Can Negative DCAD Diets Fed Prepartum Improve Reproductive Efficiency of Dairy Cows?

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Take Home Message

- Cows fed a negative DCAD diet prepartum (-24 mEq/100g of DM) had lower urine pH (pH = 5.7) than cows not fed a negative DCAD diet prepartum (+6 mEq/100g of DM) (pH > 8.0).
- Cows fed a negative DCAD diet prepartum (-24 mEq/100g of DM) had improved days to first ovulation than cows not fed a negative DCAD diet prepartum (+6 mEq/100g of DM).
- Cows fed a negative DCAD diet prepartum (-24 mEq/100g of DM) with high concentration of Ca (2.0% DM) tended to be more likely to become pregnant than cows not fed a negative DCAD diet prepartum (+6 mEq/100g of DM).
- Cows fed a negative DCAD diet prepartum (-24 mEq/100g of DM) with high concentration of Ca (2.0% DM) had improved uterine glandular epithelial cells, greater SOD activity, and lower GPX activity than cows fed a negative DCAD diet prepartum (-24 mEq/100g of DM) with low concentration of Ca (0.40% DM).
- A negative DCAD diet prepartum seems to alleviate uterine tissue damage and is amplified by increasing Ca supplementation.

Introduction

The periparturent period is defined in dairy cows as a state of near maintenance requirements in late gestation to that of rapidly increasing metabolic and nutrient demands needed for the onset of lactation. At parturition, the dairy cow is subjected to various challenges that can affect her future lactations and fertility. Failure or tardiness to adapt to these changes can leave the cow predisposed to metabolic disorders such as ketosis, acidosis, and displaced abomasum (Drackley, 1999; Vernon, 2005), or infectious disorders such as metritis and mastitis (Mulligan et al., 2006; Sheldon et al., 2002a), calving related disorders such as dystocia, retained placenta, and decreased fertility (Roche et al., 2000; Sheldon et al., 2002b). What is thought to be an underlying issue in these disorders is an imbalance in calcium homeostasis at the onset of lactation. Calcium (**Ca**), an important macromineral involved in smooth muscle contraction, milk synthesis, and immune cell activation, reaches a nadir in the first days after parturition at the onset of lactation. An estimated 5-10% (Oetzel, 2011) of dairy cows experience hypocalcemia (**HC**; milk fever, parturient paresis) at the time of parturition, with an estimated cost of $246 per case (Liang et al., 2016). The prevalence of subclinical hypocalcemia [**SCH**; estimated at 33% of all 1st lactation or greater (Reinhardt et al., 2010)] is considerably higher, therefore, its economic impact is more significant than HC. Subclinical hypocalcemia is often undiagnosed and primes the cow for other various disorders as well as decreased milk production (Horst et al., 1997; Goff, 2008). The homeostasis of Ca is often challenged at parturition due to a decrease in Ca signaling sensitivity from an oversaturation of Ca in the prepartum diet (Horst, 1986). Parathyroid hormone (**PTH**) is a calciotropic hormone that is important in absorption of dietary Ca and reabsorption of Ca stores (bones, skeletal muscle). Prepartum nutritional management is paramount in a successful transition from non-lactating to lactating cow (Drackley, 1999; Overton and Waldron, 2004). Treatments to lessen the effects of HC and SCH have been in use for decades, however, feeding management as a prevention of Ca imbalance at parturition has become more commonly used in the recent years (Reinhardt et al., 2010; Horst et al., 1997; Goff, 2008). Overfeeding Ca in the last four weeks before parturition has been associated with high incidences of SCH and HC (Horst, 1986; Overton and Waldron, 2004). However, formulating a prepartum diet that only meets and not exceeds dietary Ca is challenging.

