Interpretive Summary. Effects of feeding level, milking frequency, and single injection of cabergoline on feed intake, milk yield, milk leakage and clinical udder characteristics during dry-off in dairy cows. By Larsen et al. pages xxxx.

Abrupt and gradual dry-off strategies by reducing feeding level (normal vs. reduced energy density), reducing milking frequency (twice vs. once daily), and dopamine agonist at last milking (i.m. saline vs. cabergoline injection) were investigated for the effect on feed intake, energy balance, milk leakage, and clinical udder characteristics. Overall, gradual dry-off by reducing milking frequency without reducing feeding level decreased milk yield before dry-off and reduced udder engorgement after dry-off without inducing negative energy balance. Cabergoline injection after last milking resulted in least udder engorgement and least signs of milk leakage, but also an abrupt reduction in feed intake lasting a day.

Running head: DRY-OFF MANAGEMENT FOR DAIRY COWS

Effects of feeding level, milking frequency, and single injection of cabergoline on feed intake, milk yield, milk leakage and clinical udder characteristics during dry-off in dairy cows

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ABSTRACT

Abrupt and gradual dry-off strategies by reducing feeding level (normal vs. reduced energy density), reducing milking frequency (twice vs. once daily), and administration of a dopamine agonist after last milking (i.m. saline vs. cabergoline injection) were investigated (2×2×2 factorial arrangement) for the effects on feed intake, milk yield, energy balance, milk leakage, and clinical udder characteristics in 119 Holstein cows. In the last week before dry-off, cows were assigned to one of four combinations of feeding level and milking frequency. Within three hours after last milking, cows were injected with either saline or a dopamine agonist (cabergoline; Velactis, Ceva Santé Animale, Libourne, France; labelled for use only with abrupt dry-off, e.g. no preceding reduction in feeding level or milking frequency prior to last milking). After dry-off, all cows were fed the same diet for dry cows and data collection continued for a week. Dry matter intake (DMI) was recorded in automated feed bins and milk yield in an automatic milking system where additional concentrate was fed. Clinical udder characteristics and milk leakage were scored 10 times during the week before and the week after dry-off. Before dry-off, total DMI decreased with reduced feeding level compared to normal feeding level, but did not differ between milking frequencies. The combined effect of reduced DMI and diet energy concentration resulted in a 47% lower net energy intake with reduced feeding level compared to normal feeding level during the week before dry-off. Milk yield was approximately 30% lower during the week before dry-off when either feeding level or milking frequency was reduced as compared to no change in feeding level or milking frequency, whereas milk yield was 45% lower when both feeding level and milking frequency was reduced. The net energy balance during the week before dry-off was negative with reduced feeding level and more negative combined with twice milking. After dry-off, udder engorgement was reduced in the three gradual dry-off treatments compared to abrupt dry-off. of Cabergoline injection after last milking resulted in least udder engorgement and signs of milk leakage for 48 h, but also an abrupt reduction of DMI lasting approximately 24 h irrespective of treatment before dry-off. In conclusion, gradual cessation of lactation by reducing milking frequency to once daily without reducing the feeding level decreased milk yield before dry-off in high-yielding dairy cows and reduced udder engorgement after dry-off without inducing negative energy balance during the period of dry-off. In contrast, reduced feeding level induced negative energy balance that may compromise welfare due to metabolic stress and hunger. No clear differences in risk of milk leakage after dry-off were observed between abrupt and gradual dry-off management strategies. The use of cabergoline led to fewer signs of milk leakage and reduced udder engorgement during the first days after dry-off that may positively affect welfare at dry-off. However, the mechanism behind and the welfare consequences of the concomitant abrupt decrease in DMI lasting approximately 24 h needs further investigation to complete our understanding of dopamine agonist use for dry-off.

Key Words: dairy cows, dry-off, management, milk leakage

INTRODUCTION

After the lactation period, a period of 40 to 60 days with no milk synthesis allows the bovine mammary gland to regenerate secretory cells and prepare for the next lactation cycle (Capuco and Akers, 1999). Hence, an artificial cessation of milk production, also termed dry-off, is commonly practiced in commercial dairy farms when cows are 7 to 8 weeks before expected calving. However, dairy cows often still produce a substantial amount of milk at this stage of lactation (Rajala-Schultz et al., 2005; Gott et al., 2016). High milk yield at dry-off is considered to increase the risk of udder infection during the dry period (Dingwell et al., 2004, Rajala-Schultz et al., 2005), as milk leakage during the days after the last milking hampers the formation of a keratin plug in the teat canal (Dingwell et al., 2004). Furthermore, milk accumulation in the udder at dry-off may lead to changes in the clinical characteristics of the udder such as engorgement, which has been associated with an increased risk of discomfort and pain for cows (Chapinal et al., 2014; Zobel et al., 2015).

On farm, gradual dry-off can be practiced by reducing feeding level, daily milking frequency, or both, minimizing the risk of milk leakage and udder engorgement (Odensten et al., 2005; Tucker et al., 2009). Conceptually, reduced feeding level decreases milk synthesis as less nutrients are available to the udder. However, reduced feeding level while maintaining milking may induce undernourishment and, consequently, metabolic stress (Odensten et al., 2005) and enhanced feeding motivation (Valizaheh et al., 2008; Franchi et al., 2019). These consequences may be more pronounced if it is not combined with reduced milking frequency. Recently, i.m. injection of dopamine agonists like quinagolide and cabergoline has been observed to reduce milk synthesis likely due to inhibition of pituitary prolactin release (Ollier et al., 2013, Boutinaud et al., 2016). For instance, Bach et al. (2015) found that a single injection of cabergoline with abrupt dry-off resulted in reduced udder firmness, reduced odds of milk leakage, and increased lying time after dry-off.

The current study aimed to investigate the combined effects of reduced feeding level, reduced milking frequency, and injection of cabergoline as compared to abrupt dry-off on feed intake, milk yield, energy balance, milk leakage, and clinical udder characteristics during dry-off of high yielding dairy cows.

MATERIALS AND METHODS

All experimental procedures involving cows were approved by the Danish Animal Experiments Inspectorate (Permit No. 2017-15-0201-01230) in compliance with Danish Ministry of Environment and Food Act No. 474 (May 15, 2014). The experimental protocol (internal ref. F19-12-1919) was registered in the experimental facility data storage system at Aarhus University. The experimental work was conducted according to Good Clinical Practice Guideline VICH GL19 (VICH, 2001) and the unregistered use of cabergoline (Velactis, Ceva Santé Animale, Libourne, France) was approved by the Danish Medical Agency (Permit No. 2017064040). In countries where Velactis is registered, Velactis is labelled to be used with abrupt dry-off, e.g. no preceding reduction in feeding level or milking frequency prior to last milking; thus, use in other dry-off regimens is off-label.

Animals and Experimental Design

The experiment was conducted in the department resident Danish Holstein herd of Aarhus University from September 2017 to May 2019. The experimental design was a randomized block design with repeated measurements. Treatments were 2 × 2 × 2 factorial arrangement with the following factors: feeding level – normal lactation diet versus energy-reduced lactation diet (**NORM** or **REDU**); milking frequency – twice versus once daily (**2×** or **1×**); and dopamine agonist administration – saline versus cabergoline i.m. injection (**SAL** or **CAB**). The period -14 to -8 days relative to dry-off (**DRTD**; dry-off on day 0) was used as a baseline period for feed intake, milk variables, and body weight (**BW**). From -7 to -1 DRTD, cows were fed and milked according to one of the four combinations of feeding level and milking frequency. Within 3 h after last milking at the morning of day 0, cows were injected according to one of the two i.m. injection treatments. After dry-off, all cows were fed the same total mixed ration (**TMR**) diet for dry cows, were not further milked, and data collection continued until +7 DRTD.

Cows (experimental unit) were enrolled every second week 14 d before dry-off in batches of 1 to 6 cows depending on number of available cows fulfilling the following inclusion criteria: 1) first, second or third parity, 2) milk yield ≥15 kg/d, 3) between 210 and 240 days in pregnancy at dry-off, 4) body condition score being 2 to 4 on a 5 point scale (Ferguson et al., 1994), 5) lameness score ≤ 3 on a 5 point scale (Thomsen et al., 2008), 6) not treated for clinical disease for 2 weeks before inclusion, and 7) not previously included at an earlier dry-off. Prior to first enrollment, randomized blocks of 8 within parity group (primi- and multiparous) were prepared. Within block, 8 treatment combinations were listed in random order and cows in each batch were assigned to block and treatment after sorting according parity group and animal number. A planned sample size of 15 cows per treatment combination was based on the availability of cows in the resident herd and supported by power calculations to identify significant differences at the 5% level with a power of 90% between the two injection groups for main efficacy responses [plasma glucose concentration, SD = 0.29 mM used (Ollier et al., 2014); lying time, SD = 0.96 h/d used (Tucker et al., 2009)]. With sample size of 15 cows per treatment combination, differences of 3.3 kg/d DMI and 10.3 cm teat perimeter should be detectable at a 5% significance level with a power of 90% [DMI, SD = 2.8 kg/d (Ollier et al., 2014); teat perimeter, SD = 8.7 cm (Bertulat et al., 2012)]. Finally, a total of 119 cows were enrolled (first parity, n = 72; second parity, n = 29; third parity, n = 18) in 36 successive batches where the last block of multiparous cows had n = 7 due to lack of cows not previously included.

Management of Animals

Resident Herd. The resident herd consisted of two groups, each comprising approximately 60 Holstein cows (mixed parities), housed in a naturally ventilated barn with two adjacent mirrored free-stall pens with concrete slatted floor. Each pen had a minimum of one cubicle per cow lined with 70 mm mattresses (Cowtex, Tromborg staldudstyr og -inventar, Varde, Denmark) and bedded with a mix of sawdust and finely chopped straw, which was distributed automatically (JHminiStrø, JH Staldservice A/S, Holstebro, Denmark) twice a day. The cubicles were cleaned manually twice a day and the alleys were scraped regularly by automatic cleaning robots (Lely Discovery, Lely Holding, Maassluis, The Netherlands). Cows had ad libitum access to water from a total of four 126 cm × 60 cm (length × width) self-filling water troughs and free access to one mechanical rotating cow brush (DeLaval AB, Tumba, Sweden) in each pen.

