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A randomized controlled trial assessing the effect of intermittent and abrupt cessation of milking to end lactation on the well-being and intramammary infection risk of dairy cows

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ABSTRACT

The objectives were to compare the effects of an intermittent milking schedule with a thrice daily milking schedule during the final week of lactation on the well-being, udder health, milk production, and risk of culling of dairy cows. We hypothesized that cows subjected to an intermittent milking schedule would experience less udder engorgement and pain, lower concentrations of fecal glucocorticoid metabolites (11,17-dioxoandrostanes; 11,17-DOA concentration) after dry-off, lower risk of an intramammary infection during the dry period, higher milk production and lower somatic cell count in the subsequent lactation, and lower culling risk compared with herd mates milked 3 times daily and dried off by abrupt cessation. In a randomized controlled field study, Holstein cows (n = 398) with a thrice daily milking schedule were assigned to treatment and control groups. The treatment consisted of an intermittent milking schedule for 7 d before dry-off (gradual cessation of milking, GRAD). Gradual-cessation cows were milked once daily until the day of dry-off, whereas cows in the control group (abrupt cessation of milking, APT) were milked 3 times daily until the day of dry-off. Udder firmness and pain responses of the udder 3 d after dry-off, as well as the percentage change in fecal 11,17-DOA concentration (3 d after dry-off compared with the dry-off day), were used to assess the well-being of the animals. Compared with cows in the GRAD group, the odds [95% confidence interval (CI)] of udder firmness were 1.55 (0.99–2.42) for cows in the APT group, and the odds of a pain response were 1.48 (0.89–2.44) for cows in the APT group. The least squares means (95% CI) of the percentage change in 11,17-DOA concentration were 129.3% (111.1–150.4) for the APT group and 113.6% (97.5–132.4) for the GRAD group. Quarter-level culture results from the periods before dry-off and after calving were compared, to assess the likelihoods of microbiological cure and new infection. Cows in the APT group had lower odds of a new intramammary infection in the dry period [odds ratio, 95% CI: 0.63 (0.37–1.05)], whereas we observed no meaningful differences in the microbiological cure likelihood among groups. The least squares means (95% CI) for somatic cell counts (log_{10}-transformed) were 4.9 (4.8–5.0) in the APT group and 4.9 (4.8–5.0) in the GRAD group. The odds (95% CI) of clinical mastitis in the first 30 d postcalving were 1.32 (0.53–3.30) in the APT group compared with the GRAD group. We observed no meaningful differences in milk production at the first test date postcalving or the culling risk among groups. We conclude that the gradual-cessation protocol tested herein failed to significantly improve animal well-being, udder health, milk production, and survival in the tested study cohort. However, the observed differences in udder firmness, as well as the numerical differences in udder pain and the percentage change in fecal 11,17-DOA concentrations suggest that this line of research may be useful. Future research is needed to develop drying-off strategies that are appropriate for lowering milk production at the end of the lactation and improve animal well-being without compromising udder health.

Key words: bovine, cortisol, dry-off, gradual cessation

INTRODUCTION

Despite widespread implementation of bovine mastitis control programs and extensive research within the last decades, mastitis continues to be one of the most frequent diseases in dairy cattle. Mastitis has well-recognized negative effects on animal welfare and on the profitability of the dairy industry (Ruegg, 2017). The

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dry period has long been recognized for its importance in the dynamics of IMI in dairy cattle. Cows are more susceptible to IMI during this time, and the risk of IMI is higher during the dry period than during lactation (Bradley and Green, 2004). High milk production at dry-off significantly increases the risk of IMI during the dry period not only by causing milk leakage but also by hindering the formation of the keratin plug, thus allowing open bacterial entry to the udder and exposing the mammary gland to pathogens for a longer period (Schukken et al., 1993; Rajala-Schultz et al., 2005).

Although the beneficial effects of decreased milk yield before dry-off have been well documented, few attempts have been made to reduce the level of milk yield before dry-off, and the optimal strategy to achieve this goal remains to be determined. In previous studies, gradual cessation of milking (**GRAD**) has been shown to decrease daily milk production during the last week of lactation and reduce the risk of new IMI during the dry period (Wayne and Macy, 1933; Bushe and Oliver, 1987; Oliver et al., 2009). Although these results support the use of GRAD to end lactation as an aid to improve udder health, only a limited number of recent studies have investigated its suitability in high-producing dairy cows (Gott et al., 2016; 2017; Rajala-Schultz et al., 2018). Consequently, gradual cessation has not been implemented in the field, and no definite protocol has been recommended to dairy producers. This lack of definite recommendation is reflected by several studies showing that abrupt cessation of milking (**APT**) is used on 74% of dairy operations in the United States (USDA, 2016), 73% of dairy farms in Germany (Bertulat et al., 2015), and 83% of farms in Scotland (Fujiwara et al., 2018).

However, APT results in increased intramammary pressure and consequent tissue damage and is associated with elevated fecal glucocorticoid metabolites (Bertulat et al., 2013); therefore, increasing concerns have been posed regarding the use of this method and animal well-being, which, in addition to antimicrobial use in food animals, has become a major public concern (Zobel et al., 2015). The effect of gradual cessation on fecal glucocorticoid metabolites, an indirect index of stress or pain, has not yet been studied. Such knowledge would offer a unique opportunity to help the industry develop drying-off protocols that result in improved udder health and animal well-being (Palme, 2019). Therefore, the objectives of this study were to investigate the effect of 2 different drying-off protocols (GRAD vs. APT) on the well-being, udder health, and milk production in subsequent lactation of dairy cows. We hypothesized that cows subjected to an intermittent milking schedule (i.e., GRAD) during the final week of lactation would experience less udder engorgement and pain, exhibit lower concentrations of fecal glucocorticoid metabolites (11,17-dioxoandrostanes; **11,17-DOA**) after dry-off, have lower risk of IMI at the quarter level during the dry period, and exhibit higher milk production and lower SCC in the subsequent lactation compared with herd mates milked 3 times daily and dried off by abrupt cessation of milking. Additionally, we examined the effect of these 2 drying-off protocols on the risk of later removal from the herd.

MATERIALS AND METHODS

This randomized controlled field trial was conducted between February 2020 and April 2021 on a commercial dairy farm in New York. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Cornell University (protocol no. 2018–0079). A supporting letter from the farm owner was obtained before the start of the study.

Animals and Housing

The lactating herd consisted of approximately 1,600 Holstein cows housed year-round in freestall pens with concrete stalls covered with mattresses and bedded with wastepaper-pulp or dried sawdust (7 pens), or stalls deep-bedded with fresh sand (2 pens). Cows were fed a TMR formulated according to NRC requirements (NRC, 2001). The key herd performance indicators were average milk production (12,626 kg), bulk tank SCC (213,000 cells/mL), monthly clinical mastitis incidence (6.0%), 21-d pregnancy rate (26.0%), and culling risk (36.0%). All cows were milked 3 times per day in a 60-stall rotary milking parlor. Before the study, the milking machine settings were assessed by the investigators according to the National Mastitis Council guidelines (NMC, 2012). The farm used DHIA services, including bimonthly SCC and milk yields, and a dairy management software program (Dairy Comp 305, Valley Agricultural Software) to record mastitis and culling events.

Treatment Allocation

Cows eligible for dry-off according to the dairy farm's guidelines (first lactation cows pregnant for >220 d; ≥second lactation cows pregnant for >226 d; or all lactation cows with average daily milk production <16.3 kg and pregnant for >150 d) were included. One week before their designated dry-off day, eligible cows were randomly assigned to treatment and control groups stratified by average daily milk production and SCC using a random number generator (Urbaniak and Plous, 2013) by the first author (MW). The treatment consisted of an intermittent milking schedule for 7 d before dry-off (GRAD). Gradual-cessation cows were milked once daily, whereas cows in the control group (APT) were milked 3 times daily as usual until the day of dry-off. Gradual-cessation cows were not separated from the main milking herd and went through the parlor during every milking. To facilitate adherence to the intermittent milking schedule for these cows, they were identified with pink-colored leg bands on both hind legs. Milking technicians were instructed to milk those cows only once daily during the night milking shift. We used the data from the dairy farm management software AfiFarm (Afimilk) to monitor adherence to the modified milking schedule for gradual-cessation cows. All study cows received the same TMR, which consisted of the farm's normal lactating ration.

