Controlled trial of the effect of negative dietary cation-anion difference prepartum diets on milk production, reproductive performance, and culling of dairy cows

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ABSTRACT

Our objective was to assess the effects of feeding negative dietary cation-anion difference (DCAD) prepartum diets on milk production, reproductive performance, and culling. Cows from 4 commercial farms in Ontario, Canada were enrolled in a pen-level controlled trial from November 2017 to April 2019. Close-up pens (1 per farm) with cows 3 wk before calving were randomly assigned to a negative DCAD (TRT; −108 mEq/kg of dry matter; target urine pH 6.0–6.5) or a control diet (CON; +105 mEq/kg of dry matter with a placebo supplement). Each pen was fed TRT or CON for 3 mo (1 period), and then switched to the other treatment for the next period (4 periods per farm). Data from 15 experimental units (8 pen treatments in TRT and 7 in CON), with a total of 1,086 observational units (cows), were included. The effect of treatment on milk yield at the first 3 milk recording tests of lactation was assessed with linear regression models accounting for repeated measures. The risk of pregnancy at first artificial insemination and culling by 30, 60, and 305 d in milk (DIM) were analyzed with logistic regression models, and effects on time to first AI, pregnancy, and culling were assessed with Cox proportional hazards models. All models included treatment, parity, and their interactions, accounting for pen-level randomization and clustering of animals within farm with random effects, giving 10 degrees of freedom for treatment effects. Multiparous cows fed TRT produced more milk at the first (42.0 vs. 38.8 ± 1.2 kg/d) and second (44.2 vs. 41.7 ± 1.3 kg/d) milk tests. However, multiparous cows fed TRT tended to have 0.2 percentage units less milk fat content at these tests. Although multiparous cows fed TRT tended to have greater energy-corrected milk at the first test (least squares means ± standard error: TRT = 46.1 ± 0.9 vs. CON = 43.8 ± 1 kg/d), there were no differences observed in energy-corrected milk at the second or third tests. In primiparous cows, there was no effect of treatment on milk production. Multiparous cows fed TRT had greater pregnancy to first insemination (TRT = 42 ± 3 vs. CON = 32 ± 4%) and tended to have shorter time to pregnancy [hazard ratio (HR) = 1.20; 95% CI: 0.96–1.49]. In primiparous cows fed TRT, time to pregnancy was increased (HR = 0.76; 95% CI: 0.59–0.99). Culling by 30 DIM tended to be less in TRT (3.3 ± 1.1%) than CON (5.5 ± 1.8%). No effect of treatment on culling by 305 DIM was detected in primiparous cows, but in multiparous cows, the TRT diets decreased the odds of culling (21.3 ± 1.9 vs. 31.7 ± 2.8%) and daily risk of culling to 305 DIM (HR = 0.64; 95% CI: 0.46 to 0.89). Under commercial herd conditions, prepartum negative DCAD diets improved milk production and reproductive performance, and reduced culling risk in multiparous cows. In primiparous cows, TRT diets had no effect on milk yield or culling, but increased the time to pregnancy. Our results suggest that negative DCAD diets should be targeted to multiparous cows.

Key words: dietary cation-anion difference, pregnancy, hypocalcemia, commercial herds

INTRODUCTION

Prepartum negative DCAD diets reduce the incidence of milk fever (MF; Lean et al., 2006). This “tip of the iceberg” clinical disease affects health, production, and reproduction of dairy cattle (Curtis et al., 1983; Venjakob et al., 2017). Subclinical hypocalcemia (SCH), the unseen part of the iceberg, is associated with increased risk of disease and, depending on its degree and duration, impaired production and reproduction (Caixeta et al., 2017; McArt and Neves, 2020). A negative DCAD prepartum enhances Ca metabolism and reduces the prevalence of SCH postpartum (Shire and Beede, 2013; Martinez et al., 2018a). Negative DCAD diets are
commonly used to prevent MF, but in a meta-analysis (Lean et al., 2019), their effects on milk yield were heterogeneous. Reproductive performance outcomes were not assessed in the meta-analysis because reproductive data were not reported in most of the individual experiments.