Cows were milked by an automatic milking system (**AMS**; DeLaval AB). Primiparous and multiparous cows were allowed to be milked if time from previous milking exceeded 7 or 8 h, respectively. Milking was also allowed if predicted milk yield at next milking exceeded 9 kg. During the lactation period, cows were fed a standard partially mixed ration (**PMR**) from automated feed bins (Insentec B.V., Marknesse, the Netherlands) allocated ad libitum, and allowed 3 kg (as is form) of concentrate daily in the AMS. The PMR was filled into feed bins four times daily beginning at approximately 0630, 1030, 1430 and 2000 h, and were emptied Mondays, Wednesdays, and Fridays between 0930 and 1030 h. The stocking density for Insentec bins were two cows per bin. If cows had been used in lactation trials, they were fed with the normal lactation

PMR for at least 2 weeks before being enrolled into the dry-off treatments. In this period, cows had free access to all bins with the lactation PMR.

No teat sealant was used after dry-off, but in case a cow had both SCC >100.000 cells/mL and a positive PCR test for a mastitis related bacteria, all mammary glands were intra-mammary treated with an antibiotic (Orbenin Vet.; Zoetis, Farum, Denmark).

Experimental Pen. During the experimental period (-7 to +7 DRTD), enrolled cows were kept in an experimental pen in the same barn as the resident herd. Three batches included only one experimental cow. In these cases, a non-experimental companion dry cow was placed in the pen to avoid individual housing. The experimental pen consisted of an alley (8 m × 2.6 m) with 10 cubicles, 5 adjacent cubicles in each side (six cubicles measuring 1.8 m × 1.4 m and four cubicles measuring 1.8 m × 1.35 m; length to brisket board), and a feeding area (8.6 m × 3.25 m). The alley and feeding area had concrete slatted flooring (slats = 15 cm, slots = 4 cm), and the cubicles were lined with mattresses, bedded, and maintained as described for the resident pen. The feeding area was equipped with seven computerized feed bins (Insentec B.V., Marknesse), one mechanical rotating cow brush and one 126 cm × 60 cm self-filling water trough, all of the same type as in the resident pens. In the experimental pen, one feed bin was allocated per cow. Filling and emptying of feed bins followed the same time schedule as described for the resident herd; however, bins were emptied in between scheduled times in case feed spoilage was observed. The amount of feed filled into bins were adjusted to secure sufficient feed at all times. No adaptation period to the experimental pen could be used due to the proximity of dry-off.

Experimental Treatments

Feeding Level. The NORM feeding level treatment was obtained by ad libitum allocation of the standard lactation PMR and the REDU feeding level treatment was obtained by ad libitum allocation of the lactation PMR diluted with chopped barley straw PMR (Table 1) to obtain an energy concentration similar to the dry TMR. The diluted PMR was fortified with minerals and vitamins to reach a similar daily supplementation as with the normal lactation PMR. To reduce feeding in the AMS as well, the concentrate allowance in the AMS was set to maximally 3 or 1 kg/d (as is form) for NORM and REDU, respectively, with an equal allowance at each milking for the 2× milking frequency. On the dry-off day, all cows shifted to the standard dry TMR; however, fortified with minerals and vitamins to reach approximately 50% of the daily supplementation in the week before dry-off. The planned energy concentration of the whole diets (PMR + concentrate) were 6.75, 5.73, and 5.71MJ NE_L/kg DM for the NORM diet, REDU diet, and dry TMR, respectively. The feed mixtures were mixed daily and subject to minor adjustments due to shift of ingredients and weekly DM determination of ingredients. Forages from several silos were used due to the long course of the experiment. The forages were sampled and analyzed at a commercial laboratory (Eurofins, Vejen, Denmark) before use in diets.

Milking Frequency. In the baseline period, the AMS milking frequency was 2.09 ± 0.33/d (mean ± SD). In the experimental period, cows were also milked in the AMS but were manually moved to the AMS by barn staff. All cows were milked between 0530 and 0700 h, and cows with 2× milking frequency were milked again between 1530 and 1630 h. Cows were milked for the last time in the morning of day 0.

Injection of Cabergoline. At the day of dry-off, cows were administered with 5 mL of SAL or CAB suspension (5.6 mg cabergoline; Velactis, Ceva Santé Animale) i.m. in the neck by barn staff at approximately 0900 h using an 18G needle and 5 mL screw lock syringe. The content of the syringe was blinded to the person by wrapping a white label.

Data Collection

Feed Intake. Recording of PMR and TMR intake was made using the Insentec system. In addition, the amounts of AMS concentrate offered and refused at each milking were recorded in the AMS. The Insentec system measured the exact time of each cow entering the Insentec feed bin (placing her collar past the feed bin gate causing this to open) and leaving it (removing her head and collar from the bin thus causing the gate to close) and the weight change of the bin during each visit. The data were aggregated to day level with a day defined from 1000 h on the actual day to 0959 h on the day after. Prior to data aggregation, outliers in the Insentec data were identified and handled as follows. For data collected during the baseline period, outliers were identified as described by Bossen et al. (2009). For data collected during the week before and after dry-off, outliers in the Insentec data were identified in seven steps for each week. First, visits to a feed bin with a visit duration > 30 min and a feed intake < 0.5 kg (wet weight) were deleted (22 visits). Second, visits to a feed bin with a recorded 'end time' occurring later than the 'start time' of the subsequent visit were deleted (136 visits). Third, visits with zero duration were deleted (8 visits). Fourth, visits with eating rates (kg/min) deviating more than 7 × SD of the cow's weekly average were temporarily deleted (213 visits). Fifth, the fourth step was repeated (90 visits). Sixth, visits with an eating rate below the 0.025% quantile or above the 99.975% quantile were temporarily deleted (23 visits). Seventh, eating rates for the temporarily deleted visits (steps four to six) were replaced with the weekly median for each individual cow. After this cleaning procedure, 0.24% of total visits had been deleted leaving 67,924 visits of which 0.48% of eating rates had been adjusted.

Samples of both PMR's, TMR, and AMS concentrate were obtained weekly and stored at -20°C until chemical analysis. Before analysis, weekly samples were pooled within 5-month periods and analyzed for DM, ash, CP, crude fat, NDF and starch (Røjen et al., 2012). Contents of Ca and

Mg was determined by atomic absorption spectroscopy (Milner and Whiteside, 1981), and of P by absorbance spectroscopy (Stuffins, 1967).

Milking. Milk yield was measured during each visit in the AMS using optical milk flow measurement (DeLaval Free Flow meter MM25, DeLaval AB). In the baseline period, daily milk yield was calculated as a rolling average using the ICAR recommendation for AMS (Lazenby et al., 2003). In the last week before dry-off, daily milk yield was calculated as the sum of afternoon and the following morning milking. Milk samples were collected weekly at all milkings in a 48 h period beginning in the mornings of -10 and -3 DRTD, respectively. Milk samples were analyzed for protein, fat, lactose-monohydrate, and SCC using near-infrared spectroscopy (MilkoScan 4000, FOSS, Hillerød, Denmark) at a commercial laboratory (Eurofins, Vejen, Denmark). The weighted average milk composition was calculated for each cow by week.

Body Weight. A platform scale (Danvaegt, Hinnerup, Denmark) installed in the AMS automatically recorded BW of the cows during milking. A daily BW value was aggregated from several measurements at each visit in the AMS. Outliers of daily BW data were identified and handled as described by Bossen et al. (2009). Daily BW was averaged within week relative to dry-off.

Clinical Udder Characteristics. The measures distension of rear quarters, teat perimeter, distance from the shortest front teat end to the cubicle surface and distance from the shortest rear teat end to the cubicle surface were obtained as proxies for udder engorgement. The udder examination were conducted at -7, -6, -5, -2, 0, 1, 2 and 5 DRTD beginning at 0815 h as well as at 1130 h and 1430 h at day 0 and lasted 6 ± 2 min (mean ± SD) per cow. Distension of rear quarters was measured as the distance between two dots marked with a permanent marker on the center of each rear quarter, approximately 10 cm above the teat base (Fig. 1). The dots were marked on the udder at -7 DRTD and reinforced until 5 DRTD. Teat perimeter constituted the sum of the four distances between adjacent teats measured from the middle of each teat. These two measures were recorded manually with a ruler while each cow was kept standing in a cubicle. The distances between the cubicle floor and each of the shortest front and rear teats, both distinguished visually prior to measuring, were measured with a laser meter (GLM 50 C Professional, Bosch GmbH, Germany). Given that nine experimenters recorded distension of rear quarters, teat perimeter and distances between end of shortest front and rear teats to the floor, a one-way random effects intra-class correlation analysis [**ICC** (1,1)] (Koo and Li, 2016; 2017) was performed for each measure to check for inter-observer agreement using the package *irr* v.0.84.1 (Gamer et al., 2019). The ICC index ranges from 0 to 1, being 0 meaning no agreement and 1 meaning perfect agreement. The lowest ICC across the four measures was for distension of rear quarters [ICC = 0.85; 95% confidence interval (**CI**): 0.66 to 0.95].

Signs of Milk Leakage. Recordings of cows showing signs of milk leakage were conducted throughout the study, using similar definitions, but two different protocols. The first protocol was followed during the udder examination from -7 to 5 DRTD. The second protocol was followed by barn staff at morning milking during the pre-milking routine from -6 to 0 DRTD. The second protocol was introduced from batch 16 and onwards (total of 73 cows) to better assess milk leakage while cows were still milked. In both protocols, cows were checked for milk leakage by observing the four teats simultaneously for 30 s and recording of drop of milk hanging at the teat end, milk dripping (i.e. up to 1 drop/10 s), and milk flowing (i.e. more than 1 drop/10 s). In each protocol, if a cow had at least one teat scored with one of the milk leakage scores, the cow was scored “yes” for sign of milk leakage. If no teat showed any sign of milk leakage, the cow was scored “no” for sign of milk leakage. The inter-observer agreement for milk leakage scoring (yes/no) during the udder examination was assessed by calculation of linear-weighted kappa (κ) using R package *rel* v.1.4.2 (LoMartire, 2020). The κ statistics ranges from -1 to 1, being -1 interpreted as no agreement and 1 perfect agreement. The κ for milk leakage scoring during the udder examination was 0.53 (95% CI: 0.11 to 0.96). Milk leakage examinations during the pre-milking routine were not included in the assessments of inter-observer agreement.