In recent years, effective dry cow management has been a focal point in dairy cow research. Due to challenges faced by the periparturient cow, she is susceptible to a host of metabolic diseases (Drackley, 1999; Vernon, 2005) as well as infection from opportunistic bacteria (Mulligan et al., 2006; Sheldon et al., 2002a). This susceptibility to disease is not easily defined to a single factor, rather, it is a complex system of changing metabolic demands, resistance to bacterial contamination of the uterine lumen, and coordination of various tissues to meet the demands of lactation and a return to a heathy state. Failure to adapt can delay cow's uterine involution process, return to ovarian cyclic activity, and diminish her immune response (Sheldon et al., 2006, Griffin et al., 1974; Kimura et

al., 2006). At the time of parturition, the cow must successfully transition from fairy low energy requirements to rapid mobilization of her endogenous energy stores (Bell, 1995), as well as increasing her exogenous energy intake (Grummer et al., 2004, Vernon, 2005). Failure to transition through this period leaves the cow in a negative energy balance (**NEB**) for a prolonged period of time (Esposito, 2014), thus increasing her likelihood of disease (Hammon, 2006).

At the onset of lactation, Ca requirements increase 4-fold to meet the demands of lactogenesis (Horst et al., 1997). To satisfy this demand the cow will first increase absorption of exogenous Ca from her diet, and second, pull Ca from her own stores (bone and skeletal muscle). However, this action can be disrupted if the cow is exposed to a high dietary calcium in her prepartum diet. This is due to the actions of the calciotropic hormone, PTH. Parathyroid hormone regulates calcium homeostasis by increasing intestinal Ca absorption and renal Ca reabsorption (Goff et al., 2004; Littledike et al., 1987). If exogenous Ca does not meet the Ca demand, PTH will act upon endogenous Ca stores. If exposed to high dietary calcium in the prepartum phase, sensitivity to PTH diminishes as well as PTH receptor numbers (Goff, 2008; Horst, 1986), leaving the cow unable to adapt effectively to the increased Ca demands from colostrum and milk synthesis and instead must rely on her endogenous sources. Prolonged dependency on endogenous Ca sources puts the cow in a negative Ca balance, and in severe cases, induce HC, or more often, SCH.

An increasingly popular dry cow management practice is to feed an acidogenic or partially acidogenic diet to cows entering the close-up period up until parturition. This is achieved by manipulating the dietary cation-anion difference (**DCAD**) in the diet. Acidification of the diet is realized when the concentration of anions is greater than that of the concentration of cations. This increases anion absorption in circulating blood and leaves the cow in a slight state of metabolic acidosis. Various studies (Charbonneau et al., 2006; Goff et al., 2004; Santos et al., 2019) have examined this effect and observed that this practice decreases the chances of the cow developing HC and SCH. The mechanisms of this action is achieved by maintaining PTH sensitivity to calcium homeostasis and increasing calciotropic receptor numbers (DeGaris et al., 2008). Feeding a negative DCAD diet in the prepartum phase of the peripartiuent period has been observed to increase the subsequent reproductive performance of the cow as well as improve the calcium status of the cow (Martinez et al., 2018; Chapinal et al., 2012).

Hypocalcemia and Fertility

Hypocalcemia is one of the most prevalent and perhaps the most costly metabolic disorders to affect dairy cows (Oetzel, 2011). Dairy cows generally have 8.5-10 mg/dL of circulating Ca (Allen, 2016). Hypocalcemia, as defined by the Merck Veterinary Manual, as < 7.5 mg/dL of circulating Ca (Allen, 2016). Oetzel (2011) review of the disease observed 5-10% of all dairy cows in the periparturient period were affected by clinical hypocalcemia (milk fever), resulting in economic losses from deaths, increased culling rates, and decreased milk production, in addition to treatment costs. Symptoms of milk fever, as it is defined by Merck Veterinary Manual, can be separated into 3 stages: Stage 1: fine muscle tremors, restlessness, with excessive ear twitching and head bobbing; Stage 2: unable to stand, decreased body temperature, cold ears, and constipation; Stage 3: loss of consciousness, complete muscle flaccidity, and sever bloat. If left untreated, HC can result in cow death. Subclinical hypocalcemia effects about 33-47% of 2nd lactation or greater (Reinhardt et al., 2011) dairy cows around the time of calving. Subclinical hypocalcemia is defined as depressed Ca concentration in the blood without showing clinical signs of milk fever. While the outward effects of SCH are not as pronounced as HC, the economic effect is profound. Affecting half of the milking herd in the United States, it often goes undiagnosed and the disease can manifest into increased susceptibility to secondary diseases such as ketosis, metritis, mastitis, retained placenta, lameness, and milk fever, as well as long-term losses in production efficiency [decreased dry matter intake (**DMI**) and milk productivity; (Goff, 2008)].