Subclinical hypocalcemia is a risk factor for early lactation disease (Chapinal et al., 2012; Rodríguez et al., 2017), and diseased cows have greater risk of compromised reproductive performance (Carvalho et al., 2019). Caixeta et al. (2017) introduced the concept of chronic SCH (blood Ca $\leq 2.15$ mmol/L at all of 1, 2, and 3 DIM) and observed that these cows tended to take longer to return to cyclicity and had lower odds of pregnancy at first service compared with cows that never had low blood Ca within 3 DIM. Results from larger studies (n > 1,000 cows) support that postpartum SCH reduced pregnancy to first AI and increased time to pregnancy, whether blood Ca was assessed within 48 h (Venjakob et al., 2017) or weekly at −1, 1, 2, or 3 wk postpartum (Chapinal et al., 2012). However, there is little information on the effect of DCAD on reproductive performance. Lopera et al. (2018) and Martinez et al. (2018a) did not detect an effect by lowering the prepartum DCAD on time to pregnancy, but with fewer than 100 cows in each study, there was a lack of statistical power for assessment of reproductive outcomes.

Milk fever is detrimental to milk production (Rajala-Schultz et al., 1999; Venjakob et al., 2017), and the effect may be underestimated because cows with MF that were euthanized or culled did not contribute production data. However, the effect of SCH on milk yield depends on the time relative to calving that blood Ca was measured, the threshold applied, and parity. Some researchers have reported milk loss in hypocalcemic cows (serum Ca $\leq 2.1$ mmol/L in the first week postpartum) of up to 7.1 kg/d in the first 3 milk recording tests postpartum (Chapinal et al., 2012), but SCH measured close to parturition has been associated with greater milk production (Venjakob et al., 2017; Neves et al., 2018). Conversely, Martinez et al. (2012) did not detect any associations between SCH (plasma Ca $\leq 2.14$ mmol/L) in the first 3 DIM and milk production in the first 4 mo postpartum.

The effect of acidogenic diets on milk production is generally consistent: overall, greater milk yield is observed when lowering prepartum diets DCAD (DeGroot et al., 2010; Weich et al., 2013; Leno et al., 2017; Diehl et al., 2018). A meta-analysis of DCAD effects (Lean et al., 2019) demonstrated that lower DCAD increased milk yield by 1.1 kg/d in multiparous cows, but tended to decrease milk yield by 1.3 kg/d when fed to primiparous animals.

By improving health, production, or reproductive performance, acidogenic diets might reduce culling. Culling in early lactation might be explained by the health status of the cow, or the expectation of poor performance or reproduction (Beaudeau et al., 2000). From a management and economic perspective, dairy producers should aim to minimize uneconomical culling (Renkema and Stelwagen, 1979; De Vries, 2017), which is mainly achieved by minimizing postpartum disease incidence and, consequently, better reproductive and productive performance. There is a need to understand the effect of feeding acidogenic diets prepartum on commercial dairy farms, where numerous variables such as feeding management, grouping strategies for dry cows, and unmeasured sources of variation may influence treatment effects. The objectives of this study were to assess the effects of a negative DCAD dry cow diet on milk production, reproductive performance, and culling. We hypothesized that feeding negative DCAD for 3 wk before parturition would increase milk production in early lactation, enhance reproductive performance, and reduce culling.

MATERIALS AND METHODS

This manuscript is part of a series of 3 reports (Couto Serrenho et al., 2020, 2021). This work is reported using the REFLECT Guideline (Sargeant et al., 2010). The experimental protocol was approved by the University of Guelph Animal Care Committee (AUP 3951). “Primiparous” refers to cows that were enrolled as nulliparous (3 wk before calving) and initiated their first lactation at calving; “multiparous” refers to cows that started their second or greater lactation.