Calculations and Statistical Analyses

The ECM yield was calculated according to Sjaunja et al. (1991) using the weekly weighted milk composition in respective week. The total NE_L intake and total NE_L requirement for ECM yield, pregnancy, and maintenance was calculated using NorFor (Volden, 2011) based on observed intake of allocated feed mixtures, ECM yield, BW, and days in pregnancy. The net energy balance was subsequently calculated by difference.

The following observations were deleted: all observations for four cows were removed due to lameness (n = 2) or mastitis (n = 2) around -7 DRTD; observations from just before dry-off and onwards were removed for four additional cows due to lameness (n = 1), mastitis (n = 1), or no access to feed for 18 h (Insentec breakdown, n = 2). Thus, observations from 115 cows were used before dry-off (n = 14 to 15 per treatment combinations) and observations from 111 cows were used after dry-off (n = 13 to 15 per treatment combinations).

Feed intake, milk variables, BW, and energy variables were analyzed with linear mixed effects models using the MIXED procedure of SAS. The baseline average (day -14 to -8) was tested for difference among treatment combinations using a model with parity group (primiparous, multiparous) and treatment combination (1 to 8) as fixed effects. The models for test of treatment effects included baseline average per cow and number of cows in pen as covariates, and parity group (primiparous, multiparous), feeding level (NORM, REDU), milking frequency (once, twice), DRTD (day -7 to +7), and all possible interactions between feeding level, milking frequency and DRTD as fixed effects. For the analysis of total DMI and net energy balance, the model also included cabergoline (SAL, CAB) and all additional interactions as fixed effects. For all models, cow and batch were considered as random effects, and DRTD within cow was considered as a repeated measurement using the spatial power covariance structure. Denominator degrees of freedom was calculated using the Kenward-Roger method.

Distension of rear quarters, teat perimeter, and teat-to-cubicle distances were analyzed with linear mixed effects models in R v.3.6.1 (R Core Team, 2019) using the packages *nlme* v.3.1-140 (Pinheiro et al., 2019), *car* v.3.0-9 (Fox and Weisberg, 2019), and *emmeans* v.1.5.0 (Lenth, 2020). The model included number of cows in pen as covariate, and parity group, feeding level, milking frequency, cabergoline, DRTD, and all possible interactions between feeding level, milking frequency, cabergoline and DRTD as fixed effects. Cow and batch were included as random effects. All models contained a continuous-time autoregressive covariance structure of order 1 to account for repeated measures of each cow over days, which corresponds to the spatial power structure used in SAS.

Signs of milk leakage before (DRTD -6 to 0) and after dry-off (DRTD 0+3h to 5) were analyzed in two steps. First, the number of cows within each sign of milk leakage score (yes/no) in each assessment protocol (routine before morning milking/udder examination) was analyzed as contingency tables against the eight treatment combinations (feeding level × milking frequency × cabergoline) within each scoring time with Fisher's Exact test and Holm-adjusted post-hoc pairwise comparisons using the package *stats* v.3.6.1 (R Core Team, 2019). In the second step, signs of milk leakage was cumulated before and after dry-off, respectively, as either yes (signs of milk leakage observed at one or more scoring moments) or no (no signs of milk leakage observed during the whole period), giving 73 observations before dry-off and 111 observations after dry-off. For each period, data was analyzed with mixed effects logistic regression using the package *glmmTMB* v.1.0.2.1 (Brooks et al., 2017) and *emmeans* v.1.5.0 (Lenth, 2020). The "before dry-off" model included number of cows in pen as covariate, and parity group, feeding level, milking frequency, and interaction between feeding level and milking frequency as fixed effects. Batch was included as random effect. The “after dry-off” model also included cabergoline and all additional interactions as fixed effects. Batch was included as random effect.

Assumption of normal distribution and homoscedasticity of residuals was graphically confirmed. Somatic cell count was $\log_{10}$ transformed to obtain normal distribution of residuals. Significance was declared when $P \leq 0.05$ and tendencies were considered when $0.05 < P \leq 0.10$. Least square means were presented and separated using the Tukey-Kramer penalty method.

RESULTS

Performance

For variables measured in the baseline period, none differed among the 8 treatment combinations ($P > 0.05$) except for SCC.

Feed Intake. The shift to the REDU diet at day -7 induced an abrupt decrease in total DMI, followed by an increase and stabilization at a lower level after 3 to 4 d as compared with no change in DMI for NORM ($F_{13,1158} = 51.0$, $P_{F×D} < 0.01$; Fig. 2). Total DMI did not differ between cows milked once or twice daily within feeding level ($F_{1,99.5} = 0.06$, $P_{M} = 0.81$). During the 7 d treatment period, concentrate intake tended to be lower for NORM fed cows milked once daily as compared with NORM fed cows milked twice, and further reduced with REDU diet ($F_{1,91.2} = 3.09$, $P_{F×M} = 0.08$; Table 2).

An interaction among feeding level, cabergoline, and DRTD was observed ($F_{13,1159} = 2.44$, $P_{F×C×D} < 0.01$; Table 3 and Fig. 2). For cows injected with SAL at dry-off (Fig. 2a), the shift to the dry-cow TMR did not induce a change in total DMI for cows previously fed REDU, whereas for cows previously fed NORM, the shift to the dry-cow TMR decreased total DMI to a level similar as for cows previously fed REDU. For cows injected with CAB (Fig. 2b), total DMI was reduced to a similar level of 7.1 ± 0.38 kg/d at 0 DRTD irrespective of treatment before dry-off as compared with 13.3 ± 0.37 kg/d for SAL at 0 DRTD; after which total DMI did not differ among the eight treatments.

The shift to the REDU diet at day -7 induced an abrupt decrease in net energy intake, followed by an increase and stabilization at a lower level after 3 to 4 d as compared with no change for NORM ($F_{6,490} = 4.98$, $P_{F\times D} < 0.01$; Table 2). The net energy intake did not differ between milking frequencies ($F_{1,105} = 0.48$, $P_M = 0.49$). The net energy intake of cows fed the REDU diet was equivalent to 47% of that of NORM fed cows.

Milk Variables. The milk and ECM yield was reduced to similar level for treatment combinations NORM & 1× milked and REDU & 2× milked, and was reduced most for REDU & 1× milked (milk: $F_{1,108} = 16.0$, $P_{F\times M} < 0.01$; ECM: $F_{1,106} = 10.4$, $P_{F\times M} < 0.01$; Table 2 and Fig. 3). The milk yield decreased from -8 to -6 DRTD for REDU where after it stabilized as compared with stabile milk yield for NORM ($F_{6,482} = 22.0$, $P_{F\times D} < 0.01$; Table 2 and Fig. 3).

The milk fat and protein contents were higher for REDU as compared with NORM (fat: $F_{1,93.9} = 172$, $P_F < 0.01$; protein: $F_{1,89.1} = 24.4$, $P_F < 0.01$; Table 2), and were higher for 1× milked as compared with 2× milked (fat: $F_{1,102} = 11.2$, $P_M < 0.01$; protein: $F_{1,104} = 21.2$, $P_M < 0.01$). The SCC was higher for REDU as compared with NORM ($F_{1,103} = 10.6$, $P_F < 0.01$), and were higher for 1× milked as compared with 2× milked ($F_{1,103} = 14.1$, $P_M = 0.01$; Table 2).

Body Weight. Before dry-off, the BW was lower for cows fed REDU as compared with NORM fed cows ($F_{1,94.9} = 15.6$, $P_F < 0.01$; Table 2), but did not differ between milking frequencies.

Energy Balance. A four-way interaction among feeding level, milking frequency, cabergoline, and DRTD was observed for calculated net energy balance ($F_{13,1142} = 1.80$, $P_{F\times M\times C\times D} = 0.04$; Table 3 and Fig. 4). Before dry-off, the net energy balance of cows fed the REDU diet was lower and negative as compared with NORM fed cows, and was lower for twice milked as compared with once milked cows within feeding level. After dry-off, the shift to the TMR for dry cows and the cessation of milking increased the net energy balance for REDU cows injected with SAL to be similar and stabile level for the rest of experimental period for all SAL cows (Fig. 4a). For cows injected with CAB at dry-off (Fig. 4b), the net energy balance did not differ from SAL cows (Fig. 4a) after dry-off except at 0 DRTD where it was lower (-10 ± 2.8 MJ/d) compared with cows injected with SAL (+21 ± 2.7 MJ/d).

Clinical Udder Characteristics

Distension of Rear Quarters. From -5 to 2 DRTD, the udder of NORM & 2× milked cows had a greater distension of rear quarters compared with the other three treatment combinations, and from 0 to 2 DRTD, the distension of rear quarter increased less for REDU fed cows compared with NORM fed cows ($\chi^2_9 = 17.5$, $P_{\mathrm{F\times M\times D}} = 0.04$; Table 3 and Fig. 5a). On 1 DRTD, SAL cows displayed greater distension of rear quarters compared with CAB cows ($\chi^2_9 = 31.7$, $P_{\mathrm{C\times D}} < 0.01$; Table 3 and Fig. 5b).

Teat Perimeter. Overall, NORM & 2× milked cows had greater teat perimeter compared with cows with the other three treatment combinations ($\chi^2_1 = 5.49$, $P_{\mathrm{F\times M}} = 0.02$; Table 3 and Fig. 6a). The teat perimeter increased after dry-off for all treatment combinations of feeding level and milking frequency ($\chi^2_9 = 618$, $P_{\mathrm{D}} < 0.01$) and no triple interaction among feeding level, milking frequency, and day was observed ($\chi^2_9 = 7.22$, $P_{\mathrm{F\times M\times D}} = 0.61$). On 1 DRTD, SAL cows had greater teat perimeter compared with CAB cows ($\chi^2_9 = 56.6$, $P_{\mathrm{C\times D}} < 0.01$; Table 3 and Fig. 6b).

Distance between the Shortest Front Teat End to Cubicle Floor. Overall, NORM & 2× milked cows showed the shortest distance between the shortest front teat end to the cubicle floor compared with cows with the other three treatment combinations ($\chi^2_9 = 20.0$, $P_{\mathrm{F\times M\times D}} = 0.02$; Table

3 and Fig. 7a). After dry-off to 1 DRTD, CAB cows showed greater distance between the shortest front teat end to the cubicle floor compared with SAL cows ($\chi^2_9 = 49.49$, $P_{C\times D} < 0.01$; Table 3 and Fig. 7b).

