p = parity; W = week.
³Parity was divided into 2 parity classes: p2 = parity 2, $p \geq 3$ = parity 3 or older.
⁴Samples were collected during wk -8, -4, -2, and -1 prepartum.
⁵LSM per sample week is presented in Figure 1. NEFA = nonesterified fatty acids.
⁶BHB analysis was performed only during year 2; 4W SH, n = 13; 4W SR, n = 13; 8W SH, n = 8; 8W SR, n = 11.
⁷Statistical analysis was based on natural log-transformed data, but means are back-transformed to the original scale. The 95% CI were generally 10.2 units lower and 11.3 units higher than the mean.
⁸Statistical analysis was based on natural log-transformed data, but means are back-transformed to the original scale. The 95% CI were generally 0.12 units lower and 0.15 units higher than the mean.
⁹Statistical analysis was based on natural log-transformed data, but means are back-transformed to the original scale. The 95% CI were generally 0.02 units lower and 0.03 units higher than the mean.

Table 3. Distribution of progesterone profiles¹ for the treatments with a 4-wk (4W) and 8-wk (8W) dry period (DP) in the 2 cow breeds: Swedish Red (SR) and Swedish Holstein (SH), with percentage in parentheses

Item	DP length				DP length and breed				Breed			Risk ratio (95% CL) ²		P-value	
	4W	8W	4W SH	4W SR	8W SH	8W SR	8W SH	8W SR	SH	SR	DP	B	DP	B	
Cows, no.	43	32	20	23	14	18	14	18	34	41					
Normal (A)	30 (70)	17 (53)	16 (80)	14 (61)	10 (71)	7 (44)	10 (71)	7 (44)	26 (76.5)	21 (48.8)	0.64 (0.36–1.15)	0.48 (0.24–0.95)	0.15	0.03	
Disturbed (B + C + D)	13 (30)	15 (47)	4 (20)	9 (39)	4 (29)	11 (56)	4 (29)	11 (56)	8 (23.5)	20 (51.2)					
Profile B	3	4	1	2	0	4	0	4	1	6					
Profile C	3	3	1	2	1	2	1	2	2	4					
Profile D	7	8	2	5	3	5	3	5	5	10					

¹A = normal cyclicity; B = delayed cyclicity with low progesterone for at least 56 d after calving; C = cessation of cyclicity, with a normal start but with cyclicity interrupted with low progesterone for at least 14 d; and D = prolonged luteal phase, with a normal start but with extended periods of high progesterone for at least 20 d.

²Risk ratio and 95% confidence limits (CL), and P-values from χ^2 tests, comparing numbers of disturbed profiles to normal profiles for 4W versus 8W and SH versus SR.

Table 4. Distribution of diseases (percentage in parentheses) and LSM $\pm$ SE for commencement of luteal activity in the treatments with a 4-wk (4W) and 8-wk (8W) dry period (DP) in the 2 cow breeds (B): Swedish Red (SR) and Swedish Holstein (SH) during wk 1 to 12 postpartum

Item	DP length				DP length and breed				Breed			Risk ratio (95% CL)		P-value	
	4W	8W	4W SH	4W SR	8W SH	8W SR	8W SH	8W SR	SH	SR	DP	B	DP	B	
Cows, no.	43	34	20	23	15	19	15	19	34	43					
Fertility-related diseases ^{1,2}	5 (12)	7 (21)	3 (15)	2 (9)	3 (21)	4 (20)	3 (21)	4 (20)	6 (18)	6 (14)	0.56 (0.20–1.62)	1.65 (0.45–3.57)	0.30	0.67	
Mastitis ¹	11 (26)	3 (9)	4 (20)	7 (30)	2 (14)	1 (5)	2 (14)	1 (5)	6 (18)	8 (19)	2.90 (0.88–9.58)	0.95 (0.36–2.47)	0.06	0.92	
C-LA ³	21.8 $\pm$ 1.4	23.4 $\pm$ 1.7	23.4 $\pm$ 2.1	20.4 $\pm$ 1.9	21.5 $\pm$ 2.5	24.9 $\pm$ 2.2	21.5 $\pm$ 2.5	24.9 $\pm$ 2.2	22.7 $\pm$ 1.6	22.4 $\pm$ 1.5			0.47	0.88	

¹Risk ratio and 95% confidence limits (CL) and P-values from χ^2 tests, comparing numbers of sick animals to healthy animals for 4W versus 8W and SH versus SR.

²Endometritis, pyometra, anestrus, ovarian cyst.

³C-LA = commencement of luteal activity, LSM $\pm$ SE and P-values from the PROC MIXED in SAS 9.3 (SAS Institute Inc., Cary, NC).