Experimental Design and Treatment Groups

This pen-level randomized controlled trial was conducted on 4 commercial freestall dairy farms in Ontario, Canada. A convenience herd selection was made based on geographic location, herd size, and willingness to comply with the experimental protocol, including not administering any prophylactic calcium supplements around calving. Data were collected between November 2017 and April 2019. Details of the study herds, diets, and BCS and urine pH data are in a companion paper reporting health and serum Ca concentration outcomes (Couto Serrenho et al., 2021).

The single close-up pen on each farm was randomly assigned to a starting treatment, which was alternated approximately every 3 mo, for a total of 4 periods (2 treatment and 2 control) on each farm. The treatments were a negative DCAD [acidified with Soychlor, Landus...
Cooperative (TRT); −108 mEq/kg of DM (weighted average of the 4 farms); target urine pH 6.0–6.5; Table 1] or a control diet (CON; +105 mEq/kg of DM, weighted average, with a placebo supplement) nutritionally similar to TRT but with a positive DCAD. The DCAD level of the CON group was set similar to the DCAD in each herd’s diet before the study. Expected calving date was 280 d after the last AI date, and cows were followed until leaving the herd or the end of trial.

To maintain blinding of the farmers, the supplement was delivered in plain moisture-barrier bags labeled with “A” or “B,” and weekly measurement of urine pH was performed, even in pens receiving CON diet (urine pH results were not visible to the farmers). Feeding rate was adjusted if the average urine pH in a TRT pen was out of the target range of 6 to 6.5 for more than 2 wk. There were 2 occasions when an adjustment was required, with 2 farms making one adjustment each.

Apart from the close-up DCAD level between treatments, the pre- and postpartum TMR were as similar as possible for cows within a farm, and among the farms. Diets were formulated using feeds available or normally purchased on each farm. The diet with TRT was formulated to −100 mEq/kg of DM, and the feeding rate of TRT was adjusted if necessary to achieve urine pH of 6 to 6.5. The CON diet with the placebo supplement was formulated to mimic the diet on each farm before the study. The target DM intake for close-up cows was 14, 13, 13, and 14 kg/cow per day for farms A, B, C, and D, respectively.

Regarding reproductive management, all farms had a voluntary waiting period between 55 and 65 DIM. Farms maintained the reproductive management protocols that were in place before the trial commenced. On all farms, first AI was based primarily on automated activity monitoring or visual heat detection, supplemented with synchronization for timed AI (i.e., the Ovsynch protocol). For re-insemination, cows were monitored for estrus, and if open at pregnancy diagnosis between 28 and 42 d after AI, were synchronized for timed AI with the Ovsynch protocol.

**Data Collection and Processing**

Insemination, pregnancy diagnosis, and culling data were collected weekly from each farm’s computerized records (farms A, B, and C: Dairy Comp 305; Valley Ag Software; and farm D: DairyPlan C21; GEA).

Production data (milk, fat, and protein yields, and DIM at test day) for farms A, C, and D were extracted from the Canadian national milk recording (Lactanet, Guelph, ON, Canada) database for the first 3 test dates of the lactation during the study for each cow. Milk production data from farm B, which had a robotic milking

![Table 1. Summary of multivariable survival analysis and logistic regression models of reproductive performance of Holstein cows in a blinded randomized controlled trial of a negative DCAD diet in the last 3 wk before calving (TRT; −108 mEq/kg of DCAD) or placebo control (CON; +105 mEq/kg of DM); there were 15 experimental units (8 pen treatments in TRT and 7 in CON) Multiparous cows (TRT n = 455) vs. CON (n = 271) Primiparous cows (TRT n = 226) vs. CON (n = 134) Outcome Relative measures Relative pregnancy rate Relative measures Time to first AI HR = 0.96 0.54–1.71 0.89 First-service pregnancy risk (%) OR = 1.53 1.07–2.21 0.02 Multiparous cows TRT (n = 455) vs. CON (n = 271) Estimate 95% CI P Relative measures Estimate 95% CI P Relative measures Estimate 95% CI P Multiparous cows TRT (n = 455) vs. CON (n = 271) Time to first AI HR = 0.96 0.54–1.71 0.89 First-service pregnancy risk (%) OR = 1.53 1.07–2.21 0.02
system and did not enroll in DHIA milk recording, were obtained from the Lely T4C 3.6 dairy herd management system (Lely group). Daily sums of milk yield, fat, and protein percentages were extracted from the software. Values out of the range of biologic plausibility (outside the 10th and 90th percentiles of the data from the other 3 farms) were considered an error or artifact of milking intervals in the automatic milking system and censored (milk yield <7.5 kg; protein < 1.4 or > 4.5%; or fat < 1.46 or > 7.9%). Random selection (using the RANUNI function in SAS; SAS Institute) was used to create a random sample of 3 d per cow to correspond to milk recording test d 1, 2, and 3 in the other herds. The first day was selected between 10 and 44 DIM, the second between 48 and 86 DIM, and the third between 88 and 132 DIM. These ranges of days were defined based on the range of days for test dates 1, 2, and 3 in the herds used in the trial protocol), if the cow was intractable for sampling, or if culled before calving.