Distance between the Shortest Rear Teat End to Cubicle Floor. Throughout the experimental period, NORM fed cows had shorter distance between the shortest rear teat end to the cubicle floor compared with REDU fed cows ($\chi^2_9 = 21.9$, $P_{F\times D} < 0.01$; Table 3 and Fig. 8a). After dry-off to 1 DRTD, CAB cows showed greater distance between the shortest rear teat end to the cubicle floor compared with SAL cows ($\chi^2_9 = 38.3$, $P_{C\times D} < 0.01$; Table 3 and Fig. 8b).

Signs of Milk Leakage

Before Dry-Off. The proportion of cows showing signs of milk leakage differed among treatments (Fig. 9) on -6 DRTD ($P < 0.01$), -3 DRTD ($P < 0.01$), -2 DRTD ($P = 0.05$), and 0 DRTD ($P < 0.01$). On these 4 out of 7 days, the highest proportion of cows with signs of milk leakage was observed for NORM & 1× milked [(-6 DRTD: 71%, n = 12); (-3 DRTD: 35%, n = 6); (-2 DRTD: 32%, n = 6); (0 DRTD: 33%, n = 6)], while the lowest proportion of cows with signs of milk leakage was observed for REDU & 1× milked [(-6 DRTD: 6%, n = 1); (-3; -2; 0 DRTD: 0%, n = 0)].

When cumulating milk leakage scorings before dry-off, the probability of cows showing signs of milk leakage was lower ($\chi^2_1 = 5.09$, $P_F = 0.02$) for REDU cows (probability; 95% CI: 0.21; 0.06 to 0.51) compared with NORM cows (0.53; 0.26 to 0.79). Moreover, the probability of cows showing signs of milk leakage was lower ($\chi^2_1 = 7.63$, $P_M = 0.01$) for 2× milked cows (0.17; 0.05 to 0.47) compared with 1× milked cows (0.59; 0.30 to 0.82).

After Dry-Off. The proportion of cows showing signs of milk leakage differed among treatments on 1 and 2 DRTD ($P < 0.01$ and $P = 0.02$, respectively; Fig. 10). On 1 DRTD, the highest proportion of cows with signs of milk leakage was observed for NORM & 2× milked & SAL (54%, n = 7) and NORM & 1× milked & SAL (50%, n = 7), while the lowest proportion of cows with signs of milk leakage was observed for both NORM & 1× milked & CAB and REDU & 2× milked & CAB (0%, n = 0). On 2 DRTD, the highest proportion of cows with signs of milk leakage was observed for REDU & 2× milked & SAL (50%, n = 7) and REDU & 1× milked & SAL (47%, n = 7), whereas, the lowest proportion of cows with signs of milk leakage was observed for REDU & 2× milked & CAB (0%, n = 0).

When cumulating milk leakage scorings after dry-off, the probability of cows showing signs of milk leakage was lower ($\chi^2_1 = 12.31$, $P_C < 0.01$) for cows injected with CAB (0.21; 0.09 to 0.40) compared with SAL injected cows (0.64; 0.47 to 0.78). However, a tendency to interaction between feeding level and cabergoline was observed ($\chi^2_1 = 3.12$, $P_{F×C} = 0.08$) where the probability of cows showing signs of milk leakage was lower for REDU & CAB cows (0.09; 0.02 to 0.35) as compared with NORM & SAL (0.65; 0.39 to 0.85), NORM & CAB (0.40; 0.18 to 0.66), and REDU & SAL (0.62; 0.37 to 0.82). The probability of cows showing signs of milk leakage did not differ between milking frequencies ($\chi^2_1 = 0.26$, $P_M = 0.61$).

DISCUSSION

The current study investigated the combined effects of feeding level, milking frequency, and administration of cabergoline after the last milking on feed intake, milk yield, energy balance, clinical udder characteristics, and signs of milk leakage during dry-off of high-yielding dairy cows. Overall, we found that all gradual dry-off combinations (i.e. reduction in feeding level and milking frequency for 7 d before dry-off) lowered DMI and milk yield, which led to reduced udder engorgement after the last milking, whereas the only combination avoiding a negative energy balance was reduced milking frequency and normal feeding level. The administration of cabergoline after the last milking resulted in an abrupt reduction of DMI lasting approximately 24 h, as well as the least udder engorgement and the least signs of milk leakage for at least 48 h after injection. Effects of feeding level and milking frequency are discussed separately from effects of cabergoline due to the different time of treatment application.

Feeding Level and Milking Frequency

Before Dry-Off. In the present study, cows abruptly dried-off yielded around 25 kg/d of milk, whereas cows gradually dried-off by either reducing feeding level or milking frequency yielded approximately 30% less, whereas cows gradually dried-off by reducing both feeding level and milking frequency yielded approximately 45% less. Interestingly, the current relative reductions in milk yield are similar to those observed with grazing cows at a substantially lower yield at dry-off (9 kg/d) in a 2 × 2 factorial setup using the same experimental factors (Tucker et al., 2009). In the current experiment, the dilution of the lactation diet with barley straw targeting the energy density of the dry TMR resulted in an approximately 50% reduction in energy intake even though cows had ad libitum access to the feed mix. The change from one feed bin per two cows in the baseline period to one bin per cow in the experimental period did not appear to affect DMI when considering the cows not changing diet (normal) with the shift from the resident pen to the experimental pen (Fig. 2). Despite the concomitant reduction in milk yield, a state of negative energy balance occurred as indicated by the calculated NE_L balance, loss of BW, and higher milk fat content. Furthermore, the negative energy balance was most pronounced with the combination of reduced feeding level and twice-daily milking, as the cows milked twice daily had higher milk yields than those milked once daily. Indeed, the measured loss of BW with the reduced diet may have been caused by a lower rumen fill due to the lower feed intake; however, to which extent this could be the case is uncertain as the rumen retention time of the diet would be longer due to low rate of degradation of barley straw. For the energy balance, a difference in BW of 30 kg would change the NE_L requirement of 1.5 MJ/d (Volden, 2011) which is of minor magnitude compared to the calculated differences in energy balance. The reduction in milk yield obtained by reducing the feeding level will depend upon the extent of the reduction in energy intake. Valizaheh et al. (2008) compared ad libitum access to either grass hay or oat hay 6 days before dry-off, and in their study milk yield was reduced by 50% and 70%, respectively, likely reflecting the lowest NDF digestibility for oat hay. Ollier et al. (2015) compared ad libitum feeding of either a lactation TMR or grass hay and observed 54% reduction in milk yield with grass hay. Valizaheh et al. (2008) and Ollier et al. (2015) did not present data on energy intake, but the greater extent of reduction in milk yield combined with twice daily milking suggests greater reduction in energy intake compared to the current study and the study by Tucker et al. (2009). Negative energy balance in cows with gradual dry-off induced by reduced feeding level will likely induce metabolic stress as indicated by increased free fatty acid (**FFA**) and reduced glucose plasma concentrations with straw feeding (Odensten et al., 2005) and hay feeding (Ollier et al., 2015). In addition, cows subjected to reduced feeding level without a concomitant reduction in milking frequency or milking cessation may be experiencing hunger as suggested by increased vocalization (Valizaheh et al., 2008; Tucker et al., 2009), and increased maximum weight pushed to obtain concentrate in a feeding motivation test (Franchi et al., 2019). In the present study, as well as in the study by Tucker et al. (2009), maintained feeding level combined with reduced milking frequency resulted in a substantial reduction in milk yield. In the current study, no indications of negative energy balance were observed with this treatment and no undernutrition in the period before the dry-off day would be expected. The reduction in milk yield was established 1 to 2 days after reducing either feeding level or milking frequency (Fig. 3), indicating that less than 7 d could be sufficient for gradual dry-off, although the exact number of days required need further investigation.

The impact of milking frequency on clinical udder characteristics before dry-off was not clear in the current study. For instance, milking once daily was expected to induce greater milk accumulation in the udder and consequently cause larger udder distension due to a less frequent milk removal (Davis et al., 1999). Hence, it was expected that cows fed the normal lactation diet and milked once a day would have shown the largest udder distension due to the constant energy supply to the udder combined with a reduced milk removal. However, this was not observed in the present study and may be caused by the rapid decrease in milk yield after cows were subjected to once daily milking. Another reason could be related to the time of examination approximately 2 hours after morning milking where all experimental cows were milked. Thus, udders would likely have been scored more engorged if the examination had been performed before morning milking, where once daily milked cows would have not been milked for almost 24 hours. This is supported by the pattern of milk leakage observed before dry-off, which was scored during the morning pre-milking routine where the proportion of cows showing signs of milk leakage was greatest for cows normal fed and milked twice. Assuming that half of the daily milk yield was in the udder at morning milking for cows milked twice, milk yield at morning milking would have been approximately 12.5, 17.7, 8.7, and 13.7 kg for the NORM & 2× milked, NORM & 1× milked, REDU & 2× milked and REDU & 1× milked, respectively. Thus, the maintained energy supply to the udder combined with a reduced milking frequency likely resulted in more milk accumulating in the udder and consequently udder engorgement. This in turn led to a putative higher intra-mammary pressure causing increased signs of milk leakage (Davis et al., 1999; Klaas et al., 2005).