Table 5. Associations between dry period (DP) length [4 wk (4W), 8 wk (8W)] and colostrum quality in 2 different cow breeds (SH = Swedish Holstein, SR = Swedish Red)

Item	DP length				DP length and breed								Breed			<i>P</i> -value ²			
	4W		8W		SEM ¹	4W SH	4W SR	8W SH	8W SR	SEM ¹	SH	SR	SEM ¹	DP	B	p ³	DP × p		
	43	34	34	6.1	0.5	20	23	14	20	0.7	34	43	0.47	<0.01	0.77	0.09	—		
Cows, no.	43	34	34	6.1	0.5	20	23	14	20	0.7	34	43	0.47	<0.01	0.77	0.09	—		
Amount first milking, L	3.3	6.1	3.3	6.1	2.1	3.8	2.9	5.7	6.4	3.0	4.8	4.6	2.13	<0.01	0.01	0.57	—		
Amount milking 1–5, L	30.3	43.6	30.3	43.6	0.1	36.4	24.5	44.4	41.9	0.2	40.9	33.0	0.14	<0.01	0.72	0.84	—		
Cells first milking, log cells/mL	3.8	3.1	3.8	3.1	0.8	3.7	3.9	3.2	3.0	0.9	3.4	3.5	0.81	0.02	0.02	<0.01	—		
Fat first milking, %	4.9	6.3	4.9	6.3	0.5	5.2	4.7	7.6	5.1	0.7	6.3	4.9	0.48	<0.01	0.50	0.09	—		
Protein first milking, %	16.0	12.5	16.0	12.5	0.1	16.0	15.9	13.0	12.1	0.1	14.5	14.0	0.10	0.27	0.21	<0.01	0.04		
Cells fifth milking, log cells/mL	2.9	2.6	2.9	2.6	0.4	2.7	3.0	2.6	2.6	0.5	2.6	2.8	0.42	0.02	0.06	0.87	—		
Fat fifth milking, %	3.2	3.5	3.2	3.5	0.2	2.9	3.4	3.2	3.7	0.3	3.0	3.6	0.20	<0.01	0.02	0.07	—		
Protein fifth milking, %	6.1	5.5	6.1	5.5	0.2	5.8	6.4	5.3	5.7	0.3	5.5	6.1	0.20	<0.01	0.02	0.07	—		

¹Pooled standard error of LSM.²DP = dry period length; B = breed; *p* = parity.³Parity was divided into 2 parity classes: *p*₂ = parity 2; *p*_{≥3} = parity 3 or older.

the 4W SH breed than in the 8W SH breed, whereas it did not differ between the SR cows. It has previously been shown that cows with longer lactations than 300 d increasingly partition nutrients toward tissue gain rather than milk production aided by insulin (Marett et al., 2015). It is possible that this redistribution of nutrients was less prominent in SH cows than in the dual-purpose SR breed. Indeed, SR cows with a short DP yielded about 12% less milk than SH with a similar DP did (Andrée O'Hara et al., 2018). After parturition, the concentration of insulin in plasma of SR cows was higher than in SH cows. The basal level of insulin is apparently not related to body condition in cows during the postpartal period (Holtenius et al., 2003; Jaakson et al., 2018). Thus, the higher concentration of insulin in SR cows was probably a breed effect and not related to higher body condition. In line with our results Nyman et al. (2008) reported that SR cows had higher insulin levels than SH cows in early lactation. Ntallaris et al. (2017) observed that SR cows tended to have higher insulin and to be in a less deep NEB after calving compared with SH and the authors suggested that SR cows prioritized milk production to a lesser extent. However, this suggestion is not supported by the present study because EB postpartum was virtually equal between breeds (Andrée O'Hara et al., 2018). Only a few interactions between parity and DP length were found, unlike in other studies (Annen et al., 2004; Kuhn et al., 2006; Pezeshki et al., 2007; Sawa et al., 2015). Few metabolic differences were found between cows approaching their second calving as multiparous cows with a shortened DP. Second-parity cows had higher concentrations of IGF-1 than multiparous cows, in agreement with observations in younger cows (Wathes et al., 2007). Glucose and BHB were not affected by DP, as also shown in a systematic review by van Knegsel et al. (2013). Postpartum SR cows had a higher plasma concentration of insulin than SH cows. The SR cows were also over-conditioned to a greater extent than SH cows (Andrée O'Hara et al., 2018).

Fertility

In this study, postpartum IGF-1 tended ($P = 0.06$) to be higher in cows subjected to a 4-wk DP compared with an 8-wk DP, possibly explaining the tendency for lower incidence of disturbed progesterone profiles among 4W cows. A previous study have shown that cows with lower IGF-1 levels after calving take longer to resume their estrous cycle (Wathes et al., 2007).