The binary outcomes of pregnancy to first AI and culling by 30, 60, and 305 DIM were analyzed using logistic regression models (GLIMMIX procedure of SAS) with treatment, parity, and their 2-way interaction. These models are represented by the following equation:

\[
y_{ij} \mid \pi_{ij} \sim \text{Binary (1, } \pi_{ij})
\]

\[
\pi_{ij} = P(\pi_{ij} = 1) = \frac{\exp(\beta_i + \gamma_j + (\beta\gamma)_{ij} + F_l + F_{lp})}{1 + \exp(\beta_i + \gamma_j + (\beta\gamma)_{ij} + F_l + F_{lp})}
\]

where \(\pi_{ij}\) represents the \(P\) probability of a \(j\) parity cow on \(i\) treatment (CON or TRT) having the outcome; \(\beta_i\) = fixed effect of treatment; \(\gamma_j\) = fixed effect of parity; \((\beta\gamma)_{ij}\) = effect of treatment by parity interaction; \(F_l\) = random effect of farm; \(F_{lp}\) = random effect of farm \(\times\) treatment \(\times\) period.

Production data were assessed with linear regression models (MIXED procedure of SAS) with treatment, parity, test date, and their 2-way and 3-way interactions as covariates. For milk production variables the following linear mixed model was used:

\[
Y_{ijkl} = \mu + \beta_i + \gamma_j + \theta_k + (\beta\gamma)_{ij} + (\beta\theta)_{ik} + (\beta\gamma\theta)_{ijk} + F_l + F_{lp} + \alpha_{m(lp)} + \varepsilon_{ijklm}
\]

where \(Y_{ijkl}\) = response to treatment \(i\) (CON or TRT) at the \(k\)th test day \((k = \text{test d }1, 2, \text{ or 3 for cow number } (m)); \mu = \text{overall mean}; \beta_i = \text{fixed effect of treatment}; \gamma_j = \text{fixed effect of parity}; \theta_k = \text{fixed effect test day}; (\beta\gamma)_{ij} = \text{effect of treatment by parity interaction}; (\beta\theta)_{ik} = \text{effect of treatment by test day interaction}; (\beta\gamma\theta)_{ijk} = \text{effect of treatment by parity by test day interaction}; F_l = \text{random effect of farm}; F_{lp} = \text{random effect of farm \(\times\) treatment \(\times\) period}; \alpha_{m(lp)} = \text{repeated measures}; m = \text{cow nested within the F}_{lp}\text{ effect}; \varepsilon_{ijklm} = \text{residual error within cow } m, \text{ on treatment } i, \text{ with parity } j \text{ at test } k.\) Repeated measures of test day milk yield [cow nested in (farm \(\times\) treatment \(\times\) period)] were accounted for with an unstructured covariance structure, selected based on providing the lowest Akaike information criterion.

All logistic and linear models specified the error term for treatment with a random effect (farm \(\times\) treatment \(\times\) period term) to give the correct denominator degrees of freedom for the experimental units in the study (Bello et al., 2016; Bello and Renter, 2018) and included a second random effect term for farm to account for clustering of animals within farm and unmeasured sources of variance at the farm level. Therefore, the main effect of treatment had 10 df for all models. All models were
built using backward elimination, with covariables removed if $P > 0.05$ and interaction terms removed when $P > 0.1$. A Tukey test was used to adjust for multiple comparisons. Results are expressed as least squares means with their standard error.

For pregnancy, nonpregnant culled cows were censored on their date of culling, and cows without a record of pregnancy at the end of the study period were censored on the date of termination of data collection. The same approach was used for time to first AI. Because of inconsistent and nonstandardized recording, notations of pregnancy loss or “do not breed” were not considered. For culling, censoring was applied to cows that were in the herd at the end of the study period. Cox’s proportional hazards regression (the PHREG procedure in SAS; Collett, 1994) was used to assess time-to-event outcomes, with treatment, parity, and their interactions (treatment $\times$ parity) as covariables, accounting for clustering of animals within farm and pen-level treatment with a multiplicative frailty term (i.e., random effect of farm $\times$ treatment $\times$ period). Treatment effects on time to first AI, time to pregnancy, and time to culling are expressed as hazard ratios (HR). The Cox proportional hazards regression models are represented as follows:

$$h(t) = h_0(t) \exp[\beta_i + \gamma_j + (\beta\gamma)_{ij} + F_{ijp}],$$

where $h(t)$ is the expected hazard at time $t$; $h_0(t)$ = baseline hazard and represents the hazard when all of the predictors are equal to zero; $i$ = treatment (CON or TRT); $j$ = cow parity; $\beta_i$ = fixed effect of treatment; $\gamma_j$ = fixed effect of parity; $(\beta\gamma)_{ij}$ = effect of treatment by parity interaction; $F_{ijp}$ = random effect of farm $\times$ treatment $\times$ period (multiplicative frailty term). Proportional hazard assumptions were inspected using graphical diagnostics (inspection that plots of the $-\log[\log(\text{Survival function})]$ against the natural log of time to event were parallel (Collett, 1994); absence of lines crossing in Kaplan-Meier curves; and plots of the Schoenfeld residuals). For all models, when interactions with treatment were detected, stratified models were produced.

**Deviation from Study Design**

The study design included 4 periods of 3 mo each per farm (e.g., TRT-CON-TRT-CON). Farm C started the trial on TRT, and after 3 mo, was switched to CON. This diet was only offered for 25 d (October 1–25) instead of 3 mo as planned. When cows fed CON started calving, early lactation disease incidence and mortality dramatically increased. Of the 39 cows that calved, 14 suffered from MF, 1 of which died. Because of this unexpected and unacceptable incidence of disease, the second study period (feeding CON) was stopped, and the farm was switched to the third period, with TRT. Because such an occurrence was not anticipated, we had not set out formal trial stopping rules in advance, but an early-stopping rule was applied. This is a common and required practice in human medicine trials (Bassler et al., 2010), where early evidence of an inferior treatment outcome with harm to the study subjects justifies study protocol changes. Therefore, on farm C, only 3 periods were applied, resulting in 15 experimental units: 8 for TRT and 7 for CON. The reduced number of experimental periods on this farm is accounted for in the statistical models with the correct number of experimental units.

**RESULTS**

A total of 15 experimental units (8 TRT and 7 CON) with 1,368 Holstein cows were enrolled in the study. After applying exclusion criteria (detailed in Couto Serrenho et al., 2021), a total of 15 experimental units with 1,086 observational units (360 primiparous and 726 multiparous cows) were included in the analyses. There were 85 ± 41 cows per pen-treatment for TRT and 58 ± 24 for CON. At enrollment, 32% of primiparous and 30% of multiparous cows had BCS ≥3.75, with no differences detected between treatment groups ($P > 0.6$). The average urine pH was 8.1 ± 0.4 and 6.3 ± 0.6 in CON and TRT groups, respectively. In the TRT group, during the 3 wk before parturition, 85% of urine samples had pH ≤7, and 72% were ≤6.5.

**Effect of Treatment on Milk Production**

The effects of treatment on milk yield and ECM depended on parity ($P < 0.01$), but this interaction was not observed for fat or protein percentage. Multiparous cows fed TRT produced more milk at the first (TRT = 42.0 ± 1.2 vs. CON = 38.8 ± 1.3 kg/d, $P < 0.01$) and second (TRT = 44.2 ± 1.2 vs. CON = 41.7 ± 1.3 kg/d, $P = 0.02$) test days. Milk yield was not different at the third test day (TRT = 41.4 ± 1.2 vs. CON = 40.6 ± 1.3 kg/d, $P = 0.39$; Figure 1). Primiparous cows produced 29.7 ± 1.2 (TRT = 30.2 ± 1.3 vs. CON = 29.2 ± 1.5), 34.2 ± 1.2 (TRT = 34.9 ± 1.3 vs. CON = 33.5 ± 1.4), and 33.8 ± 1.2 kg/d (TRT = 34.2 ± 1.3 vs. CON = 33.5 ± 1.4) at test d 1, 2, and 3, respectively, with no differences between treatment groups ($P > 0.29$; Figure 1).

Cows fed TRT tended to have lower milk fat percentage (Figure 2a) at test d 1 (TRT = 3.7 ± 0.1 vs. CON = 3.9 ± 0.2%, $P = 0.05$) and test d 2 (TRT = 3.3 ± 0.1 vs. CON = 3.4 ± 0.2%, $P = 0.06$), with no differ-
No effect of treatment was detected for milk protein percentage (Figure 2b), which averaged 3.21 ± 0.05, 3.07 ± 0.05, and 3.15 ± 0.05% at test d 1, 2, and 3, respectively. Combining milk yield and components, multiparous cows fed TRT tended to produce more ECM at test d 1 (TRT = 46.1 ± 0.9 vs. CON = 43.8 ± 1 kg/d, \( P = 0.07 \)); no differences were identified at test d 2 or 3 (Figure 3). In primiparous cows, treatment had no effect on ECM (\( P > 0.35 \); Figure 3).

**Effect of Treatment on Reproduction**

Table 1 summarizes reproductive outcomes. Time to first AI was not different between treatments for primiparous (\( P = 0.36 \)) or multiparous cows (\( P = 0.35 \)). In multiparous cows, TRT diets increased the odds of pregnancy at first AI [odds ratio (OR) = 1.53, 95% CI: 1.07–2.21, \( P = 0.02 \)], and TRT tended to reduce time to pregnancy (HR = 1.20, 95% CI: 0.96–1.49, \( P = 0.10 \); Table 1). In primiparous cows, treatment had no effect on pregnancy to first AI (\( P = 0.20 \)), but TRT increased the time to pregnancy (HR = 0.76, 95% CI: 0.59–0.99, \( P = 0.04 \); Table 1).

**Effect of Treatment on Culling**

Culling within 30 DIM tended to be greater in CON than TRT, but no differences were detected at 60 DIM. An interaction was observed between treatment and parity (\( P = 0.03 \)) for the risk of culling to 305 DIM. Although treatment had no effect on culling in
primiparous cows, TRT decreased the odds of culling in multiparous (\( OR = 0.58, 95\% \text{ CI: 0.41–0.82, } P < 0.01; \) Table 2). Similarly, culling by 305 DIM was reduced in multiparous cows on TRT (HR = 0.64, 95\% CI: 0.46–0.89, \( P < 0.01 \)), but was not different between treatments in primiparous cows.

### DISCUSSION

Our results demonstrated that the effects of a negative DCAD diet fed 3 wk before parturition differed between multiparous and primiparous cows. In multiparous cows, treatment increased milk production in early lactation, tended to reduce time to pregnancy, and reduced whole lactation culling. In primiparous cows fed the negative DCAD diet, milk yield and culling were unchanged, but time to pregnancy was increased.