Caixeta et al. (2017) examined the effect of Ca status through the first 3 days postpartum and observed that cows that had SCH (cows with blood Ca ≤ 8.6 mg/dL for at least one of the first 3 days postpartum) had an elevated number of cows in NEB when compared to eucalcemic animals. The study also revealed that chronic SCH (cSCH) cows (cows with blood Ca ≤ 8.6 mg/dL for all first 3 days postpartum) had lower odds of being pregnant at first service than eucalcemic cows. Moreover, the authors observed that cows that had SCH also had higher incidences of disease when compared to eucalcemic cows and this effect was more pronounced in cSCH. Feeding behavior of the cow as well as feeding strategies employed by the producer in the close-up dry period can influence the cows' ability to affectively use Ca in the days following parturition. Bell (1995) estimation of nutrient and energy demands to increase dramatically from day 250 of gestation to 4 days postpartum (tripling the demand for glucose, doubling the demand for amino

acids, and approximately quintupling the demand of fatty acids). In addition to this, Ca requirements increase about fourfold on the day of parturition (Horst et al., 1997). It has been reported by multiple studies (Caixeta et al., 2017; Kamgarpour et al., 1999; Horst, 1986) for Ca concentrations in the blood to reach a nadir on the first day of lactation as the cow adapts to the metabolic changes. However, as was examined by Caixeta et al. (2017), the ability of the cow to adapt to these changes, i.e. failure to do so, will negatively affect the reproductive performance and incidence of disease of the cow. In a meta-analysis, Santos et al. (2019) concluded that Reducing the DCAD of diets fed to prepartum cows reduced DMI prepartum but increased postpartum intake. Multiparous cows produced more milk, fat-corrected milk, fat, and protein when fed acidogenic diets prepartum, but a similar response was not observed in nulliparous cows. The authors hypothesized that it was possible that the limited number of experiments reporting data on nulliparous precluded a more precise estimate of the effects of manipulating the DCAD on that group of cows. Their findings support the recommendation of feeding acidogenic diets to multiparous cows to improve Ca metabolism around calving, reduce the risk of milk fever and uterine diseases, and improve lactation performance (Santos et al., 2019).

Calcium metabolism in the periparturent period is extremely important for the cow to have a successful transition to lactation. Horst (1986) examined calcium homeostasis during the transition period and observed that to minimize incidence of clinical hypocalcemia, he recommended to keep dietary Ca intake at ≤ 50 g/d in the prepartum phase. At the onset of lactation, the cow will secrete up to 50 g/d of Ca (Allen, 2016) in the production of colostrum and milk. This sudden outflow of Ca into milk will cause plasma Ca levels to drop and to increase the release calciotropic hormones such as PTH and 1,25-dihydroxyvitamin D3. The parathyroid hormone first promotes Ca absorption in the intestines and Ca reabsorption through the renal system, however if PTH continues to be released, it will stimulate the resorption of Ca from skeletal muscle and bones (Oetzel, 2011; Horst et al., 1994; DeGaris and Lean, 2008). High circulating plasma Ca concentrations (due to high dietary Ca intake) in the prepartum phase can decrease the sensitivity of PTH on target cells in the intestines and renal system (decreasing dietary Ca absorption/reabsorption), thus increasing Ca release from reservoirs in the bones and skeletal muscles (DeGaris and Lean, 2008). The loss of sensitivity of the PTH can be unsustainable for the cow at onset of lactation as she pulls more Ca from her own reserves to meet the demands of milk production and will result in parturient paresis (Goff, 2008).

At the onset of HC, there are various options for treatment. Due to the paralysis experienced as a result of HC, the weight of the cow will cut off blood supply to the down side of the cow which will begin to cause necrosis in as little as 4 hours, therefore, treatment should begin at first signs of HC. Goff (2008) discussed the efficacy of these different treatments of HC extensively. The most effective method is to administer an IV injection on Ca salts (Ca borogluconate being the most common) at a dosage of 2 g Ca/100 kg of body weight at a rate of 1 g/min. Other methods include subcutaneous injection of Ca salts, however these have falling out of favor as they require 6-10 separate, 50-75 mL shots to be administered for an effective dosage. