Prepartum Negative DCAD Diets – They're Not Just for Milk Fever Anymore

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Introduction

Hypocalcemia is presented in two forms, the less common clinical disease called milk fever and the more common form called subclinical hypocalcemia (**SCH**). Depending upon how it is defined and the frequency of blood sampling for diagnosis, SCH can affect 25 to 40% of primiparous and 45 to 80% of the multiparous cows. Cows with SCH have reduced dry matter (**DM**) intake, suppressed measures of innate and acquired immune function, compromised energy metabolism, and increased incidence of other periparturient diseases. This is why hypocalcemia is considered a "gateway" disease for dairy cattle.

Cows develop hypocalcemia because of the sudden irreversible loss of calcium (**Ca**) during synthesis and secretion of colostrum and milk. Most cows are able to cope with the loss of Ca with the onset of lactation, but many, if not all, will experience a decline in blood concentrations of total (**tCa**) and ionized (**iCa**) Ca in the first 2 to 3 days of lactation. Multiple strategies are available to minimize the risk or prevent hypocalcemia in dairy cows and they often include manipulations of the prepartum diet. One of such strategies is the manipulation of the mineral content of prepartum diets to induce a compensated metabolic acidosis. Extensive research has characterized the benefits of feeding acidogenic diets prepartum on reducing the incidence of milk fever, but less has been known about the potential benefits of these diets on productive performance and incidence of other periparturient diseases. One would think that preventing milk fever would consistently result in improved yields of milk and milk components and reduced risk of other common diseases in early lactation. This manuscript reviews recent research on methods to control and reduce the impact of hypocalcemia in dairy cows and the extended benefits of acidogenic diets prepartum.

Hypocalcemia

During the last month of gestation, the absorbable Ca requirements in the typical dairy cow are estimated at 16 g/day to meet the needs for accretion into the pregnant uterus and endogenous fecal losses. As lactation initiates and cows synthesize colostrum, the needs for Ca increase substantially. The concentration of Ca in

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colostrum is range from 2.2 to 2.4 g/L and it remains elevated during the period in which the cow produces transition milk.

Dairy cows develop hypocalcemia because of the inability to promptly restore blood concentrations of Ca with the onset of colostrogenesis and lactation. In general, blood concentrations of Ca start to decline 1 or 2 days before calving in old Jersey cows fed an alkalogenic diet that predisposes to hypocalcemia (Goff et al., 2002) or at least 9 h before calving in multiparous Holstein cows fed an acidogenic diet to prevent hypocalcemia (Megahed et al., 2018). This decline in blood concentrations of Ca before calving is thought to be mediated by the sequestration of Ca in the gland for synthesis of colostrum. When Jersey cows underwent mastectomy, they did not experience any noticeable decline in blood tCa despite the typical decline in DM intake on the days preceding calving (Goff et al., 2002). These findings by Goff and colleagues (2002) reinforced the concept that changes in blood Ca concentrations around calving are the result of irreversible loss of Ca for synthesis of colostrum and milk, and not related to the endocrine changes associated with parturition. As cows age, they become more susceptible to hypocalcemia (DeGaris and Lean, 2008), in part because older cows produce more colostrum (Martinez et al., 2018a) and, therefore, have greater loss of Ca immediately after calving. Multiparous cows secrete larger quantities of Ca in the first milking (52% more or 9 g) than primiparous cows, which poses increased strain on Ca homeostasis. Risk of hypocalcemia depends, in part, on the amount of colostrum synthesized and secreted by cow. Depending on the amount produced, the irreversible loss of Ca in the first milking might represent 5 to 10 times the total plasma Ca pool. If milked twice in the first day postpartum, the total Ca loss might be as much as 22 to 30 g. This means that a dairy cow has to replenish the equivalent of total amount of circulating Ca at least 5 to 10 times daily to be able to supply all the Ca for colostrum and transition milk synthesis on the day of calving.