The study farm had a designated weekly dry-off day. The farm's standard procedures, including selection of intramammary dry-cow antibiotics and use of internal teat sealant, were not altered, although sampling procedures were accommodated. During the morning milking shift, dry-off cows were separated from their herd mates after exiting the rotary parlor with automatic sorting gates and held in a pen to await dry-off procedures. The dry-off procedures were as follows: after cows were moved to the parlor, subjected to premilking teat sanitization and stimulation, and milked, sterile-quarter milk samples were obtained by study personnel according to the *Procedures for Collecting Milk Samples* outlined by the National Mastitis Council (NMC, 2004). Briefly, teat ends were scrubbed vigorously with individual gauze swabs moistened with 70% alcohol until no more dirt appeared on the swab. Following forestripping to discard several streams of milk, 1–3 streams of milk were collected in a sterile vial and promptly placed on ice. Immediately after milk sample collection, all cows received 1 tube of intramammary dry-cow antibiotic (400 mg of Novobiocin sodium and 200,000 IU of Penicillin G procaine, Albadry Plus, Zoetis) administered into each lactating quarter according to label directions. After the application of the intramammary dry-cow antibiotic, an internal teat sealant (2.6 g of bismuth subnitrate, ShutOut, Merck & Co. Inc.) was administered into each teat according to label directions. Subsequently, an iodine-based teat disinfectant (Eco-Plus 50 S. A., Ecolab) was applied to all teats with a teat dip applicator cup. The pink-colored leg bands were removed from gradual-cessation cows, and a red leg band was placed on each hind leg of every cow (i.e., both gradual-cessation and abrupt-cessation cows). All dry-off procedures were performed by farm personnel. Subsequently, cows were moved to the palpation rail, where a fecal sample from each cow was collected by study personnel as previously described (Bertulat et al., 2013). Briefly, approximately 50 to 80 g of feces was obtained manually from the rectum using disposable obstetrical sleeves (VetOne OB Sleeves, #603042, MWI), placed into sample vials (90 mL, #244010, Parter Medical Products Inc.), stored on ice immediately, transported, and frozen at −20°C until analysis. Finally, cows were moved to the dry-cow pen.

Three days after dry-off, a second fecal sample was obtained from each cow following the procedure described above. In addition, udder firmness, udder pain, and milk leakage were assessed by 1 investigator blinded to the treatment groups. To facilitate fecal sampling and examinations, cows were penned in their stalls by attaching a rope between the stall partitions that prevented them from leaving. Udder firmness was evaluated by palpation using a 4-point ordinal scale (1, soft; 2, slightly firm; 3, firm; and 4, extremely firm) in accordance with Rees et al. (2014). Briefly, palpation was performed by pressing all fingers of 1 hand (except the thumb) into the tissue of both hind mammary glands at the middle aspects between the caudal base of the udder and the hind teats. Each hind mammary gland was palpated once, and the highest score was documented. For subsequent analyses, we binarized this variable as follows: scores of 1 and 2, udder firmness absent; and scores of 3 and 4, udder firmness present. The presence or absence of udder pain was assessed during udder firmness estimation and defined as present if a cow exhibited avoidance behavior such as flinching, vocalizing, raising their leg, or swishing their tail as previously described by Bertulat et al. (2017). Milk leakage was assessed visually and defined as present if milk was dripping from 1 or more teats at the time of examination, as previously described (Zobel et al., 2013; Bach et al., 2015; Bertulat et al., 2017).

In the subsequent lactation following the dry period, sterile quarter-level milk samples were obtained between 2 and 8 DIM after milking in the rotary parlor in accordance with the procedures outlined above. Hereafter, the 2 milk samples are referred to as the dry-off sample and fresh sample (i.e., from the subsequent lactation).

Microbiological Examination

Upon sampling, milk samples were transported on ice to the Animal Health Diagnostic Center (Cornell University, Ithaca, NY) for microbiological examination. Standard culture techniques of milk samples were performed according to the *Laboratory Handbook on Bovine Mastitis* (NMC, 2017). Briefly, samples were plated on tryptic soy agar with 5% sheep blood and 0.1% esculin (Northeast Laboratory Services) using a disposable loop (10-μL inoculating loops, #12000–810, VWR International) for samples processed from February 26 to November 30, 2020, or a cotton swab (Puritan Medical Products Co.; cotton swab dimensions, 16 × 5 mm) for samples processed from December 1 to January 29, 2021, resulting in approximately 0.01 mL and 0.03 mL of milk streaked per plate, respectively. Plates were incubated aerobically at 37°C and were read after 18 to 24 h and again after 48 h. Mixed growth was considered if the sample grew 2 distinct organisms. From pure cultures (i.e., growth of 1 distinct organism from the sample) and cultures with mixed growth (i.e., growth of 2 phenotypically different colony types from the sample), a representative colony was subjected to MALDI-TOF using MALDI Biotyper Microflex LT (Bruker Daltonics). Adaptation and maintenance of the MALDI-TOF library, sample preparation, and interpretation of the MALDI score were performed according to Randall et al. (2015). Briefly, the instrument reports a logarithmic score between 0 and 3 that quantifies the similarity to known database entries (i.e., MALDI Biotyper Library #8468, Bruker). A score ≥1.8 was the threshold for identification at the genus level, and a log (score) ≥2.0 was used as the threshold for species identification. If ≥3 phenotypically different types of colonies were present, the sample was considered contaminated, and no MALDI-TOF analysis was performed. Culture results were interpreted according to Dohoo et al. (2011). A quarter was defined as infected if ≥1 colony was isolated from 0.01 mL of the milk sample for all pathogens except non-*aureus Staphylococcus* spp. For non-*aureus Staphylococcus* spp., isolation of ≥2 colonies from 0.01 mL of milk was necessary to establish the presence of an infection.

A quarter was eligible for the analysis of new IMI if the culture from the dry-off sample yielded no growth or only 1 organism. We excluded quarters if the dry-off or fresh sample exhibited mixed growth or was contaminated. A new IMI in a quarter was defined as isolation of an organism from the fresh sample that was not present in the dry-off sample. Quarters that yielded growth of the same organism at both sampling times (i.e., dry-off and fresh samples) were included but not defined as new IMI. A quarter was eligible for bacteriological cure analysis if 1 organism was isolated from the dry-off sample. A quarter was not eligible for bacteriological cure analysis if the dry-off sample exhibited no growth or the dry-off or fresh sample exhibited mixed growth or was contaminated. We defined bacteriological cure of a quarter as the absence of the organism isolated from the dry-off sample in the fresh sample. Quarter-level data from cows that were culled or died during the dry or postcalving period or were lost to follow-up for both new IMI and bacteriological cure for any reason after calving excluded.

Fecal Glucocorticoid Metabolites

For extraction of the fecal glucocorticoid metabolites, samples were thawed at 4°C and stirred vigorously. Subsequently, 0.5 g of feces was weighed (MXX-123, Denver Instrument Inc.), added to 15-mL tubes (15-mL centrifuge tube, #430791, Corning Science Mexico), and dispersed in 5 mL of 80% methanol (MX0490–4, EMD Millipore Corporation). The samples were then vortexed for 30 min using a multitube vortexer (#02–215–450, Thermo Fisher Scientific) and subsequently centrifuged at 2,500 × *g* for 15 min at 20°C (TJ-6, Beckman Coulter Inc.). Finally, a 0.5-mL aliquot of the supernatant was transferred into a 1.7-mL tube (VWR Microcentrifuge Tubes, #87003–294, VWR International) and dried at 60°C with a heat block (Dry Bath, #BSH1002, Benchmark Scientific Inc.) until further analysis. To determine the concentration of 11,17-DOA, a group of fecal cortisol metabolites, an 11-oxoetiocholanolone enzyme immunoassay validated for use in cattle (Palme et al., 1999) was performed as previously described (Palme and Möstl, 1997). Concentrations of 11,17-DOA are presented in nanograms per gram of fresh feces.