Among the disturbed progesterone profiles, a prolonged luteal phase was the most common disturbance. Similarly, Chen et al. (2015b) found numerically fewer disturbances among cows with a 30-d DP than cows

Table 6. Associations between dry period (DP) length [4 wk (4W), 8 wk (8W)] and plasma content in the calf in 2 different cow breeds (SH = Swedish Holstein, SR = Swedish Red), mean $\pm$ SD

Item ¹	DP length		Breed		P-value	
	4W	8W	SH	SR	DP	B
Total protein 1 calves, no.	15	14	14	15		
Total protein 1, g/L	58.2 $\pm$ 5.4	56.5 $\pm$ 5.3	59.6 $\pm$ 7.8	55.3 $\pm$ 5.0	0.61	0.12
Total protein 2 calves, no.	13	14	14	13		
Total protein 2, g/L	58.0 $\pm$ 2.9	58.9 $\pm$ 4.2	57.3 $\pm$ 3.5	59.7 $\pm$ 3.5	0.41	0.08
IgG calves, no.	11	12	12	11		
IgG, g/L	8.0 $\pm$ 4.9	7.4 $\pm$ 4.2	8.8 $\pm$ 5.1	6.5 $\pm$ 3.4	0.79	0.26

¹Samples were taken during d 2 to 9 (protein 1 and IgG) or d 56 to 64 (protein 2) of life.

with a 60-d DP and also that prolonged luteal phase was the most frequent disturbed profile. Despite less cyclicity disturbances and a tendency for fewer fertility-related diseases in 4W cows than in 8W cows, the commencement of luteal activity was not significantly different, and varied by less than 2 d, despite the higher EB, higher IGF-1, and lower concentrations of NEFA in 4W cows. Gümen et al. (2005) and Chen et al. (2015b) also did not observe any differences in days to luteal activity when the DP was shortened or omitted, whereas Watters et al. (2009) found that cows with a shortened DP had fewer days to first ovulation. The SR cows in our study had an unexpectedly high rate of disturbed progesterone profiles. Atypical profiles and delayed cyclicity have been shown to be related to NEB, great loss in BW, and increases in fertility-related diseases (Petersson et al., 2006), none of which was observed in SR cows in the present study. The high BCS in SR cows (Andrée O'Hara et al., 2018) could be a reason for their poorer reproduction performance (Roche et al., 2007).

Udder Health

The proportion of cows diagnosed with mastitis postpartum was approximately 3-fold higher among 4W cows compared with 8W cows; however, mastitis had only a tendency to be more common among 4W cows. Furthermore, the SCC in milk collected during 3 milkings at 12 wk postpartum was numerically higher in 4W cows and the SCC at the first and fifth milkings postpartum was higher in the 4W group. This finding was somewhat unexpected because DP length, normal or short, was reported in several studies not to affect udder health (Rastani et al., 2005; Watters et al., 2008; Klusmeyer et al., 2009). In the present study, SCC was calculated as an average of 3 measures over 12 wk and should therefore be interpreted with caution. However, in contrast with previous studies, the cows in the present study were not treated with antibiotics at drying off. van Hoeij et al. (2018) studied a short (30 d) DP versus no DP in cows not treated

with preventive antibiotics at drying off and found that the DP length did not affect the SCC in early lactation. However, their study did not include cows with a conventional DP length, so their results are difficult to compare with ours. Although a shorter DP could decrease milk yield at drying off, and thereby reduce the risk of IMI at calving (Rajala Schultz et al., 2005), a 4-wk DP may not be long enough for the mammary tissues to adapt to the coming lactation (Capuco et al., 1997), especially not in this setup where the dry off procedure claimed a few days of the DP. Thus a 4-wk DP might not only lead to reduced milk production, but possibly also to an increased risk of IMI during the DP (Oliver and Sordillo, 1989). This in turn might increase the risk of mastitis postpartum (Green et al., 2002). It is possible that a short DP is more hazardous if cows are not treated with antibiotics at drying off. de Vries et al. (2016) observed increased concentrations of proteins involved in host defense and cell generation in colostrum from cows not treated with antibiotics at drying off and subjected to a 4-wk DP compared with a conventional 8-wk DP.

Colostrum Quality and Calves

The 4W cows produced less colostrum, but colostrum quality was not affected, confirming findings in other studies (Rastani et al., 2005; Watters et al., 2008; Klusmeyer et al., 2009; Cermakova et al., 2014; Mayasari et al., 2015). The IgG content in colostrum was not analyzed, but the higher protein content in colostrum of 4W cows than of 8W cows presumably reflects higher IgG content (Fleenor and Stott, 1980). The concentration of IgG in plasma showed no difference between calves born to cows subjected to a 4- or 8-wk DP.

CONCLUSIONS

A 4-wk DP improved metabolic status, as reflected by lower plasma NEFA concentration in early lactation compared with an 8-wk DP. However, the concentra-

tions of insulin, glucose, and BHB were not affected by shortening DP length. Generally, no breed differences were present in response to DP length, but the concentration of insulin was higher in SR cows before calving. The incidence of mastitis during the first 12 wk after calving had a tendency to be higher among cows subjected to a 4-wk DP. Cows with a 4-wk DP yielded a lower volume of colostrum with a higher protein and fat content. The IgG content in plasma did not differ in calves that received milk from cows subjected to a 4- or 8-wk DP.

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