After Dry-Off. With the change to the dry cow diet after last milking, DMI for previously NORM fed cows decreased to a similar extent as the DMI observed for the REDU fed cows at the start of the gradual dry-off period. However, the impact on energy balance of the reduction in feed intake at this time was substantially less due to the concomitant cessation of milking and hence the requirement for energy. The change to the dry cow diet and milking cessation did not affect DMI for the REDU fed cows, indicating that the negative energy balance before dry-off was alleviated by cessation of milking. The current observations on clinical udder characteristics indicate that cows subjected to the reduced feeding level had less milk accumulating in the udders irrespectively of the milking frequency. Similarly, Tucker et al. (2009) observed reduced udder firmness in the beginning of the dry period when restricting the amount of feed allocated, suggesting that limiting the energy supply to the udder prevents accumulation of milk and hence limits intra-mammary pressure. After dry-off, clinical characteristics of the udder for cows previously fed the normal lactation diet and milked twice daily (abrupt dry-off), indicated most engorged udders, likely reflecting that the high milk yield led to a greater milk accumulation in the first days after the last milking. However, the increment in teat perimeter after dry-off did not differ among treatment combinations, as there was three-way interaction among feeding level, milking frequency, and day. Indeed, this three-way interaction was significant for distension of rear quarters indicating less incrementing distension with the reduced feeding after dry-off. To which extent the cows experienced discomfort from udder engorgement in relation to rapid increments after dry-off or to absolute engorgement need further investigation for example by use of motivation tests for lying down (Tucker et al., 2018) and the recording of lying postures (Tucker et al., 2007). No clear difference between abrupt dry-off and the tested gradual dry-off practices (i.e. reducing feeding level or milking frequency) was evident for signs of milk leakage after dry-off in the current study neither based on single scoring times nor based on cumulated scorings. This was surprising as Tucker et al. (2009) observed reduced feeding level before dry-off to lower the risk of milk leakage at the beginning of the dry period with cows at a substantially lower milk yield (i.e. 9.3 kg/d). Also Zobel et al. (2013) observed decreased risk of milk leakage after dry-off in high-yielding cows at dry-off (24.0 kg/d) subjected to gradual cessation of milking and concomitantly allocated a reduced feeding level before last milking.

Overall, gradual dry-off by combining normal feeding level and reduced milking frequency decreased milk yield before dry-off in high-yielding dairy cows and reduced udder engorgement after dry-off without inducing negative energy balance during the period of dry-off. In contrast, reduced feeding level induced negative energy balance that may compromise welfare. On the other hand, the increase in distension of rear quarters and teat perimeter after dry-off was approximately the same for the four feeding level by milking frequency combinations, thus the welfare implications of abrupt dry-off are not readily separable from the gradual dry-off. However, no clear differences in risk of milk leakage after dry-off were observed between abrupt and gradual dry-off management strategies.

Dopamine Agonist

Irrespective of dry-off management practice applied before last milking, cabergoline injection caused a clear abrupt decrease in DMI at the dry-off day lasting approximately 24 h equivalent to 53% of the intake of saline cows. Dry-off using cabergoline have been investigated before (Bach et al., 2015; Boutinaud et al., 2016; Hop et al., 2019), but this is the first time, to our knowledge, that the DMI of cows treated with cabergoline is reported. With dopamine agonist quinagolide treatment on the day of dry-off, abrupt reductions in DMI of 24 to 31% have been reported (Ollier et al., 2013, 2014, 2015). The latter studies reported that there were no signs of negative energy balance associated with the decreased DMI based on FFA plasma concentrations. In the current study, the calculated NE_L balance was slightly negative at the dry-off day for cabergoline injected cows, which indicate that the reduction in DMI did induce a short and moderate period of negative energy balance. As discussed in the previous section, the BW used in the calculation of maintenance requirement could have been biased by a lower rumen fill caused by the lower feed intake. The mechanism underlying the abrupt decrease in DMI could be either a decreased nutrient requirement due to rapid inhibition of milk synthesis, or a decreased appetite caused by other dopaminergic effects than decreased stimulation of milk synthesis. The reduced udder engorgement and signs of milk leakage after dry-off observed in cabergoline injected cows indicate that cabergoline is able to induce a rapid reduction in milk synthesis leading to a concomitant reduction in the mammary uptake of nutrients, which could have down-regulated feed intake via changes in plasma concentrations of appetite regulating metabolites and hormones. Alternatively, the decreased DMI could be related to lower prolactin concentrations in the bloodstream. An increased prolactin level in blood is known to stimulate hyperphagia during lactation (Woodside, 2007, Lacasse et al., 2016). Therefore, the putative reduction in blood prolactin concentrations after cabergoline injection (Bach et al., 2015, Boutinaud et al., 2016) could inversely cause hypophagia (i.e. decreased DMI). Another possible explanation for the decreased DMI in cows treated with cabergoline could be a feeling of nausea. In humans, nausea is a known adverse effect of therapeutic treatment with dopamine agonists (Martins et al., 2017). However, whether the same adverse effects occur in cows remains unknown. Hence, further studies will be necessary to elucidate the consequences and mechanisms behind the abrupt decrease in feed intake observed in cows treated with cabergoline at dry-off, including potential effects on animal welfare.

As cabergoline is registered for use with abrupt dry-off, the current study is the first to investigate the use in connection with gradual as well as abrupt dry-off management practices in dairy cows. The injection of cabergoline after the last milking reduced udder engorgement for 1 to 2 days after dry-off irrespective of abrupt or gradual dry-off management. In cows subjected to abrupt dry-off, treatment with cabergoline after the last milking have been observed to reduce udder firmness (Bach et al., 2015) and reduced teat perimeter (Bertulat et al., 2017). Cabergoline injection has also been reported to reduce the risk of milk leakage after the last milking (Bach et al., 2015; Bertulat et al., 2017; Hop et al., 2019) as also observed in the current study, especially for those cows subjected to gradual dry-off. Similar to these studies, we observed that the effect of cabergoline on clinical udder characteristics and signs of milk leakage was most evident during the first day after injection after which the effect declined during subsequent days. The tendency that cabergoline did not clearly reduce the signs of milk leakage with abrupt dry-off when cumulating milk leakage scores may indicate that level of milk yield at dry-off affects the efficacy of cabergoline treatment in preventing milk leakage as these cows yielded substantially more milk than those with gradual dry-off. Previously, Bach et al. (2015) and Hop et al. (2019) observed dedreased risk of milk leakage with cabergoline treatment in abrupt dry-off for average milk yields of 18.5 and 24.5 kg/d, respectively, in comparison with 25.1 kg/d for abruptly dried-off cows in the current study. Indeed, a larger number of cows were used in these studies. Reduced risk of milk leakage with cabergoline treatment at dry-off have recently been associated with reduced the risk of mastitis during the dry period and subsequent lactation (Hop et al., 2019); however, the sample size of the current study was insufficient to assess risk of subsequent mastitis.

Overall, cabergoline injection after the last milking appeared to decrease milk synthesis and milk accumulation as interpreted from udder engorgement and signs of milk leakage. The observed abrupt decrease in DMI lasting approximately 24 h with cabergoline was noticeable and the impact of this on animal welfare and metabolism requires further investigation.

CONCLUSION

Gradual cessation of lactation by reducing milking frequency to once daily without reducing the feeding level decreased milk yield before dry-off in high-yielding dairy cows and reduced udder engorgement after dry-off without inducing negative energy balance during the period of dry-off. In contrast, reduced feeding level induced negative energy balance that may compromise welfare. On the other hand, the increase in udder engorgement after dry-off was approximately the same irrespective of feeding level or milking frequency used; thus, the welfare implications of abrupt dry-off is not clearly separable. In accordance with this, no clear differences in risk of milk leakage after dry-off were observed between abrupt and gradual dry-off management strategies.

The use of cabergoline to facilitate the cessation of milk synthesis led to fewer signs of milk leakage and reduced udder engorgement during the first days after dry-off that may positively affect welfare at dry-off. However, it also caused a concomitant abrupt decrease in DMI lasting approximately 24 h, of which the welfare consequences and the mechanism behind requires further investigation to complete the understanding of dopamine agonist use for dry-off.

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Table 1. Composition of feed mixes used (g/kg DM unless otherwise noted).

	Lactation			
	Normal PMR	Reduced PMR	AMS concentrate[1]	Dry TMR
Ingredients				
Maize silage	325.4	205.3		528.5
Grass-clover silage	282.1	178.0		132.2
Barley straw, chopped		302.7		246.7
Barley grain	104.1	65.7	141.5	
Wheat grain			141.5	
Sugar beet pulp, dried	104.1	65.7	172.5	
Rape seed cakes	86.8	54.8		35.3
Rape seed meal			169.3	
Soybean meal, 54% CP	86.8	54.8	90.8	35.3
Citrus pulp, dried			72.5	
Sunflower meal			71.0	
Grass pellets			52.4	
Wheat bran			49.6	
Molasses, sugar beet			18.6	
Vegetable fat, saturated			9.0	
Premixes	5.5[2,3]	13.0[2,3]	2.3[4]	9.7[5]
$NaHCO_3$	2.4	7.8		
NaCl	1.7	7.4	8.0	3.5
$CaCO_3$	1.1	7.0		4.4
$Ca(H_2PO_4)_2$		31.5		4.4
MgO		6.3		
$MgSO_4$			1.0	
Nutrients (mean ± SD, n = 4)				
DM, g/kg	396 ± 23	455 ± 27	901 ± 2	407 ± 26
Ash	70 ± 3	106 ± 4	66 ± 3	64 ± 3
CP	168 ± 3	124 ± 4	214 ± 1	115 ± 9
Crude fat	37 ± 2	27 ± 1	35 ± 2	28 ± 0.5
NDF	312 ± 8	429 ± 16	235 ± 4	443 ± 25
Starch	172 ± 5	108 ± 9	199 ± 10	188 ± 10
Ca	7.5 ± 0.3	14.2 ± 0.5	7.8 ± 0.4	7.7 ± 0.7
P	3.7 ± 0.2	9.4 ± 0.7	5.6 ± 0.2	3.7 ± 0.1
Mg	2.6 ± 0.1	6.4 ± 0.4	3.3 ± 0.2	3.3 ± 0.2
NE_L, MJ/kg DM[6]	6.59	4.96	6.76	5.40
MP[6]	101	78	111	79

[1] Commercial pelletized concentrate (SL395044, DLG, Copenhagen, Denmark).

[2] Micro-mineral and vitamin premix lactation (Type 3, ViloFoss, Gråsten, Denmark).

[3] E-vitamin premix (Suplex E-50000, ViloFoss, Gråsten, Denmark).

[4] Micro-mineral and vitamin premix (ViloFoss, Gråsten, Denmark).

[5] Micro-mineral and vitamin premix dry (Komix Top Gold d-alfa Org Se, ViloFoss, Gråsten, Denmark).

[6] Calculated according to NorFor (Volden, 2011).