It is known that milk fever is a problem that affects primarily older cows and prepartum nulliparous cows are almost never affected by the clinical form of hypocalcemia. It is thought that the greater colostrum synthesis associated with a less dynamic bone Ca resorption mechanisms and, perhaps, less active Ca transport in the gastrointestinal tract favors a greater incidence of hypocalcemia in multiparous than primiparous cows. The risk of milk fever increases 9% for every lactation in the life of a cow (DeGaris and Lean, 2008). Although milk fever can be life-threatening, the most common presentation of hypocalcemia is the subclinical form, in which blood Ca concentrations are below a particular threshold that consequently predisposes cows to other diseases. Most commonly, SCH characterized by blood tCa ≤ 2.0 m*M* (Reinhardt et al., 2011), although the rationale for such a cut-off has not been clearly defined. Others have proposed different thresholds such as tCa of < 2.15 m*M* (Martinez et al., 2012) based on increased risk of metritis and other diseases, or iCa < 1 m*M* (Oetzel et al., 1988). Prevalence of SCH within the first 2 days postpartum, based on serum tCa ≤ 2.0 m*M*, was 25% in primiparous and 45% in multiparous cows (Reinhardt et al., 2011), whereas incidence can vary not only with threshold selected, but also frequency of sampling of blood postpartum.

Impacts of Hypocalcemia

One of the imminent effects of milk fever is the risk of death. Cows that suffer from milk fever are more likely to die either because of the consequence of low blood Ca and recumbence with subsequent muscle-skeletal lesions, but also because of the risk of therapy with Ca, particularly intravenously, might result in cardiac arrest.

The importance of hypocalcemia goes beyond its clinical symptoms. It is well established that hypocalcemia increases the risk of other diseases. Subclinical hypocalcemia reduces DM intake and rumination, and impairs insulin secretion (Martinez et al., 2014), leading to increased lipolysis. Those effects likely explain the increased risk of displaced abomasum, ketosis, dystocia and uterine prolapse. Furthermore, hypocalcemic cows have increased plasma concentrations of cortisol (Horst and Jorgensen, 1982), reduced proportion of neutrophils with phagocytic activity (Martinez et al., 2012; 2014) and reduced concentrations of cytosolic iCa in neutrophils (Martinez et al., 2014) and mononuclear cells (Kimura et al., 2006). Therefore, reducing the risk of hypocalcemia is thought to minimize its consequences, i.e., by reducing the incidence of SCH and milk fever, one expects that the incidence of the periparturient problems mentioned above would also be reduced.

Dietary Methods to Reduce Hypocalcemia

There are three main dietary strategies evaluated to reduce the risk of SCH and milk fever in dairy cows. They include limited gastrointestinal Ca absorption either by feeding limited amounts of Ca or by sequestering Ca in the lumen of the digestive tract with the use of zeolites; supplementing Ca orally or through intravenous or subcutaneous administration immediately after calving; and manipulation of the mineral content of prepartum diets. For this manuscript, we will focus on the effects of altering the dietary-cation anion difference (**DCAD**) of prepartum diets.

Altering the dietary cation-anion difference

Although clear statistics are not available, it is likely that in the US today, manipulations of the DCAD are the most widely implemented dietary method to reduce the risk of hypocalcemia in dairy cows. Feeding acidogenic diets induce a state of compensated metabolic acidosis that increases the concentrations of iCa in blood through different pathways. The reduction in blood pH increases the ionized fraction of Ca by displacing it from albumin. Metabolic acidosis increases parathyroid hormone secretion (Lopez et al., 2002), increases sensitivity of tissues to PTH (Goff et al., 2014), and increases the expression of PTH receptor in the kidney of cattle (Rodriguez et al., 2016). In general, the maximal PTH response to Ca chelation occurs during metabolic acidosis compared with normal blood pH (Lopez et al., 2002).