Culling, Clinical Mastitis, Milk Production, and SCC

Study cows were followed up for removal from the herd before 30 DIM, clinical mastitis events before 30 DIM, and milk production and SCC at first test date after calving. Culling events were recorded by farm personnel in DairyComp 305. Clinical mastitis was detected by farm personnel during premilking udder preparations. A cow was defined as having clinical mastitis if milk from 1 or more quarters was abnormal with or without signs of local inflammation of the affected quarter as previously described (Erskine et al., 2003). Clinical mastitis events were also recorded in DairyComp 305 by farm personnel. Milk production and SCC at the first test after calving were assessed by DHIA services. Mastitis and culling data were retrieved from DairyComp 305.

Sample Size Calculation

The sample size calculation was based on the risk of new IMI at freshening, which has a documented prevalence of 6.4 to 25% at the quarter level (Godden et al., 2003; Pantoja et al., 2009; Arruda et al., 2013). Using a prevalence of 25%, a significance level of 0.05, a power of 0.95, and the ability to detect a 15% change in risk, the sample size was calculated to be 604 quarters (151 cows). To account for the clustering of quarters within cows, an adjustment to the original sample size was made with the formula $n' = n[1 + \rho(m - 1)]$ as described by Dohoo et al. (2009), where n' equals the adjusted sample size, n equals the original sample size, ρ equals the intraclass correlation coefficient, and m equals the cluster size. Using an intraclass correlation coefficient of 0.25 resulted in an adjusted sample size of 1,060 quarters (265 cows). After accounting for a 25% loss due to an assumed IMI at dry-off (at the quarter level), the sample size was 1,328 quarters (332 cows). The adjusted sample size of 332 cows was then inflated by a factor of 0.20 because of an assumed attrition rate of 20%, resulting in a pre-exclusion sample size of 400 cows. This calculation was based on a presumed coefficient of determination value of 0.45 and an equal sample proportion [G*Power version 3.1.9.2 (Faul et al., 2007)]. The resulting sample size of 400 cows was sufficient to detect a minimum difference of 75% increase in 11,17-DOA concentration relative to the baseline concentrations between the 2 groups at a significance level of 0.05 with a power of 96%. This calculation was based on a 2-tailed test and a presumed standard deviation of 200%.

Analytical Approach

We compiled the data in Excel (Microsoft Office Excel 2019; Microsoft Corp.) and JMP (version 14, SAS Institute Inc.). For subsequent analyses, SCC values were $\log_{10}$-transformed. Hereafter, $\mathbf{logSCC_0}$ refers to the $\log_{10}$-transformed SCC value from the last test before enrollment, and $\mathbf{logSCC_1}$ refers to the $\log_{10}$-transformed SCC value from the first test date after calving.

Descriptive Statistics

Differences in the baseline characteristics between groups were assessed with Pearson's chi-squared test (for categorical variables) and with Student's *t*-tests (for continuous variables) in JMP. All subsequent analyses were performed with SAS (version 9.4, SAS Institute Inc.).

Udder Firmness, Udder Pain, and Milk Leakage

To assess the effect of treatment on the binary outcome variables udder firmness, udder pain, and milk leakage, we fitted 3 separate generalized linear models with a logit link and a binomial distribution with PROC LOGISTIC. The following steps were consistent for all 3 models. Treatment was added to the model as a fixed effect. The following independent variables were considered for inclusion in each model and screened through univariate analysis: lactation number at enrollment (first, second, or ≥third lactation), stage of lactation at enrollment, average daily milk production at enrollment, and $logSCC_0$. We included all variables with a P-value < 0.20 from this step in the initial model. Collinearity among eligible variables was assessed by calculating Spearman r with PROC CORR. We considered a coefficient of $>|0.60|$ as indicative of collinearity. We performed manual backward elimination until each of the variables had a P-value < 0.05 and assessed confounds by observing regression coefficient changes. Variables that modified regression coefficients by >20% were considered confounding factors. Two-way interactions between treatment and the remaining variables were tested individually, and a variable was retained in the final model if the P-value < 0.05. We calculated adjusted probabilities (95% CI) using the LSMEANS statement. We used Pearson goodness-of-fit statistics to assess the fit of the final model. To assess the association of udder firmness with pain response and milk leakage, we used Pearson's chi-squared test.

Fecal Glucocorticoid Metabolites

To determine differences in the percentage change in fecal 11,17-DOA concentrations among groups, we fitted a general linear model with PROC GLM. Treatment was added to the model as a fixed effect. Lactation number at enrollment (first, second, or ≥third lactation), stage of lactation at enrollment, average daily milk production at enrollment, and $logSCC_0$ were considered independent variables and screened for inclusion initially through univariate analysis. All variables with a P-value < 0.20 from this step were included in the initial model. Collinearity among eligible variables was assessed by calculating Spearman r in PROC CORR and considered present if the coefficient was $>|0.60|$. Manual backward elimination was used to reach the final model. We assessed the assumptions of homoscedasticity and normality of residuals of the final model by the inspection of residual plots versus corresponding predicted values and the examination of quantile–quantile residual plots. Finally, we assessed the associations of udder pressure and the occurrence of a pain response with the percentage change in 11,17-DOA concentration (log-transformed) with ANOVA and Student's *t*-tests, respectively.

Culling and Mastitis Events

To determine the effect of treatment on rates of culling and clinical mastitis incidence, we fitted 2 separate generalized linear models with a logit link and a binomial distribution in accordance with the procedure

Table 1. Baseline characteristics of 398 Holstein cows subjected to abrupt (APT) or gradual (GRAD) cessation of milking at the end of lactation[1]

Item	APT	GRAD	Total	*P*-value
Lactation number[2] (n, %)				0.13
First	75 (38.1)	95 (47.3)	170 (42.7)	
Second	43 (21.8)	43 (21.4)	86 (21.6)	
≥Third	79 (40.1)	63 (31.3)	142 (35.7)	
DIM at the day of enrollment (d)	311 ± 39	309 ± 34	309 ± 37	0.53
$\log SCC_0$[3]	4.9 ± 0.51	4.9 ± 0.50	4.9 ± 0.50	0.50
Average daily milk production[4] (kg)	24.7 ± 8.4	25.7 ± 8.7	25.2 ± 8.5	0.23

[1]Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. The results are presented as the mean and SD unless otherwise stated. *P*-values were derived from Pearson's chi-squared test for the categorical variable of lactation number and from Student's *t*-tests for the continuous variables.

[2]Lactation number at the time of enrollment.

[3]$\log SCC_0$: SCC from the last test before enrollment, $\log_{10}$-transformed.

[4]Daily average milk production for the last 7 d before enrollment.

outlined above. In addition to lactation number at enrollment (first, second, or ≥third lactation), stage of lactation at enrollment, average daily milk production at enrollment, and $\log SCC_0$, we also considered milk production at the first test day after calving, as well as $\log SCC_1$, for inclusion.

Milk Production and SCC

To compare differences in milk production and SCC at the first test date after calving, we fitted 2 separate general linear models with PROC GLM in accordance with the protocol outlined above, except for the following item. For the dependent variable of milk production, we considered DIM at the first test date and $\log SCC_1$ for inclusion in addition to the lactation number at enrollment (first, second, or ≥third lactation), average daily milk production at enrollment, and $\log SCC_0$.

Bacteriological Cure and New IMI

To investigate the effect of treatment on bacteriological cure and new IMI at the quarter level, we fitted 2 separate generalized linear mixed models with PROC GLIMMIX. We included cow as a random effect to account for the clustering of quarters within a cow. Treatment was added to both models as a fixed effect. We considered lactation number at the time of enrollment (for the dependent variable of bacteriological cure), average daily milk production at enrollment, $\log SCC_0$, and the dry period length for inclusion in the model and screened them through univariate analysis. Variables with $P < 0.20$ from this step were included in the initial model. Manual backward elimination was performed until each of the variables had a *P*-value < 0.05. Based on results from Gott et al. (2016), who found that lactation number modified the effect of gradual cessation on new IMI, we added lactation number to the model and tested the interaction between treatment and lactation number for the dependent variable of new IMI.

RESULTS

Description of the Study Population

A total of 398 (APT, n = 197; GRAD, n = 201) cows were enrolled in the study. At the time of enrollment, cows were in their first (170, 42.7%), second (86, 21.6%), or third or greater lactation (142, 35.7%). Table 1 shows the baseline characteristics stratified by group. We observed no differences in lactation number, DIM at the day of enrollment, $\log SCC_0$, or average daily milk production in the week before enrollment ($P \geq 0.07$). Seven cows (APT, n = 2; GRAD, n = 5) were excluded during the treatment week because of clinical mastitis. Two cows (APT, n = 2; GRAD, n = 0) were sold during the treatment week. Three cows in the GRAD group were excluded due to lack of compliance. Three cows were missed on the dry-off day and not dried (APT, n = 0; GRAD, n = 3). Twenty cows (APT, n = 10; GRAD, n = 10) were excluded due to COVID-19 restrictions. This resulted in a total of 363 cows (APT, n = 183; GRAD, n = 180) that were enrolled and successfully entered the dry period. Among the 363 cows that entered the dry period, 5 cows (APT, n = 3; GRAD, n = 2) died or were sold during the dry period. This resulted in a total of 358 cows (APT, n = 180; GRAD, n = 178) that entered the next lactation.

Udder Firmness, Pain Response, and Milk Leakage

Data from 2 cows in the GRAD group were missing. Thus, data from 361 cows (APT, n = 183; GRAD, n

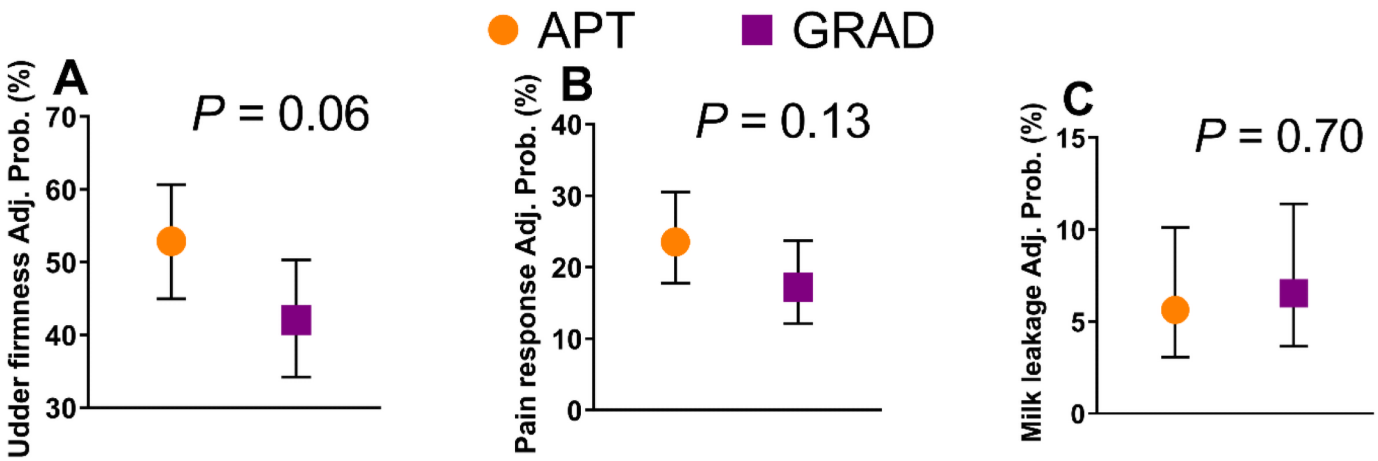

Figure 1. Adjusted probabilities (Adj. Prob.) from generalized linear models showing the effect of gradual cessation (GRAD) versus abrupt cessation (APT) of milking at the end of lactation on udder firmness (A), udder pain (B), and milk leakage (C) 3 d after dry-off. Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. Error bars show 95% CI.

= 178) were available for analyses of udder firmness, udder pain, and milk leakage. The results of generalized linear models for each outcome variable are described separately below. Adjusted probabilities are shown in Figure 1.

The assessment of udder firmness in the APT and GRAD groups had the following frequency distributions: score 1, 6 (3.3%) and 11 (6.2%) cows; score 2, 79 (43.2%) and 84 (47.2%) cows; score 3, 68 (37.2%) and 59 (33.2%) cows; and score 4, 30 (16.4%) and 24 (13.5%) cows, respectively. Using udder firmness as a binary variable with scores 1 and 2 as no firmness present and scores 3 and 4 as firmness present, the final model included lactation number at dry-off (P = 0.04), average daily milk production at enrollment ($P < 0.0001$), and treatment ($P = 0.06$). Compared with cows in their third or higher lactation, the odds ratio (**OR**) (95% CI) of udder firmness at 3 d after dry-off was 0.97 (0.57–1.64) for first lactation animals and 0.47 (0.25–0.87) for second lactation animals. A 1-kg increase in average daily milk production at enrollment increased the odds of udder firmness by 8% [OR, 95% CI: 1.08 (1.05–1.12)]. Compared with the GRAD group, cows in the APT group had an OR (95% CI) of udder firmness of 1.55 (0.99–2.42).

A pain response was observed in 46 (25.1%) and 33 (18.5%) cows in the APT and GRAD groups, respectively. The final model included lactation number (P = 0.22), which was retained in the model to achieve model fit, and treatment ($P = 0.13$). The OR (95% CI) of a pain response at 3 d after dry-off for cows in the APT group was 1.48 (0.89–2.44) relative to the GRAD group. Among the 180 cows without udder firmness, 15 (8.3%) showed a pain response, whereas a pain response was documented in 64/181 (35.4%) cows with udder firmness. Udder firmness was associated with a pain response (Pearson chi-squared test, $P < 0.0001$).

Milk leakage was observed in 12 (6.6%) and 15 (8.4%) cows in the APT and GRAD groups, respectively. The final model included average daily milk production at enrollment ($P = 0.001$) and treatment ($P = 0.70$). The odds of milk leakage increased by 9% for each 1-kg increase in average daily milk production at enrollment [OR (95% CI): 1.09 (1.04–1.15)]. Compared with cows in the GRAD group, the OR (95% CI) of milk leakage in the APT group was 0.85 (0.38–1.91). Pearson goodness-of-fit statistics for the final 3 models revealed $P \geq 0.25$. Thus, we accepted the null hypothesis that the models fit the data. We documented milk leakage in 7/180 (3.9%) cows without udder firmness, whereas among the 181 cows with udder firmness, 20 (11.1%) exhibited milk leakage. The Pearson chi-squared test revealed an association between udder firmness and milk leakage ($P = 0.008$).

Fecal Glucocorticoid Metabolites

Among the 363 cows entering the dry period, fecal samples from 6 cows (APT, n = 3; GRAD, n = 3) were missing. Thus, a total of 357 (APT, n = 180; GRAD, n = 177) paired samples were available for analyses. The average (mean ± SD) concentrations of fecal 11,17-DOA for the APT and GRAD groups were 53.2 ± 30.5 and 57.8 ± 27.5 ng/g, respectively, at dry-off and 120.4 ± 59.6 and 128.2 ± 65.4 ng/g at 3 d after dry-off. The mean ± standard deviation (SD) percentage change in

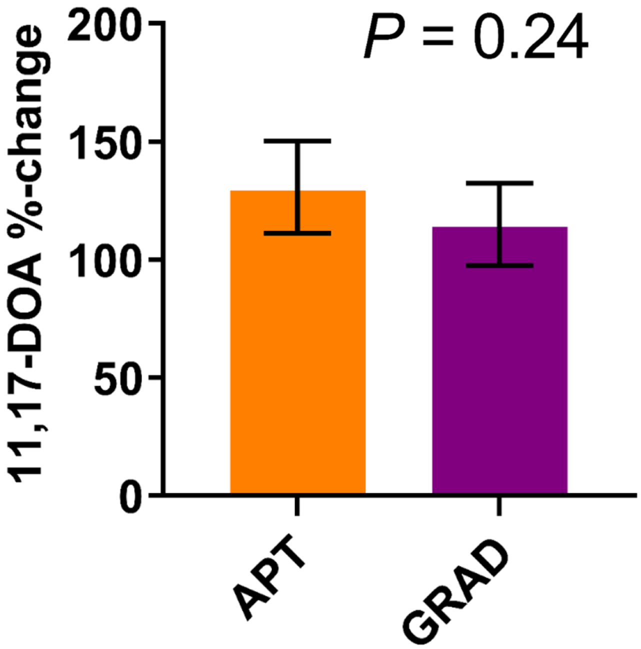

Figure 2. Least squares means from the general linear model showing the effect of gradual cessation of milking (GRAD) versus abrupt cessation of milking (APT) at the end of lactation on the relative change in the concentration of fecal glucocorticoid metabolites (11,17-dioxoandrostane; 11,17-DOA). Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. Error bars show 95% CI.

11,17-DOA concentrations was 173.8 ± 174.1% in the APT group and 156.0 ± 149.9% in the GRAD group. The final model included stage of lactation at enrollment ($P = 0.02$) and treatment ($P = 0.24$). To satisfy the assumption of homoscedasticity and normality of residuals, the dependent variable of percentage change in 11,17-DOA concentrations was log-transformed using the natural logarithm. Least squares means (LSM) and corresponding 95% confidence intervals (CI) were subsequently back-transformed. The LSM (95% CI) for the percentage change in 11,17-DOA concentrations were 129.3% (111.1–150.4) for the APT group and 113.6% (97.5–132.4) for the GRAD group (Figure 2).

The percentage change in 11,17-DOA concentrations (mean ± SD) among the 4 scores of udder firmness were 191.1 ± 40.6% (score 1), 182.3 ± 12.7% (score 2), 144.9 ± 124.4% (score 3), and 151.9 ± 135.2% (score 4). The ANOVA yielded no association between udder firmness and 11,17-DOA concentrations ($P = 0.20$). The percentage change in 11,17-DOA concentrations (mean ± SD) for cows with and without a pain response was 168.4 ± 170.0% and 153.2 ± 133.4%, respectively. We observed no association between the occurrence of a pain response and the percentage change in 11,17-DOA concentrations (Student's *t*-test, $P = 0.68$).

Bacteriological Cure and New IMI

Data from 4 (APT, n = 3; GRAD, n = 1) out of 358 cows that entered the subsequent lactation were lost because no dry-off sample was collected. Six cows (APT, n = 3; GRAD, n = 3) died during the early fresh period before postcalving milk samples were obtained. Furthermore, no postcalving samples were collected from 135 (APT, n = 70; GRAD, n = 65) cows due to COVID-19 restrictions. Thus, 213 cows (APT, n = 104; GRAD, n = 109) were subjected to sampling at dry-off and postcalving.

Among the 213 cows, 10 cows (APT, n = 5; GRAD, n = 5) had a nonlactating quarter. Sixty samples (dry sample, 33; fresh sample, 27) were contaminated and thus excluded. Additionally, the results from 1 fresh cow sample were missing. Thus, samples from 781 quarters of 213 cows were available for analyses. Tables 2 and 3 show the distributions of culture results from dry-off and postcalving, respectively.

Ninety-three quarters met the inclusion criteria and were used for the analyses. A quarter was not eligible for bacteriological cure analysis (multiple listings possible) if the dry-off sample did not exhibit growth (n = 681) or if the dry-off or fresh sample exhibited mixed growth (n = 11). We documented bacteriological cure in 91 (97.9%) of the quarters; of these, 46 (97.9%) were from cows in the APT group and 45 (97.8%) were from cows in the GRAD group. The final model included

Table 2. Quarter-level culture results at dry-off for 781 quarters from cows subjected to abrupt (APT, n = 382) or gradual (GRAD, n = 399) cessation of milking at the end of lactation[1]

Item	APT (n, %)	GRAD (n, %)	*P*-value[2]
Negative	331 (86.6)	350 (87.7)	0.67
Staphylococcus spp.	31 (8.1)	30 (7.5)	0.79
Staphylococcus aureus	3 (0.8)	4 (1.0)	>0.99
Lactococcus spp.	5 (1.3)	1 (0.3)	0.12
Mixed growth	3 (0.8)	3 (0.8)	>0.99
Streptococcus dysgalactiae	2 (0.5)	2 (0.5)	>0.99
Enterococcus spp.	2 (0.5)	2 (0.5)	>0.99
Pseudomonas spp.	2 (0.5)	0 (0)	0.24
Prototheca spp.	1 (0.3)	0 (0)	0.49
Other[3]	2 (0.5)	7 (1.8)	0.18

[1]Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. Etiological classification is based on aerobic culture results and MALDI-TOF.

[2]*P*-values derived from Fisher's exact test from bivariate analysis or each etiology by group.

[3]Includes *Aerococcus* spp., *Corynebacterium* spp., *Enterobacter* spp., and *Klebsiella* spp.

treatment (P = 0.99). The OR (95% CI) of bacteriological cure was 1.02 (0.05–19.42) for quarters from cows in the APT group relative to those in the GRAD group. Figure 3A shows the adjusted probabilities from the final model.

We defined a new infection as the presence of new or different growth on the fresh quarter sample compared with the dry-off quarter sample. Quarters with samples that exhibited mixed growth were not included in the analysis. Quarters with samples that exhibited growth of the same organism at both time points were included but not defined as new infections. A total of 770 [APT, n = 375 (48.7%); GRAD, n = 395 (51.3%)] quarter observations were analyzed. We documented 77 (10.0%) new infections [APT, n = 29 (7.7%); GRAD, n = 48 (12.2%)]. The final model included lactation number at the time of enrollment (P = 0.66) and treatment (P = 0.08). The interaction between treatment and lactation number was nonsignificant (P = 0.68) and thus was not retained in the final model. Compared with quarters from cows in the GRAD group, the odds (95% CI) of a new infection in quarters from cows in the APT group were 0.63 (0.37–1.05). The adjusted probabilities (95% CI) of a new IMI for the APT and GRAD groups are shown in Figure 3B.

Culling and Mastitis Events

We included all 363 cows that entered the dry period in the analyses for culling and mastitis events. The results of generalized linear models for each outcome variable are described separately below. Adjusted probabilities are shown in Figure 4.

Overall, 92.8% of the 363 cows that entered the dry period remained in the herd until they reached 30 DIM in the subsequent lactation. A total of 26 cows were culled or died, 16 in the APT group and 10 in the GRAD group. The final multivariable logistic regression model included average milk production before enrollment (P = 0.002) and treatment (P = 0.33). A 1-kg increase in average milk production before enrollment decreased the odds of culling by 0.93 (95% CI: 0.88–0.97). Compared with cows in the GRAD group, the odds of a cow being culled before the first 30 DIM were 1.52 (95% CI: 0.66–3.48) in the APT group. Pearson goodness-of-fit statistics for the final model revealed P = 0.80.

We documented a total of 32 (8.8%) clinical mastitis cases in the first 30 DIM, with 17 cases in the APT group and 15 cases in the GRAD group. All cases were observed between 1 and 30 DIM. The final model included milk production on the first test day after calving (P = 0.03), $\log SCC_1$ ($P < 0.0001$), and treatment (P = 0.56). A 1-kg increase in average milk production decreased the odds of clinical mastitis in the first 30 DIM by 0.95 (95% CI: 0.91–0.99). A 1-unit increase in $\log SCC_1$ increased the odds of clinical mastitis in the first 30 DIM by 3.66 (95% CI: 1.94–6.93). The odds of clinical mastitis in the first 30 DIM were 1.32 (0.53–3.30) in the APT group compared with the GRAD group. Pearson goodness-of-fit statistics revealed P = 0.11.

Table 3. Quarter-level culture results after calving for 781 quarters from cows subjected to abrupt (APT, n = 382) or gradual (GRAD, n = 399) cessation of milking at the end of lactation[1]

Item	APT (n, %)	GRAD (n, %)	P-value[2]
Negative	346 (90.6)	347 (87.0)	0.11
Staphylococcus spp.	2 (0.5)	14 (3.5)	0.004
Staphylococcus aureus	7 (1.8)	13 (3.3)	0.26
Mixed growth	4 (1.0)	1 (0.3)	0.21
Streptococcus dysgalactiae	2 (0.5)	3 (0.8)	>0.99
Pseudomonas spp.	1 (0.3)	0 (0)	0.49
Prototheca spp.	2 (0.5)	0 (0)	0.24
Candida spp.	0 (0)	1 (0.3)	>0.99
Other[3]	18 (4.7)	20 (5.0)	0.87

[1]Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. Etiological classification is based on aerobic culture results and MALDI-TOF.

[2]P-values derived from Fisher's exact test from bivariate analysis or each etiology by group.

[3]Includes *Aerococcus* spp., *Citrobacter* spp., *Corynebacterium* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Kocuria* spp., *Lelliottia* spp., and *Raoultella* spp.

Milk Production and SCC

A total of 347 milk production values and 334 SCC values from the first test day after calving were available for analyses. The mean (±SD) milk production on the first test date was 40.4 ± 10.2 kg (APT, 40.4 ± 10.2; GRAD, 40.3 ± 10.2). The final multivariable model included $\log SCC_1$ (P = 0.02), DIM at the first test day after calving ($P < 0.0001$), and treatment (P = 0.52); none of the tested interactions remained in the model. A 1-unit increase in $\log SCC_1$ decreased the milk production at the first test date by 1.7 kg (95% CI: −3.0, −0.3). A 1-d increase in DIM at the first test date increased milk production by 0.7 kg (95% CI: 0.6–0.7). Controlling for these covariates, milk production (LSM, 95% CI) at the first test date was 40.2 (39.0–41.4) kg for cows in the APT group and 40.8 (39.6–41.9) kg for cows in the GRAD group (Figure 5A).

The mean (±SD) $\log SCC_1$ was 4.8 ± 0.6 (APT, 4.9 ± 0.6; GRAD, 4.8 ± 0.6). The final multivariable model included lactation number at the time of enrollment (P = 0.03), $\log SCC_0$ (P = 0.03), and treatment (P = 0.99). None of the tested interactions were retained in the model (Table 4). When controlling for these covariates,

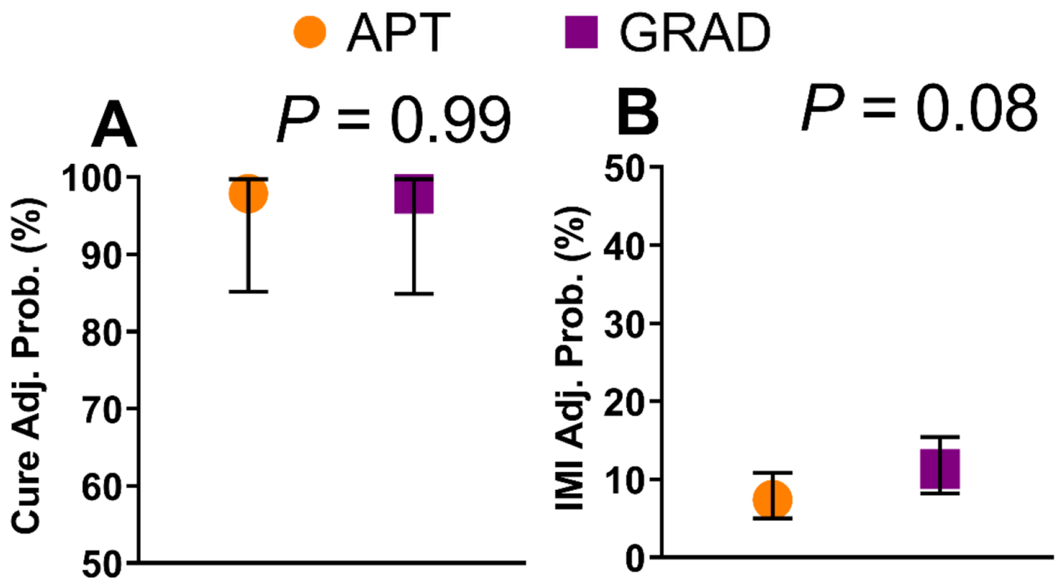

Figure 3. Adjusted probabilities (Adj. Prob.) from generalized linear mixed models showing the effect of gradual cessation of milking (GRAD) versus abrupt cessation of milking (APT) at the end of lactation on bacteriological cure (A) and new IMI (B) over the dry period. Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. Error bars show 95% CI.

$logSCC_1$ (LSM, 95% CI) was 4.9 (4.8–5.0) in the APT group and 4.9 (4.8–5.0) in the GRAD group (Figure 5B). The assumptions of homoscedasticity and normality of residuals were met for both models.

DISCUSSION

In this study, we compared the effect of GRAD with APT at the end of lactation on the (1) well-being, (2) udder health, (3) milk production, and (4) risk of culling of Holstein dairy cows. To assess the well-being of the animals, we used udder firmness, udder pain, and the percentage change in fecal 11,17-DOA concentrations as indirect indicators of stress. Data on milk leakage, bacteriological cure and new IMI over the dry period, SCC at the first test date after calving, and clinical mastitis incidence within the first 30 DIM in the subsequent lactation were used to determine the effect of gradual cessation on udder health. To evaluate the effect on herd economics, we examined differences in milk production and risk of culling using the milk production data from the first test date after calving and culling risk within the first 30 d postcalving, respectively.

Animal Well-Being

We found that the odds of udder firmness at 3 d after dry-off were 55% higher in cows in the APT group than in those in the GRAD group. The probability that these differences were due to chance was 6% ($P = 0.06$). Increased udder pressure after dry-off, caused by a slow decrease in milk secretion and cessation of milking, has been thought to cause discomfort and pain (Bertulat et al., 2013; Silanikove et al., 2013) and was shown to be associated with increased 11,17-DOA concentrations (Bertulat et al., 2013). Our findings therefore suggest that GRAD during the last week of lactation by omitting 2 out of 3 daily milkings can alleviate the pain and discomfort of dairy cows after dry-off by decreasing intramammary pressure. Our results are consistent with those reported by Tucker et al. (2009), who found that cows milked once daily for 14 d before dry-off had reduced udder firmness compared with those that were milked twice daily until dry-off. In previous works, researchers reported a decreased udder pressure in cows that received a single administration of the prolactin-release inhibitor cabergoline at dry-off compared with animals that received a placebo (Bach et al., 2015; Bertulat et al., 2017). Maynou et al. (2018) found lower udder pressure in cows that received 2 acidogenic oral boluses on the day before dry-off. Leitner et al. (2007) tested the effect of intramammary infusion of casein hydrolysate on the comfort behavior of cows after dry-off and reported that a single treatment with casein hydrolysate was sufficient to prevent the increase in udder pressure. The average daily milk production at enrollment was positively associated with udder firmness,