Milk protein percentage did not change with treatment, but fat percentage tended to be reduced in TRT, by 0.17 and 0.16 percentage points at the first 2 DHI tests, respectively. Martinez et al. (2018b) assessed daily milk yield and weekly milk fat and protein content of 79 cows and did not detect any DCAD treatment effect on milk yield, ECM, or true protein percentage, but they reported approximately 0.23 percentage points greater fat in the first 49 DIM when feeding negative DCAD prepartum. Leno et al. (2017) compared 3 diets (DCAD of +183, +59, or −74 mEq/kg of DM) and described a linear increase in milk yield (40.8, 42.4, and 43.9 kg/d, respectively) and ECM (46.2, 48.1, 49.6 kg/d, respectively) in the first 3 wk postpartum by lowering prepartum DCAD. Although Leno et al. (2017) found that daily TS tended to increase (5.42, 5.65, and 5.86 kg/d, respectively), percentage of TS decreased (13.63, 13.61, and 13.27\%, respectively) following the decreased percentage of both milk fat and protein. Contrary to our results, an interaction between DCAD and parity group for both milk fat and protein content was detected by Santos et al. (2019). In primiparous cows, milk fat was reduced and protein tended to be reduced with negative DCAD diets; however, in multiparous cows, fat content was not different between DCAD groups, but milk protein content increased by feeding negative DCAD prepartum (Santos et al., 2019).

In our study, multiparous cows had greater milk yield at the first 2 DHI tests, and thus calculation of ECM was important to distinguish between a dilution effect and a net change in production. In multiparous cows, ECM tended to be 2.2 kg/d greater at the first test, but there were no differences in ECM between treatments at test d 2 or 3. Our results agree with a meta-analysis (Santos et al., 2019) that described improved milk yields (+1.7 kg/d) and increased FCM (+1.1 kg/d) in multiparous cows. As in meta-analyses (Lean et al., 2019; Santos et al., 2019), we did not detect a significant difference in milk yield in primiparous cows fed the TRT diets. However, in the meta-analysis, milk yield of primiparous cows was numerically lower in primiparous cows fed negative DCAD diets. In our study, milk yield and ECM was numerically greater than the CON group. Additional larger studies will be needed to evaluate the effect of DCAD on milk production in primiparous cows.

Treatment improved reproductive performance in multiparous cows. As expected, the time to first AI was not different between treatment groups. In multiparous cows, the odds of pregnancy were increased by 1.53 times in the TRT group, as reflected in 10 percentage points greater pregnancy to first AI compared with the CON group. Although not statistically significant (\( P = 0.11 \)), a 20\% greater pregnancy rate was observed in multiparous cows fed TRT diets compared with the CON group. These are meaningful effects on reproductive performance. However, the opposite effect was observed in primiparous cows: treatment reduced relative pregnancy rate by 24\% (\( P = 0.04 \)). Martinez et al. (2018a) did not observe effects of DCAD on pregnancy at first AI or median time to pregnancy in 28 primipa-

### Table 2. Effect of DCAD fed prepartum\(^1\) on culling risk in a controlled trial of negative DCAD diets for close-up dry cows; there were 15 experimental units (8 pen treatments in TRT and 7 in CON)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>CON LSM(^2)</th>
<th>CON SE</th>
<th>TRT LSM</th>
<th>TRT SE</th>
<th>OR(^3) (95% CI)</th>
<th>DCAD Parity</th>
<th>DCAD × parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culling by 30 DIM</td>
<td>5.5</td>
<td>1.8</td>
<td>3.3</td>
<td>1.1</td>
<td>0.59 (0.31–1.14)</td>
<td>0.11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Culling by 60 DIM</td>
<td>9.0</td>
<td>1.5</td>
<td>7.5</td>
<td>1.0</td>
<td>0.79 (0.48–1.28)</td>
<td>0.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Culling by 305 DIM(^4)</td>
<td>6.7</td>
<td>0.67</td>
<td>6.7</td>
<td>0.56</td>
<td>0.67 &lt;0.01 0.03</td>
<td></td>
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</tr>
</tbody>
</table>

\( ^1\)From 3 wk before expected parturition cows were fed control (CON; +105 mEq/kg of DM) or treatment (TRT; −108 mEq/kg of DCAD).