Table 2. Feed intake, milk yield, milk composition, SCC, and BW before dry-off in dairy cows allocated to a 2 × 2 factorial arrangement of the factors feeding level (F, NORM vs. REDU) and milking frequency (M, 2× vs. 1×) during the 7 d (D) before dry-off treatment period.

		7 d before dry-off											
		NORM		REDU			*P*-values						
	Baseline[1]	2×	1×	2×	1×	SEM[2]	F	M	F×M	D	F×D	M×D	F×M×D
Intake													
PMR, kg DM/d	17.8 ± 2.4	17.5	17.6	12.0	12.1	0.41	<0.01	0.84	0.90	<0.01	<0.01	0.28	0.76
Concentrate, kg DM/d	2.3 ± 0.4	2.1[a]	1.6[b]	0.8[c]	0.6[c]	0.09	<0.01	<0.01	0.08	0.26	0.22	0.59	0.35
NE_L, MJ/d[3]	134 ± 14.5	132	129	67	67	2.2	<0.01	0.49	0.41	<0.01	<0.01	0.31	0.70
Milk yield, kg/d													
Milk	25.9 ± 5.8	25.1[a]	17.7[b]	17.3[b]	13.7[c]	0.48	<0.01	<0.01	<0.01	<0.01	<0.01	0.18	0.73
ECM	28.4 ± 5.8	27.7[a]	20.5[b]	21.9[b]	18.0[c]	0.52	<0.01	<0.01	<0.01	0.15	<0.01	0.03	0.82
Milk composition, g/kg													
Fat	45 ± 5.7	46	50	61	64	1.2	<0.01	<0.01	0.50	-	-	-	-
Protein	39 ± 3.2	40	42	42	44	0.5	<0.01	<0.01	0.74	-	-	-	-
Lactose	49 ± 1.7	49	48	48	47	0.4	<0.01	0.01	0.68	-	-	-	-
SCC, ×1,000 cells/mL[4]	93 ± 129	68	114	109	149	-	<0.01	0.01	0.38	-	-	-	-
		[55-85]	[92-141]	[88-135]	[118-187]								
BW, kg	752 ± 78	739	739	724	728	3.5	<0.01	0.54	0.61	-	-	-	-

[1] Baseline in week before trial used as covariate; n = 115; mean ± SD.

[2] n = 29.

[3] Net energy for lactation calculated according to NorFor (Volden, 2011).

[4] SCC data were $\log_{10}$ transformed for analysis to obtain normality. Values presented are $\log_{10}$ LSM [95% CI] back-transformed to median.

[a, b, c] Signify LSM to differ within row ($P < 0.05$).

Table 3. Test statistics and *P*-values for total DMI (Fig. 2), NE_L balance (Fig. 4), distension of rear quarters (Fig. 5), teat perimeter (Fig. 6), and teat to floor distances (Fig. 7 and 8) in Holstein cows used in a 2 × 2 × 2 factorial arrangement of treatment factors feeding level (F, NORM vs. REDU), milking frequency (M, 2× vs. 1×), and cabergoline (C, SAL vs. CAB) during 7 d (D) before and 7 d after dry-off.

	Statistics	F	M	C	D	F×M	F×C	M×C	F×D	M×D	C ×D	F×M×C	F×M×D	F×C×D	M×C×D	F×M×C×D
Total DMI	*P*-value	<0.01	0.81	<0.01	<0.01	0.70	0.05	0.50	<0.01	0.03	<0.01	0.68	0.12	0.03	0.06	0.09
	F-test	152[1]	0.06[1]	10.9[1]	72.9[2]	0.15[1]	3.93[1]	0.45[1]	51.0[2]	1.84[2]	14.6[2]	0.18[1]	1.47[2]	2.44[2]	1.67[2]	1.55[2]
NE_L balance	*P*-value	<0.01	<0.01	0.01	<0.01	0.39	0.05	0.36	<0.01	<0.01	<0.01	0.67	0.57	0.04	<0.01	0.04
	F-test	214[1]	27.0[1]	6.42[1]	101[2]	0.76[1]	4.08[1]	0.85[1]	41.6[2]	3.70[2]	9.61[2]	0.18[1]	0.89[2]	1.82[2]	2.78[2]	1.80[2]
Distension of rear quarters	*P*-value	<0.01	0.62	0.29	<0.01	0.13	0.72	0.18	<0.01	0.31	<0.01	0.55	0.04	0.18	0.80	0.19
	χ^2-test	9.71[1]	0.24[1]	1.11[1]	160[3]	2.35[1]	0.12[1]	1.78[1]	36.4[3]	10.6[3]	31.7[3]	0.35[1]	17.5[3]	12.7[3]	5.39[3]	12.4[3]
Teat perimeter	*P*-value	0.01	0.14	0.52	<0.01	0.02	0.57	0.05	<0.01	0.10	<0.01	0.97	0.61	0.55	0.22	0.43
	χ^2-test	6.83[1]	2.12[1]	0.40[1]	618[3]	5.49[1]	0.32[1]	3.71[1]	28.0[3]	14.8[3]	56.6[3]	0.001[1]	7.22[3]	7.87[3]	11.8[3]	9.04[3]
Front teat to floor distance	*P*-value	0.42	>0.99	0.96	<0.01	0.07	0.57	0.01	<0.01	0.91	<0.01	0.74	0.02	0.23	0.57	0.18
	χ^2-test	0.65[1]	<0.001[1]	0.002[1]	158[3]	3.27[1]	0.32[1]	6.94[1]	24.6[3]	4.08[3]	49.5[3]	0.11[1]	20.0[3]	11.7[3]	7.66[3]	12.6[3]
Rear teat to floor distance	*P*-value	0.28	0.96	0.88	<0.01	0.15	0.97	0.02	0.01	0.31	<0.01	0.27	0.36	0.85	0.50	0.33
	χ^2-test	1.15[1]	0.002[1]	0.02[1]	204[3]	2.04[1]	<0.001[1]	5.66[1]	21.9[3]	10.5[3]	38.3[3]	1.23[1]	9.83[3]	4.79[3]	8.31[3]	10.2[3]

[1] Degrees of freedom (numerator for F-test) = 1

[2] Degrees of freedom (numerator for F-test) = 13

[3] Degrees of freedom (numerator for F-test) = 9

Figure 1, Larsen et al.

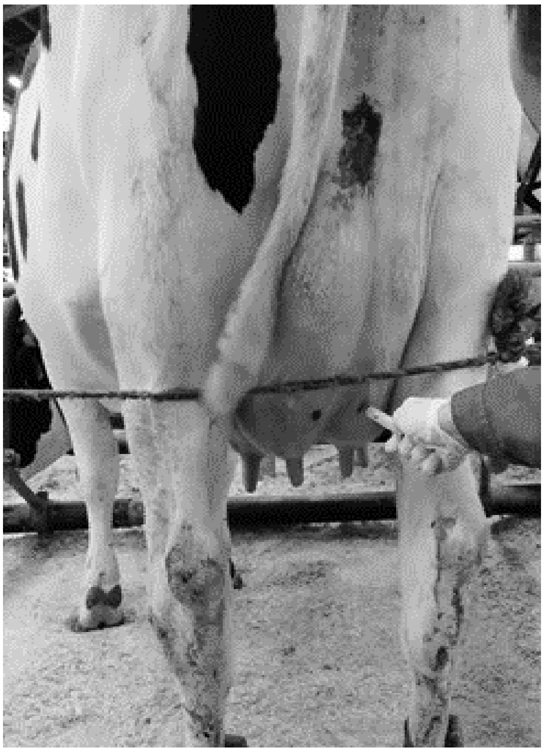

Figure 1. Representation of the measure "distension of rear quarters" used as proxy of udder engorgement. On the first udder examination, the experimenter marked one dot in the center of each rear quarter, approximately 10 cm above the teat base, using a permanent marker. Then, the experimenter measured the distension of rear quarters (i.e. the distance between the marks) with a ruler. Dots were reinforced daily.

Figure 2, Larsen et al.

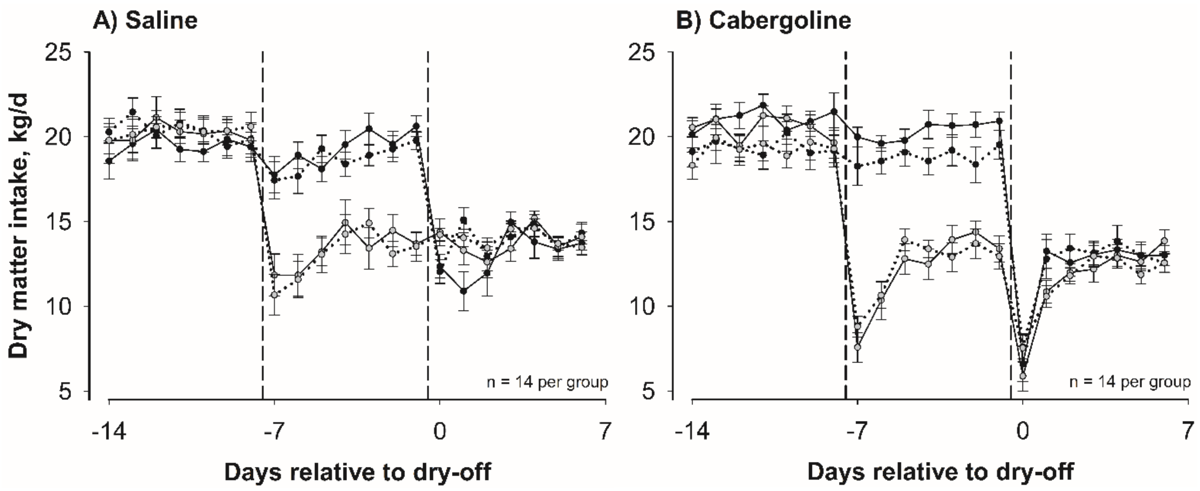

Figure 2. Total DMI in Holstein cows during baseline period (-14 to -8 d) and during 7 d before and after dry-off. From -7 to 0 d cows were ad libitum fed either a normal lactation (black) or a reduced lactation diet (grey) and either milked twice (solid line) or once (dotted line) daily, and injected with either saline (panel A) or cabergoline (panel B) 3 h after last milking in a 2 × 2 × 2 factorial arrangement of treatment factors. After dry-off, all cows were fed the same TMR for dry cows. Each data point is mean ± SE.