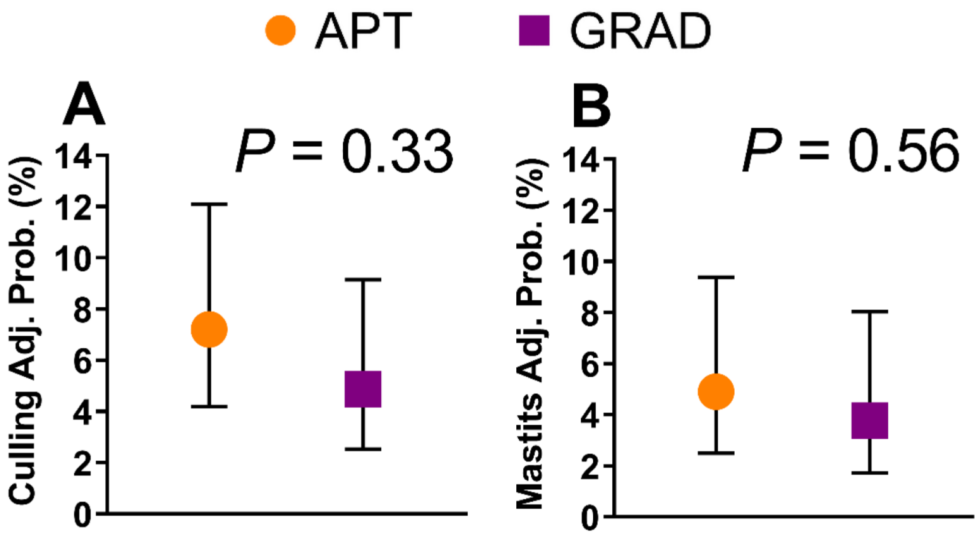

Figure 4. Adjusted probabilities (Adj. Prob.) from generalized linear models showing the effect of gradual cessation of milking (GRAD) versus abrupt cessation of milking (APT) at the end of lactation on the risk of culling (A) and the risk of mastitis (B) over the first 30 DIM. Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. Error bars show 95% CI.

which is consistent with previous results (Bertulat et al., 2013). In accordance with Bertulat et al. (2017), we observed an association between lactation number and udder firmness such that first- and second-lactation animals had lower odds of udder firmness. However, these findings are in contrast with those reported by others who found no relationship between lactation number and udder firmness (Bertulat et al., 2013; Bach et al., 2015). We believe that differences in study populations, measuring techniques, and measuring protocols account for the differences among studies. The numerical difference in the pain response we observed between the groups was likely due to chance ($P = 0.13$). Given the meaningful differences in udder firmness found between the groups and the observed association between udder firmness and pain response, this was an unexpected and difficult-to-explain finding. Bertulat et al. (2017) also reported an association of udder firmness with the occurrence of a pain response and found that cows that received a single injection of cabergoline at dry-off were less likely to exhibit a pain response.

We observed an increase in 11,17-DOA concentrations at 3 d after dry-off in both groups, suggesting that animals in both groups suffered from stress because of the dry-off procedure. Contrary to our expectations, we did not observe a meaningful difference in the percentage change in 11,17-DOA concentrations between the APT and GRAD groups ($P = 0.24$). Fecal 11,17-DOA concentrations are an established indicator of stress (Palme, 2019), and their increase following dry-off in dairy cows has been related to discomfort due to higher udder pressure (Bertulat et al., 2013). Taken together, our results suggest that the gradual-cessation protocol studied here failed to provide a measurable reduction in stress, discomfort, and pain associated with dry-off in dairy cows. Our results are somewhat consistent with those reported by Rajala-Schultz et al. (2018). The researchers investigated the effects of GRAD and APT on the behavioral activity of 95 dairy cows after dry-off. As in our study, gradual-cessation cows were milked once daily for the last week of lactation, whereas cows that were dried off abruptly were milked 3 times daily until the end of lactation. Among the 4 evaluated measures (i.e., daily lying duration, number of lying bouts per day, average duration of lying bouts, and steps taken per day), only the duration of lying bouts appeared to be affected by the cessation method [$P = 0.07$ (Rajala-Schultz et al., 2018)].

Udder Health

Milk leakage has been associated with an increased risk of clinical mastitis (Schukken et al., 1993; Elbers et al., 1998; Waage et al., 2001) and an increased risk of IMI (De Prado-Taranilla et al., 2020). We included milk leakage in our study to help explain possible differences in the udder health indices (i.e., new IMI, clinical mastitis incidence) and SCC. Our data do not support a measurable difference between the 2 groups. This finding is consistent with results reported by Gott et al. (2016), who investigated the effects of APT and GRAD at the end of lactation on milk leakage following dry-off and IMI at calving in 285 dairy cows from 5 Ohio dairy herds. The researchers evaluated

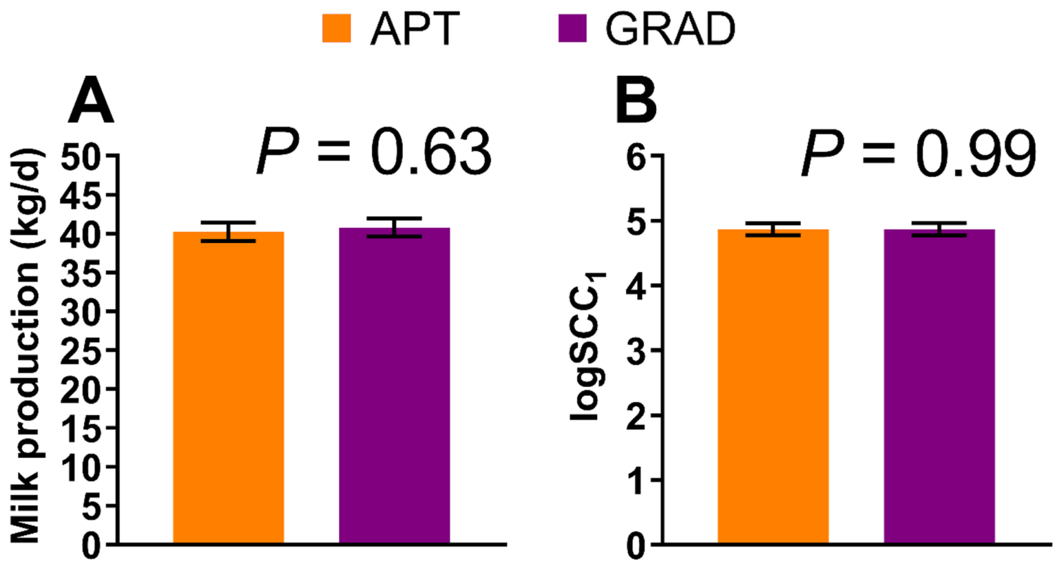

Figure 5. Least squares means from general linear models showing the effect of gradual cessation of milking (GRAD) versus abrupt cessation of milking (APT) at the end of lactation on milk production at the first test date after calving (A) and the SCC ($\log_{10}$-transformed, $\log SCC_1$; B) at the first test date after calving. Cows in the APT group were milked 3 times daily until the day of dry-off, whereas cows in the GRAD group were milked once daily for 7 d before dry-off. Error bars show 95% CI.

milk leakage in 107 cows from 1 herd in which cows did not receive an internal teat sealant and observed no differences in the proportion of quarters or cows with milk leakage at least once after dry-off ($P = 0.72$, $P = 0.27$, respectively). Tucker et al. (2009) reported that reducing the milking frequency from 2 to 1 daily milkings during the last 8 d before dry-off did not result in less milk leakage. In contrast, Zobel et al. (2013) reported a higher frequency of milk leakage in cows that were dried off abruptly (9/12) than in those that were subjected to GRAD at the end of lactation (3/11, $P = 0.04$). In a recent observational study, the investigators assessed the incidence of milk leakage after dry-off and its relationship with new IMI and clinical mastitis in 8 European countries (De Prado-Taranilla et al., 2020). They found that the reduction in the number of daily milkings in the final week of lactation was associated with a decreased risk of milk leakage between 20 and 52 h after dry-off (De Prado-Taranilla et al., 2020). We speculate that differences in study populations, frequency of observations, study designs (including specifics of the gradual-cessation method), and discrepancies in dry-off protocols (i.e., the use of internal teat sealant) account for the discrepancies among studies. The positive association between average daily milk production and milk leakage found in our study is consistent with results from other studies (Bertulat et al., 2013; Zobel et al., 2013; Gott et al., 2016; Hop et al., 2019). Milk leakage is a consequence of increased intramammary pressure due to the accumulation of milk following dry-off. Once the pressure exceeds the strength of the teat sphincter muscle, milk leakage can occur (Gott et al., 2016; De Prado-Taranilla et al., 2020). It is plausible that cows with higher milk production at the time of dry-off accumulate more milk faster, increasing the risk of milk leakage after dry-off. In addition to milk production at the time of dry-off, other cow factors, such as teat and milking characteristics, have been associated with the