\( ^2\)LSM and SE are expressed as percentage (%).

\( ^3\)OR = odds ratio.

\( ^4\)LSM and SE of the significant interactions are presented in Table 3.
rous and 52 multiparous cows. In a prospective cohort study of 1,000 cows, DeGaris et al. (2010) reported a positive association between length of exposure to a prepartum diet that included a negative DCAD and improved time to first AI and a tendency to increase the odds of pregnancy.

Calcium metabolism and responses to DCAD differ between primiparous and multiparous cows, and thus the interactions of treatment with parity were not surprising. We hypothesized that negative DCAD diets would improve blood calcium concentrations postpartum, consequently improve health outcomes, and in turn improve production and reproduction. As reported elsewhere (Couto Serrenho et al., 2021), treatment increased serum Ca concentrations and decreased the incidence of MF, displaced abomasum, and clinical mastitis. These effects would plausibly favor greater milk production, improved reproduction, and decreased culling, as reported for multiparous cows. Conversely, a mechanism by which treatment impaired reproductive performance in primiparous animals, without affecting their milk yield, is not clear. A negative DCAD diet before calving is expected to reduce prepartum DMI in heifers (Santos et al., 2019) and, presumably for that reason, decrease their milk yield. A negative DCAD diet may also have effects on energy metabolism. Rodney et al. (2018a) did not observe a reduction in DMI in primiparous cows fed a negative DCAD diet, but the authors (Rodney et al., 2018b) observed differences in bone and energy metabolism (e.g., osteocalcin and insulin like growth factor-1) between parity groups. Further research should evaluate the effects of metabolic acidification prepartum and performance of primiparous versus multiparous cows.

We acknowledge several limitations of this study. The fact that farm B was not enrolled on milk recording required us to sample from the daily data generated by the farm’s automatic milking system units. The greatest challenge in recruiting herds for this trial was finding owners who were willing to not routinely or prophylactically administer calving supplements at calving; therefore, we chose to include a herd with milk production and component data that was not from official milk recording. It would have been ideal to impose the same reproductive management protocol on all farms, but that would have made herd recruitment that much more difficult. Despite these limitations, there was no differential bias in reproductive management by treatment, as the owners and farm personnel were blinded to treatment. Culling reasons were not recorded in a consistent or interpretable way. Knowing the reason why each cow left the herd could potentially have added more information to our understanding of treatment effects on culling.

Table 3. Summary of multivariable survival analysis and logistic regression models of culling of Holstein cows in a blinded randomized controlled trial of a negative DCAD feed in the last 3 wk before calving (TRT: −108 mEq/kg of DCAD) or placebo control (CON: +105 mEq/kg); there were 15 experimental units (8 pen treatments in TRT and 7 in CON)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Multiparous cows</th>
<th>Primiparous cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRT (n = 455) vs. CON (n = 271)</td>
<td>TRT (n = 226) vs. CON (n = 134)</td>
</tr>
<tr>
<td>Culling hazard over time HR = 0.64 0.46–0.89</td>
<td>&lt;0.01</td>
<td>0.30</td>
</tr>
<tr>
<td>Culling risk (to 305 DIM) OR = 0.58 0.41–0.72</td>
<td>&lt;0.01</td>
<td>0.32</td>
</tr>
</tbody>
</table>

HR = hazard ratio; OR = odds ratio.
CONCLUSIONS

Under commercial conditions, the negative DCAD diets resulted in increased milk yield in early lactation, better reproductive performance, and reduced whole lactation culling in multiparous cows. In primiparous cows, we did not detect differences between treatments in milk production or culling, but treatment was detrimental for time to pregnancy. Although challenging for practical implementation, our results suggest that negative DCAD diets in the close-up dry period should be targeted to cows calving for the second or greater time. However, we encourage additional large-scale studies to assess the effects of negative DCAD diets on milk yield and reproductive performance in heifers.

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