When salts containing strong anions are fed, the premise is that the rate and extent of absorption of the anion is greater than that of the cation in the salt or that the cation is metabolized such that it is not absorbed as a cation. In the latter case, the

cation in the salt can be utilized by the rumen microbes such as in the case of ammonium chloride. The strong anions most commonly supplemented in diets are chloride (**Cl^-**) and sulfur (**S^{2-}**) in the form of sulfates (**SO_4^{2-}**). It is thought that Cl^- is more bioavailable than S^{2-} so it has greater acidifying power. When salts of Cl and SO_4 are fed, for instance $CaCl_2$, more equivalents of the anion (Cl^-) from the molecule is absorbed than the cation (Ca^{2+}). This causes an imbalance in charges in the epithelial cell of the gut (increase in negative charges), which forces secretion of bicarbonate (**HCO_3^-**) into the intestinal lumen or retention of H^+ ions. The end result is a loss of HCO_3^- and an increase in H^+ concentration which ultimately results in a state of metabolic acidosis. If compensated, then minor changes in blood pH occur, with changes in blood HCO_3^- and the partial pressure of carbon dioxide (**CO_2**) caused by changes in respiration rate. A common finding is aciduria because of the increased proton excretion in urine as part of the compensatory mechanism.

Overwhelming evidence from controlled experiments demonstrate that feeding acidogenic diets prepartum reduce the risk of milk fever and SCH in dairy cows (Ender et al., 1971; Block et al., 1984; Martinez et al., 2018b); however, less certainty exists about the effects of acidogenic diets on lactation performance and incidence of diseases other than hypocalcemia.

We recently completed a meta-analysis of the published literature with randomized controlled experiments with transition cows in which prepartum diets fed as totally mixed rations had the mineral composition altered to manipulate the DCAD or cows were fed diets with acidogenic products in which the contents of Ca, phosphorus (**P**), or magnesium (**Mg**) were manipulated (Santos et al., 2018).

A total of 42 experiments were included in the data base, with 5 experiments reporting data for nulliparous totaling 151 cows and 41 experiments that reported data for 1,652 parous cows. The DCAD ([mEq of K+ + mEq of Na+] – [mEq of Cl− + mEq of S^{2-}]) of prepartum diets ranged from -246 to 1,094 mEq/kg and concentrations of dietary Ca (0.16 to 1.98%), P (0.18 to 1.58%), and Mg (0.09 to 0.68%) had wide ranges that allowed us to evaluate their associations with production and health. Diets were fed for an averaged (± SD) of 21.9 ± 1.8 and 25.6 ± 1.0 d for nulliparous and parous cows, respectively.

Effects of altering the prepartum DCAD on DM intake pre- and postpartum

Data were analyzed with 115 treatment means from 36 of the 42 experiments that reported DM intake prepartum. Manipulating the DCAD resulted in a quadratic response with a decrease in prepartum DM intake. As the DCAD decreased, DM intake prepartum also decreased in both nulliparous and parous cows with no interaction between DCAD and parity group. Reducing the prepartum DCAD from 200 to -100 mEq/kg resulted in a 0.7 and 0.4 kg/d reduction in DM intake in nulliparous and parous cows. Concentrations of Ca, P, and Mg in prepartum diets did not influence DM intake prepartum. We then analyzed data on prepartum DMI incorporating type of acidogenic product fed as none for diets without any acidogenic product, salts for diets in which

acidogenic salts were used, or commercial products for diets in which the acidogenic product was a designed commercial product to determine if the depression was induced by use of a source of strong ions. Inclusion of salts or commercial product reduced (P < 0.01) DMI prepartum irrespective of the source of strong ions fed (none = 9.0 ± 0.4 vs. salts = 8.5 ± 0.4 vs. commercial products = 8.4 ± 0.4 kg/d).

Intake postpartum was reported in 26 experiments with 86 treatment means. As opposed to prepartum intake, a reduction in prepartum DCAD increased postpartum DM intake in both parity groups. An increment of approximately 1 kg/d in DM intake was estimated by reducing the prepartum DCAD from 200 to -100 mEq/kg (Santos et al., 2018).

It is often debated the reason acidogenic diets influence prepartum DM intake in dairy cows. In some cases, authors suggested that palatability of acidogenic salts is the culprit for such depression, although the decrease is observed even when cows are fed commercial products. We recently completed an experiment to address this question (Zimpel et al., 2018). We used 10 nulliparous pregnant non-lactating Holstein cows that were subjected to a replicated 5 x 5 Latin square design. The experiment was composed by 5 periods of 14 days each and all 10 cows received all 5 treatments. Diets were fed as total mixed rations and composed of corn silage, Bermuda hay, and concentrates. Diets were manipulated by replacing a portion of the grain in the concentrates with an acidogenic product or salts containing potassium (**K**), sodium (**Na**), and Cl. Dietary treatments were:

T1. (K = 1.42%, Na = 0.04%, Cl = 0.26% of DM) a base diet containing 55% corn silage, 10% grass hay, and 35% concentrate that resulted in a DCAD of +200 mEq/kg;
T2. (K = 1.83%, Na = 0.42%, Cl = 1.23% of DM), the control diet with 2% added mixture of 1:1 NaCl and KCl to result in a DCAD of +200 mEq/kg;
T3. (K = 1.71%, Na = 0.54%, Cl = 0.89% of DM), the control diet with added acidogenic product and a mixture of K_2CO_3 and $NaHCO_3$ to result in a DCAD of +200 mEq/kg;
T4. (K = 1.29%, Na = 0.13%, Cl = 0.91% of DM), the control diet with added acidogenic product to reduce the DCAD to -120 mEq/kg; and
T5. (K = 1.78%, Na = 0.53%, Cl = 2.03% of DM), the control diet with added acidogenic product, KCl, and NaCl to result in a DCAD of -120 mEq/kg.

Therefore, T1, T2 and T3 had different contents of Cl and addition or not of acidogenic product, but the same positive DCAD, whereas T4 and T5 had distinct amounts of Cl, but the same negative DCAD. Intake of DM and water was monitored daily and feeding behavior was evaluated for 48 h in each period. Blood and urine samples were collected multiple times from each cow in each period for measurements of acid-base status and urinary excretion of minerals.

A summary of some important findings in the experiment is presented in **Table 1**. Adding chloride salts, including the acidogenic product without altering the acid-base status of cows did not affect dry matter intake (see T1, T2 and T3); however, when the

acidogenic product reduced the DCAD from +200 to -120 mEq/kg in treatments T4 and T5, then cows experienced a compensated metabolic acidosis with reduced blood and urinary pH, increased respiratory rate, and reduced blood bicarbonate (HCO_3^-) and partial pressure of CO_2 (**pCO_2**), which reduced dry matter intake. It is important to note that addition of acidogenic product per se, as in T3, did not reduce dry matter intake. In fact, if one compares intake in treatments T1, T2 and T3, it is clear that not only they did not differ statistically, but they were numerically very similar, ranging from 10.2 to 10.3 kg/day (or 1.76 to 1.74% of body weight). On the other hand, when adding the acidogenic product induced a metabolic acidosis, like in the case of T4 and T5, regardless of level of Cl in the diet, then DM intakes decreased to 9.7 and 9.5 kg/d (1.68 and 1.64% of body weight), respectively (Zimpel et al., 2018). These results demonstrate that depression in intake is not necessarily related to the inclusion of acidogenic products but caused by the metabolic acidosis induced by the acidogenic diet.

Effects of altering the prepartum DCAD on production performance

Numerous experiments have evaluated the impact of manipulating the prepartum DCAD on postpartum performance in dairy cows and, in many cases, numerical differences were observed without statistical effect.

Interactions between level of DCAD and parity group were observed for yields of milk, fat-corrected milk, fat, and protein. Reducing the DCAD increased yields of milk, fat-corrected milk, fat, and protein in parous cows; however, a similar manipulation in DCAD either did not influence yields of milk but tended to reduce those of fat-corrected milk and protein and reduced that of milk fat in nulliparous cows (**Table 2**; Santos et al., 2018).

It is clear that parous cows respond positively to acidogenic diets with increased yields of milk and milk components. Nevertheless, heterogeneity in response to manipulations in DCAD prepartum also have been reported by others (Lean et al., 2014), and productive responses of nulliparous to acidogenic diets do not seem to be the same as that observed for parous cows. Parous cows are more prone to disturbances of Ca metabolism with the onset of lactation (Lean et al., 2006), and hypocalcemia is known to depress appetite (Martinez et al., 2014), which can compromise lactation performance. One of the limitations of the meta-analysis by Santos et al. (2018) is that only 5 experiments with 15 treatment means reported production performance for nulliparous cows. This limited data base might have precluded detection of a clearer productive response of nulliparous to manipulation of prepartum DCAD.

Effects of altering the prepartum DCAD on incidence of diseases in early lactation

Milk fever and retained placenta were reported in most experiments, whereas metritis, mastitis and displaced abomasum were reported in only half of the experiments reviewed in the meta-analysis. Because milk fever affected only parous cows, nulliparous were not included in the statistical models. As expected, DCAD had a

profound effect on incidence of milk fever. The predicted incidence in parous cows reduced from 11.7 to 2.8% by reducing the DCAD from 200 to -100 mEq/kg (**Table 3**). Moreover, the benefits of diets with negative DCAD were also observed for retained placenta and metritis. Incidence of retained placenta and metritis decreased with a reduction in DCAD and the benefits were observed in nulliparous and parous cows (**Table 3**). The incidence of mastitis and displaced abomasum were not influenced by DCAD of prepartum diets and no interaction between DCAD and parity group were observed for those two diseases. Number of disease events per cow declined with a reduction in DCAD of prepartum diets in both nulliparous and parous cows.

We had previously shown that cows that develop SCH have suppressed innate immune function and increased risk of uterine diseases (Martinez et al., 2012). When SCH was induced in dry cows, neutrophil function was suppressed for at least 72 h after concentrations of tCa and iCa had been reestablished in blood of cows. It is well described that innate immunity is critical for shedding of the placental tissues and protection of the reproductive tract against invading pathogens. Because cows fed acidogenic diets maintain increased tCa and iCa concentrations on the day of calving and the first days postpartum, they are less likely to develop SCH, which likely improves innate defenses of the uterus, thereby minimizing the risk of retained placenta and metritis.

Although cows fed acidogenic diets had reduced incidence of milk fever and metritis and consumed more DM postpartum, the risk of displaced abomasum did not change. It is important to mention that only 14 experiments reported displaced abomasum incidence. Also, some experiments removed cows from the data base if they developed issues at calving. Therefore, it is possible that lack of differences in risk of displaced abomasum might have been influenced by either the limited data base or by potential exclusion of cows from experiments.

Duration of feeding acidogenic diets and level of DCAD

To our knowledge, only three experiments have evaluated the impact of duration of feeding of acidogenic diets prepartum (Weich et al., 2013; Wu et al., 2014; Lopera et al., 2018). Sixty cows were fed one of 3 treatments starting at 42 d relative to the expected calving date. Treatments were a control diet (+120 mEq/kg), a positive DCAD in the first 21 d followed by a negative DCAD diet in the final 21 d of gestation (+120 mEq/kg followed by -160 mEq/kg), or 42-d of feeding a negative DCAD diet (-160 mEq/kg). The authors found feeding acidogenic salts for the last 21 d of gestation improved Ca homeostasis and milk yield (5.6 kg/d). They also found that extending the feeding of acidogenic salts from 21 to 42 d had no statistically significant effect on the subsequent lactation, although cows fed the diet for the extended period produced 2.3 kg less milk (44.8 vs. 42.5 kg/d). Wu et al. (2014) showed no differences in postpartum performance when prepartum cows were fed a diet with a DCAD of -210 mEq/kg for the last 3, 4 or 6 weeks of gestation.