Table 4. Multivariable general linear model showing the effect of gradual cessation of milking at the end of lactation on SCC ($\log_{10}$-transformed) at the first test date after calving[1]

Item[2]	β[3] (SE)	*P*-value	LSM (95% CI)
Treatment		0.99	
APT	0.0 (0.1)		4.9 (4.8–5.0)
GRAD	Referent		4.9 (4.8–5.0)
Lactation number[4]		0.03	
First	−0.2 (0.1)		4.8 (4.7–4.8)
Second	0.0 (0.1)		4.9 (4.8–5.1)
≥Third	Referent		4.9 (4.8–5.0)
$\log SCC_0$[5]	0.1 (0.1)	0.03	—

[1]Cows subjected to abrupt cessation of milking (APT) were milked 3 times daily until the day of dry-off, whereas cows subjected to gradual cessation of milking (GRAD) were milked once daily for 7 d before dry-off.

[2]Intercept omitted for clarity.

[3]Linear regression coefficient.

[4]Lactation number at the time of enrollment.

[5]$\log SCC_0$: SCC from the last test before enrollment, $\log_{10}$-transformed.

risk of milk leakage (Klaas et al., 2005); these characteristics were not examined in our study.

We found no differences in rates of bacteriological cure between groups, suggesting that both drying-off strategies worked equally well. However, we caution against overinterpreting these results, as only a small number of quarters were included in the analyses. The odds of a new IMI were 37% lower for cows in the APT group, and the likelihood that these differences were due to chance was 8% ($P = 0.08$). Gott et al. (2016) documented an interaction between parity and treatment, such that APT decreased the risk of new IMI in cows at the end of their first lactation, whereas GRAD increased the risk of new IMI at calving in multiparous cows. In our study, we detected no interaction between treatment and lactation number ($P = 0.68$), suggesting that lactation number did not modify the effect of treatment in the current study population. Newman et al. (2010) reported no differences in new IMI between cows subjected to GRAD or APT at the end of lactation. In contrast, older works showed a beneficial effect of gradual cessation on the risk of new IMI (Natzke et al., 1975; Oliver et al., 1990; Oliver et al., 2009). The differences in IMI across studies may be attributable to the differences in study populations (including milk production), drying-off protocols (including the type of gradual-cessation method), and the application of intramammary treatments at dry-off (i.e., intramammary antimicrobial drugs and teat sealant) as well as differences in dry-cow management strategies among study herds.

We observed no meaningful differences in the occurrence of clinical mastitis between groups. To our knowledge, data on the association between different dry-off strategies (i.e., gradual cessation vs. abrupt cessation) and clinical mastitis incidence are scarce. De Prado-Taranilla et al. (2020) documented the incidence of clinical mastitis within the first 30 DIM in their study. They reported an average incidence of 9.9%; however, the investigation of the relationship between the method of dry-off and clinical mastitis incidence was not reported (De Prado-Taranilla et al., 2020). In agreement with findings reported by Gott et al. (2017), our data show that treatment had no effect on $\log SCC_1$. The observed associations of lactation number and $\log SCC_0$ with $\log SCC_1$ in our study are consistent with results from previous studies (Green et al., 2008; Gott et al., 2017; Vasquez et al., 2018). The absence of meaningful differences in the clinical mastitis incidence and $\log SCC_1$ in the presence of differences in new IMI may be attributable to the differences in the number of *Staphylococcus* spp. causing the infections, which may have a protective effect on udder health (Vanderhaeghen et al., 2014; De Buck et al., 2021).

Milk Production and Culling

Our data indicate no differences in milk production at the first test date after calving between groups and therefore support results reported by others (Gott et al., 2017). Finally, we found no differences in culling risk between groups.

Study Limitations and Future Directions

Our study had some limitations that the reader should consider. First, we conducted our study on a single New York dairy farm with Holstein cows that were milked 3 times per day. Our results therefore likely reflect what would happen in a similar commercial operation in this region. Thus, the external validity of our study may be limited to similar operations employing a similar milking routine. Second, gradual-cessation cows were kept with their herd mates until the day of dry-off. This housing likely diminished possible confounding effects, such as discrepancies in feed access or stall and alleyway cleanliness, that may have occurred if gradual-cessation cows had been segregated in a separate pen. However, as a result, gradual-cessation cows were brought to the milking parlor at each milking during the last week before dry-off. Based on reports from the farm personnel and our own observations, this resulted in milk leakage of cows, which in turn could have influenced the efficacy of the intermittent milking protocol and the measured outcome variables. However, because we did not document milk leakage of cows in the GRAD group in the milking parlor, we can only speculate on its possible effects on the measured outcome variables. Some mention should be made on the COVID-19 restrictions that led to interruption of the animal enrollment and loss to follow-up diminishing the number of animals that were subjected to the final analyses. Thus, the reader should consider the possibilities of Type I and Type II errors. We also recognize that the absence of statistically significant differences does not conclude noninferiority among groups due to lack of statistical power. For example, using an α-level of 0.05, a power of 0.95, a ratio among groups of 1:1, an expected incidence of 9% in both groups, and an acceptable difference of cases, the margin of noninferiority (Δ) of 4.5%, a total sample size of 876 cows would have been needed for testing noninferiority among groups for the outcome variable clinical mastitis during the first 30 DIM. This calculation was performed with the package "SampleSize4ClinicalTrials" (Qi and Zhu, 2021) in R Statistical Software (R Core Team, 2021). In this study, we investigated the outcome variables new IMI and bacteriological cure at the quarter level, whereas all other variables were studied at the cow level. Thus, the reader should consider the possibility of atomistic bias when drawing conclusion from the results presented here.

The gradual-cessation protocol used in our study included the identification and marking of GRAD cows with leg bands. This facilitated the omission of the premilking udder preparation and attachment of the milking unit when GRAD cows entered the milking parlor during milking sessions 1 and 2. Despite being effective, the use of leg bands was time-consuming and costly, likely diminishing their likelihood of implementation in the field. Conversely, a gradual-cessation protocol that facilitates cows entering the milking parlor only once per day through, for example, segregation in a separate pen, may result in the frustration of the cows due to preventing them from participating in a habitual activity (Munksgaard and Simonsen, 1996; Zobel et al., 2013). Developing new technologies that facilitate the GRAD to end lactation will therefore provide future research opportunities. A step-down program for reducing the degree of udder emptying through automated early removal of the milking cluster, as described by Martin et al. (2020), could be a viable solution and subject of future studies. Such studies should also investigate herd- and cow-level factors that may customize management practices near dry-off, as suggested by previous authors (Gott et al., 2016; Vilar and Rajala-Schultz, 2020).

CONCLUSIONS

Gradual cessation of milking decreased udder firmness at 3 d after dry-off. We observed no meaningful effect on udder pain or the percentage change in fecal 11,17-DOA concentrations. Cows that were subjected to GRAD during the last week of lactation had a higher risk of IMI at the quarter level over the dry period compared with cows that were dried off abruptly, whereas no meaningful differences in bacteriological cure rates were observed. The observed differences in IMI between the 2 groups were in large part attributable to *Staphylococcus* spp. and not reflected in the cow-level udder health indices of clinical mastitis incidence within the first 30 DIM or SCC or milk production at the first test date after calving. No differences in culling risk were observed. We conclude that the gradual-cessation protocol tested herein failed to significantly improve animal well-being, udder health, milk production, or survival in the tested study cohort. However, the observed differences in udder firmness, as well as the numerical differences in udder pain and the percentage change in fecal 11,17-DOA concentrations suggest that this line of research may be useful. Future research is needed to develop drying-off strategies that are appropriate for lowering milk production at the end of lactation and improve animal well-being without compromising udder health.

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