Figure 3, Larsen et al.

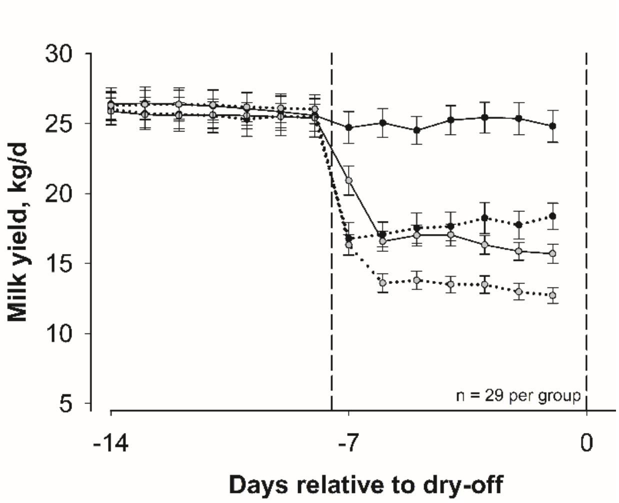

Figure 3. Milk yield in Holstein cows during baseline period (-14 to -8 d) and during the last 7 d before dry-off where ad libitum fed either a normal lactation diet (black) or a reduced lactation diet (grey) and either milked twice (solid line) or once (dotted line) daily in a 2 × 2 factorial arrangement of treatment factors. Each data point is mean ± SE.

Figure 4, Larsen et al.

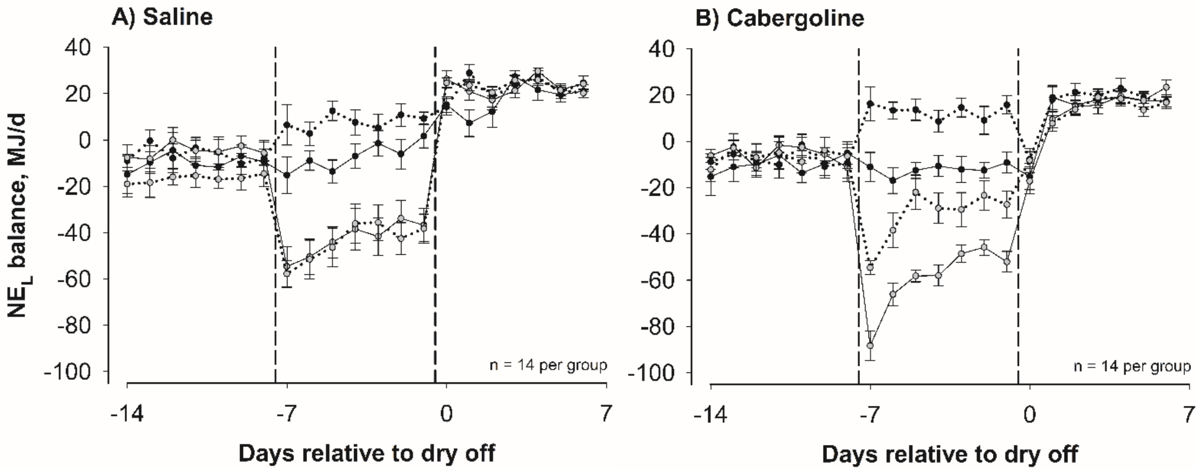

Figure 4. NE$_L$ balance in Holstein cows during baseline period (-14 to -8 d) and during 7 d before and after dry-off. From -7 to 0 d cows were ad libitum fed either a normal lactation (black) or a reduced lactation diet (grey) and either milked twice (solid line) or once (dotted line) daily, and injected with either saline (panel A) or cabergoline (panel B) 3 h after last milking in a 2 × 2 × 2 factorial arrangement of treatment factors. After dry-off, all cows were fed the same TMR for dry cows. Each data point is mean ± SE.

Figure 5, Larsen et al.

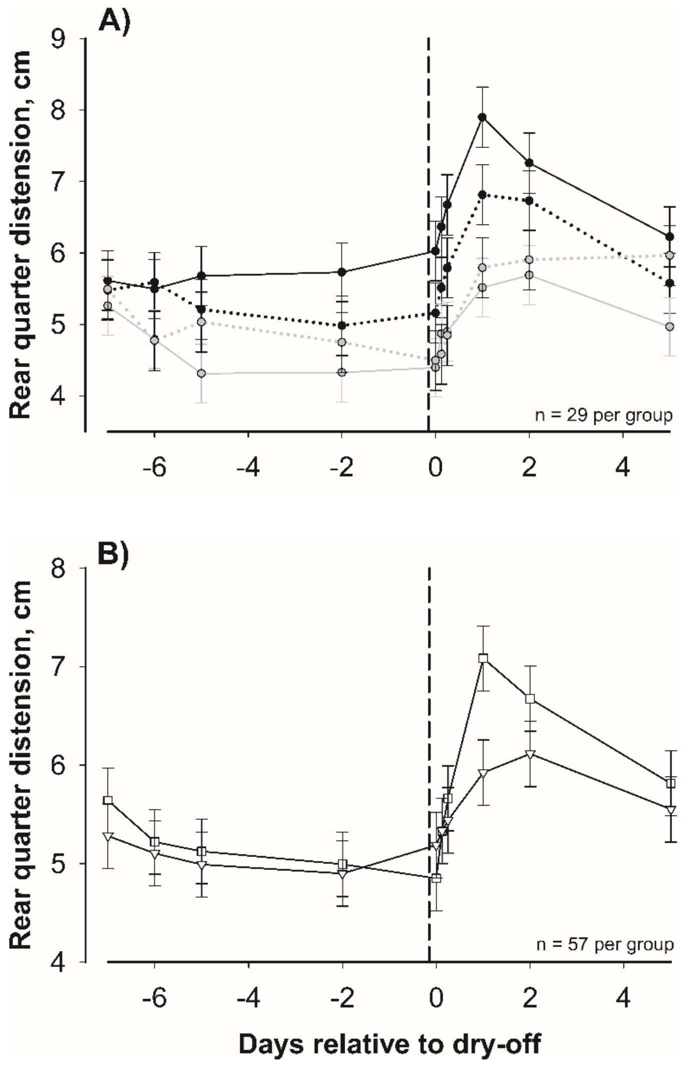

Figure 5. Distension of rear quarters during dry-off in Holstein cows used in a 2 × 2 × 2 factorial arrangement of treatment factors feeding level, milking frequency, and dopamine agonist. Panel A shows the 2 × 2 of diet and milking frequency with cows ad libitum fed either normal lactation (black) or reduced lactation diet (grey) and either milked twice (solid line) or once (dotted line) daily in the last 7 d before dry-off. Panel B shows the dopamine agonist with cows injected with either saline (squares) or cabergoline (triangles) 3 h after last milking. Each data point is LSM ± SEM.

Figure 6, Larsen et al.

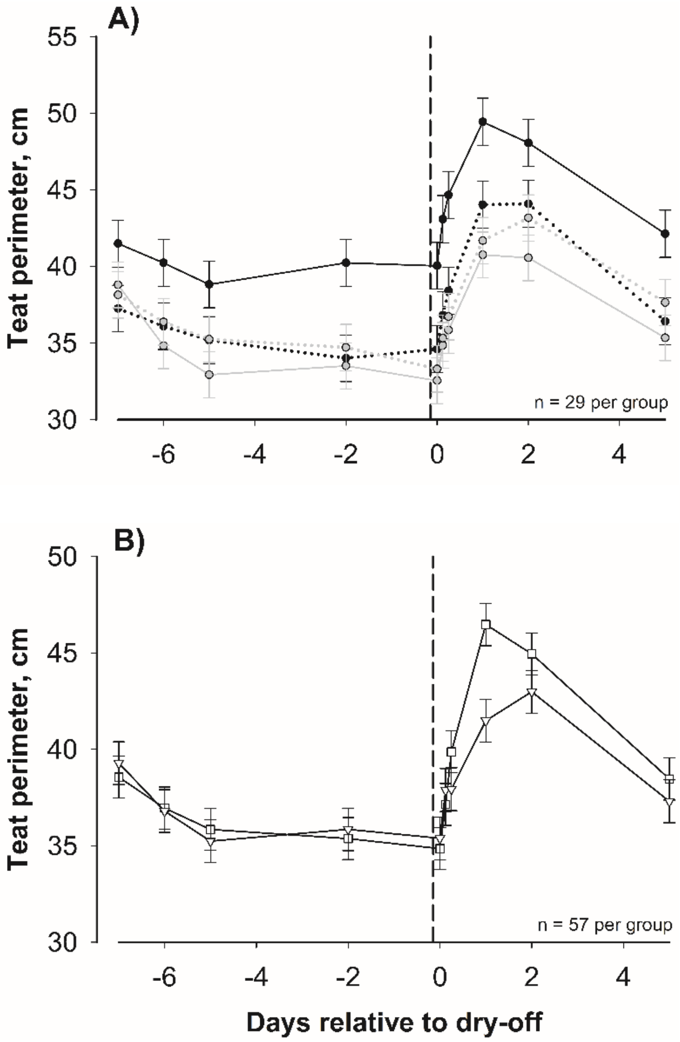

Figure 6. Teat perimeter during dry-off in Holstein cows used in a 2 × 2 × 2 factorial arrangement of treatment factors feeding level, milking frequency, and dopamine agonist. Panel A shows the 2 × 2 of diet and milking frequency with cows ad libitum fed either normal lactation (black) or reduced lactation diet (grey) and either milked twice (solid line) or once (dotted line) daily in the last 7 d before dry-off. Panel B shows the dopamine agonist with cows injected with either saline (squares) or cabergoline (triangles) 3 h after last milking. Each data point is LSM ± SEM.

Figure 7, Larsen et al.