We have recently explored this question and evaluated the effects of feeding acidogenic diets for the last 21 or 42 d of gestation at two levels of DCAD, -70 or -180 mEq/kg (Lopera et al., 2018). We enrolled 114 parous Holstein cows at 230 d of gestation, 48 that completed lactation 1 and 66 that completed lactation > 1. Cows were randomly assigned to 1 of 4 treatments arranged as a 2 x 2 factorial; treatments varied by level of DCAD, -70 or -180 mEq/kg, and by length of feeding, the last 21 d (Short) or the last 42 d (Long) prepartum. Therefore, the 4 treatments were Short -70 (n = 29), Short -180 (n = 29), Long -70 (n = 28) and Long -180 (n = 28). Cows in the Short treatments were fed a diet with positive DCAD of +110 mEq/kg of DM from -42 to -22 d relative to calving. After calving, cows were fed the same diet and lactation performance and incidence of diseases were evaluated for the first 42 DIM, whereas reproduction and survival was evaluated for 305 d postpartum. Reducing the DCAD linearly decreased prepartum DM intake between -42 and -22 d relative to calving (+110 mEq/kg = 11.5 vs. -70 mEq/kg = 10.7 vs. -180 mEq/kg =10.2 ± 0.4) and the diet with -180 mEq/kg fed in the last 21 d of the dry period reduced intake by 1.1 kg/d compared with the diet containing -70 mEq/kg (-70 mEq/kg = 10.8 vs. -180 mEq/kg = 9.7 ± 0.5 kg/d). Cows fed the -180 mEq/kg diet had increased concentrations of iCa in blood on the day of calving (-70 mEq/kg = 1.063 vs. -180 mEq/kg = 1.128 ± 0.020 m*M*), but no differences were observed in the days following calving. Extending the duration of feeding the diets with negative DCAD from 21 to 42 d reduced gestation length by 2 d (Short = 277.2 vs. Long = 275.3 d), milk yield by 2.5 kg/d (Short = 40.4 vs. Long = 37.9 ± 1.0 kg/d) and tended to increase days open because of reduced pregnancy per AI after all inseminations (Short = 35.0 vs. Long = 22.6%).

Although other experiments would suggest some flexibility in how long acidogenic diets should be fed prepartum, the results of Lopera et al. (2018) suggest that feeding these diets for longer than 21 d might not be advised. In any case, there seems to be no advantage of extending the feeding of acidogenic diets for the entire dry period and a potential loss in production and, perhaps, reproduction. Although the meta-analysis did not reveal a value of negative DCAD that optimized postpartum performance and health (Santos et al., 2018), the results of Lopera et al. (2018) suggest that there is no need to reduce the DCAD to -180 mEq/kg.

Conclusions

Hypocalcemia is a prevalent metabolic disorder in dairy cows in early lactation. Cows develop SCH and milk fever because of inability to either mobilize Ca from bones or readjust gastrointestinal absorption at the onset of colostrogenesis and lactation to replenish the blood Ca pool. Hypocalcemia increases the risks of numerous other health problems resulting in economic losses to dairy producers. Dietary manipulation by feeding acidogenic diets remains the method of choice for prevention of hypocalcemia. The foundation of acidogenic diets should be based on limiting the intakes of Na, K, at the same time that strong anions, particularly Cl, are supplemented. The metabolic acidosis induced by acidogenic diets is expected to depress DM intake prepartum, but prevention of clinical and SCH have long-last benefits to dairy cows, particularly those that become multiparous cows. Acidogenic diets reduce the risk of milk fever, SCH,

retained placenta, and metritis; they increase DM intake postpartum, and productive performance in parous cows. The ideal DCAD remains to be established, but it is likely that for parous cows it does not need to be less than -150 mEq/kg, whereas for nulliparous the data are scarce, and the potential benefits remain unclear.

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Table 1. Effect of manipulating the dietary cation-anion difference on acid-base status and intake in dry cows[1]

Item	T1	T2	T3	T4	T5	SE[2]
Intake DM, kg/d*§	10.3	10.2	10.2	9.7	9.5	0.2
Intake DM, % of BW*§	1.76	1.75	1.74	1.68	1.64	0.03
Intake of water, L/d§‡ †	25.4	31.0	30.5	26.5	32.3	0.8
Urine pH*§‡	8.1	7.9	7.9	5.7	5.6	0.06
Blood						
pH*§‡	7.450	7.436	7.435	7.420	7.416	0.005
Base excess, m*M* *§	1.85	1.20	1.45	-0.20	-0.95	0.32
HCO_3^-, m*M* *§	25.9	25.5	25.8	24.3	23.7	0.3
pCO_2, mm Hg§	37.4	38.2	38.4	37.0	36.6	0.7
Respiratory rate, n/min§	27.6	27.3	26.8	28.4	29.0	0.4