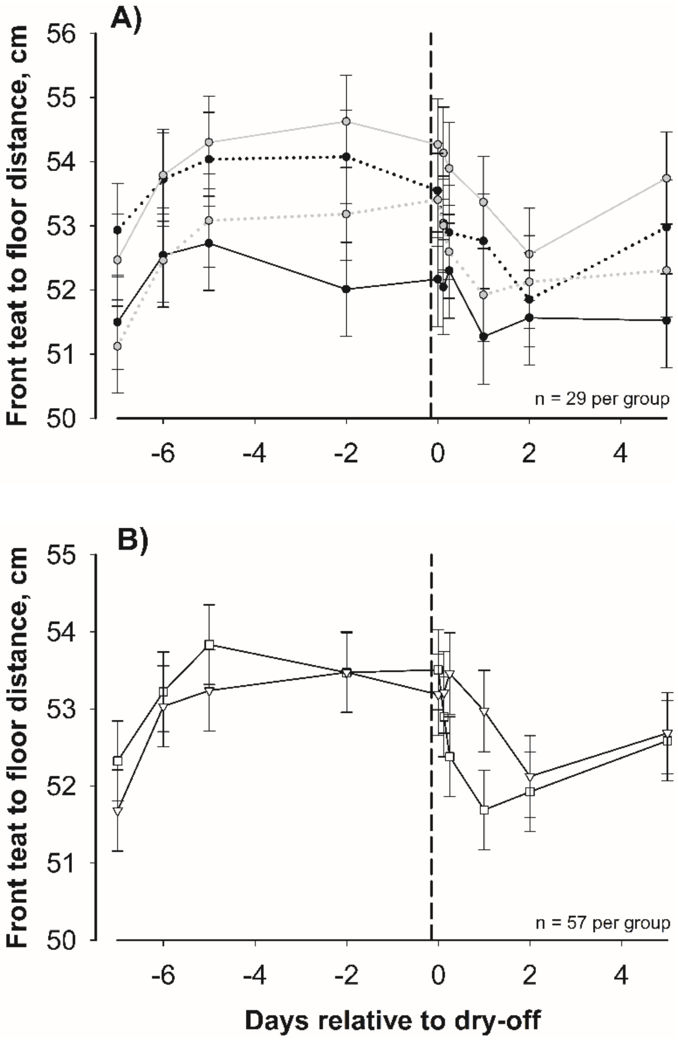

Figure 7. Distance between the shortest front teat end to cubicle floor during dry-off in Holstein cows used in a 2 × 2 × 2 factorial arrangement of treatment factors feeding level, milking frequency, and dopamine agonist. Panel A shows the 2 × 2 of diet and milking frequency with cows ad libitum fed either normal lactation (black) or reduced lactation diet (grey) and either milked twice (solid line) or once (dotted line) daily in the last 7 d before dry-off. Panel B show the dopamine agonist with cows injected with either saline (squares) or cabergoline (triangles) 3 h after last milking. Each data point is LSM ± SEM.

Figure 8, Larsen et al.

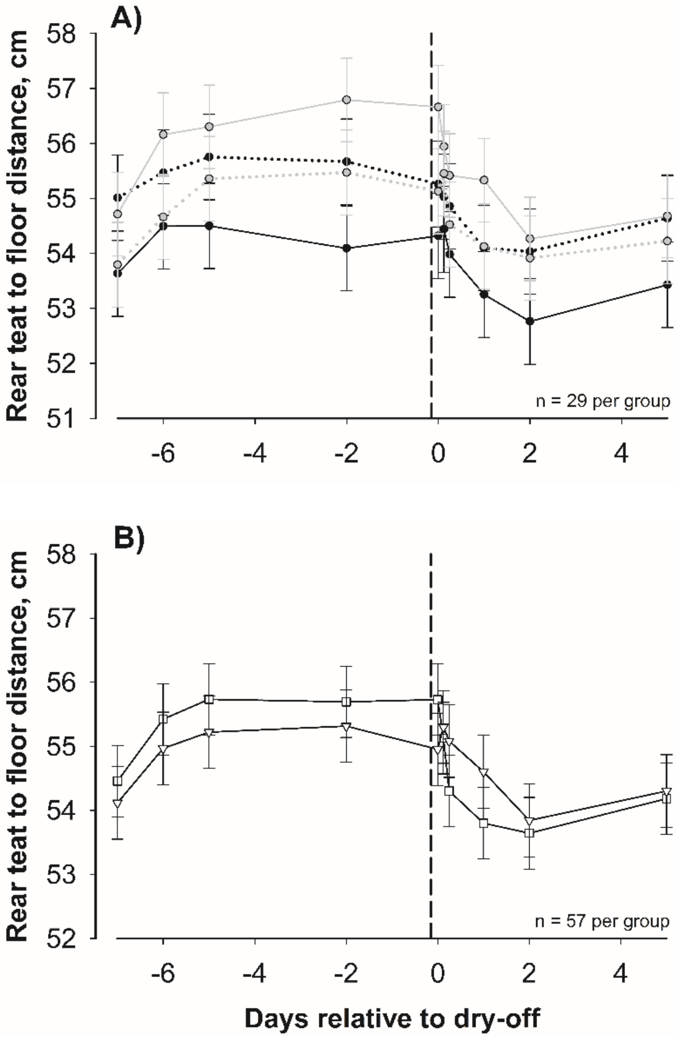

Figure 8. Distance between the shortest rear teat end to cubicle floor during dry-off in Holstein cows used in a 2 × 2 × 2 factorial arrangement of treatment factors feeding level, milking frequency, and dopamine agonist. Panel A shows the 2 × 2 of diet and milking frequency with cows ad libitum fed either normal lactation (black) or reduced lactation diet (grey) and either milked twice (solid line) or once (dotted line) daily in the last 7 d before dry-off. Panel B shows the dopamine agonist with cows injected with either saline (squares) or cabergoline (triangles) 3 h after last milking. Each data point is LSM ± SEM.

Figure 9, Larsen et al.

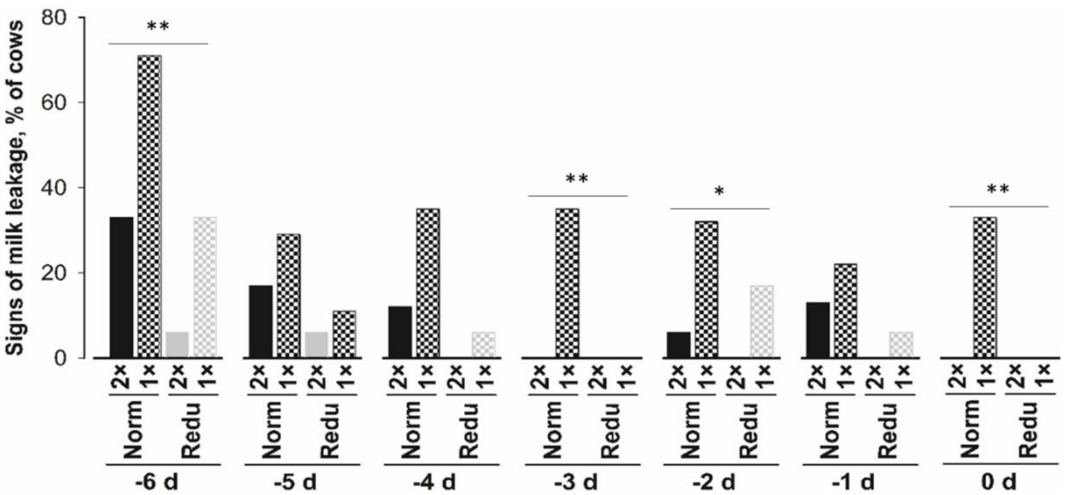

Figure 9. Signs of milk leakage before morning milking in Holstein cows ad libitum fed either a normal lactation diet (black) or a reduced lactation diet (grey) and either milked twice (solid bars) or once (checkered bars) daily in the last 7 d before dry-off in a 2 × 2 factorial arrangement of treatment factors. Each bar is the percentage of cows in each treatment where * and ** signify a statistical difference among treatments within day at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Figure 10, Larsen et al.

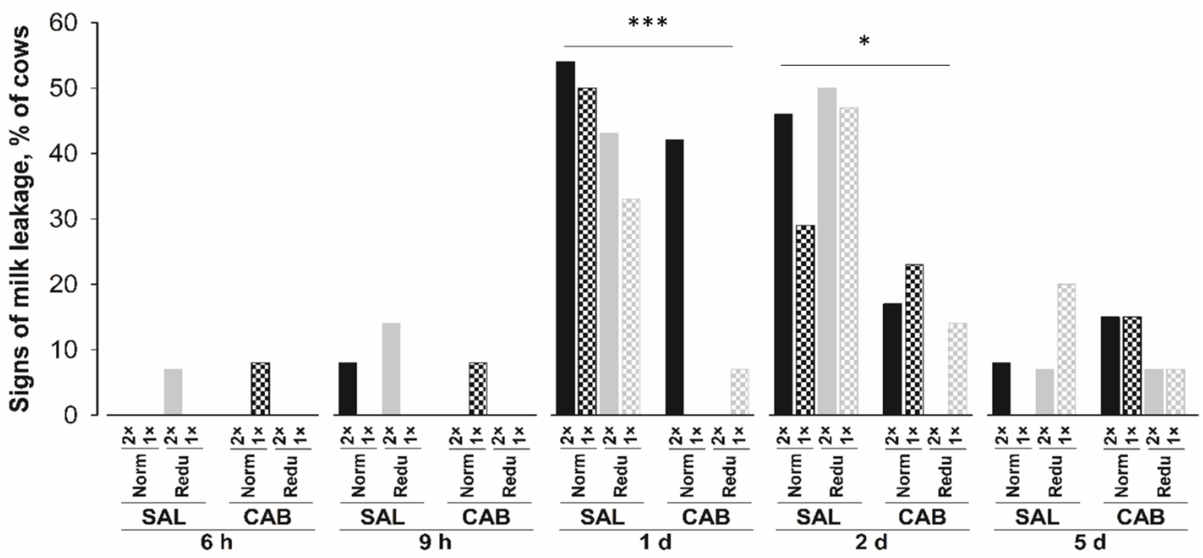

Figure 10. Signs of milk leakage from 6 h to 5 d after dry-off in Holstein cows ad libitum fed either a normal lactation diet (black) or a reduced lactation diet (grey) and either milked twice (solid bars) or once (checkered bars) daily in the last 7 d before dry-off, and injected with either saline (SAL) or cabergoline (CAB) 3 h after last milking in a 2 × 2 × 2 factorial arrangement of treatment factors. Each bar is the percentage of cows in each treatment where * and *** signify a statistical difference among treatments within day at $P \leq 0.05$ and $P \leq 0.001$, respectively.