[1] Data from Zimpel et al. (2018)
[2] SE = standard error.
* Effect of adding acidogenic product: T1 vs. T4 ($P < 0.05$).
§ Effect of metabolic acidosis: T2 + T3 vs. T4 + T5 ($P < 0.05$).
‡ Effect of adding Cl salts to alkalogenic diet: T1 vs. T2 ($P < 0.05$).
† Effect of adding Cl salts to acidogenic diet: T4 vs. T5 ($P < 0.05$).

Table 2. Estimated effect (means and SE) of reducing the DCAD from +200 to -100 mEq/kg on intake and yields of milk and milk components in Holstein cows[1]

		Nulliparous		Parous		*P*-value[2]	
Item	Means (Exp.)[3]	+200	-100	+200	-100	DCAD	DCAD x parity
DM intake, kg/d							
Prepartum	115 (36)	10.3 ± 0.5	9.6 ± 0.5	12.4 ± 0.4	12.0 ± 0.4	0.02	0.49
Postpartum	86 (26)	12.9 ± 0.9	13.7 ± 0.9	17.6 ± 0.7	18.6 ± 0.7	0.03	0.81
Yield, kg/d							
Milk	90 (28)	25.9 ± 1.3^{c}	24.5 ± 1.3^{c}	36.2 ± 1.1^{b}	37.9 ± 1.1^{a}	0.74	0.03
Fat-corrected milk	84 (25)	26.6 ± 1.9^{c}	24.5 ± 1.9^{d}	38.8 ± 1.8^{b}	39.9 ± 1.8^{a}	0.90	0.002
Fat	84 (25)	0.995 ± 0.073^{c}	0.888 ± 0.073^{d}	1.438 ± 0.066^{b}	1.512 ± 0.066^{a}	0.60	0.006
Protein	84 (25)	0.755 ± 0.053^{c}	0.695 ± 0.053^{c}	1.115 ± 0.047^{b}	1.139 ± 0.047^{a}	0.44	0.07

^{a,b,c,d} Within a row, superscripts differ ($P < 0.05$).

[1] Data from Santos et al. (2018).

[2] DCAD = effect of DCAD; DCAD x parity = interaction between DCAD and parity.

[3] Number of treatment means and number of experiments (Exp.) for each response analyzed.

Table 3. Estimated effect (means and SE) of reducing the DCAD from +200 to -100 mEq/kg on incidence of diseases in Holstein cows[1]

		Nulliparous		Parous		*P*-value[2]	
Item (% ± SEM)	Means (Exp.)[2]	+200	-100	+200	-100	DCAD	DCAD x parity
Milk fever, parous cow	99 (35)	0	0	11.7 ± 2.8	2.8 ± 0.9	< 0.001	NE[3]
Retained placenta	73 (22)	12.7 ± 2.7	3.5 ± 2.7	17.0 ± 1.6	9.0 ± 1.6	0.05	0.61
Metritis	42 (12)	34.4 ± 5.6	12.0 ± 5.6	16.3 ± 2.7	9.9 ± 2.7	0.02	0.34
Cases per cow, n ± SEM	108 (35)	0.34 ± 0.07	0.20 ± 0.07	0.39 ± 0.04	0.17 ± 0.04	< 0.001	0.56

[a,b,c,d] Within a row, superscript differ ($P < 0.05$).

[1] Data from Santos et al. (2018).

[2] DCAD = effect of altering the DCAD; DCAD x parity = interaction between DCAD and parity (nulliparous or parous).

[3] Number of treatment means and number of experiments (Exp.) for each response analyzed.

[4] Non-estimable because of no incidence in nulliparous cows

SESSION NOTES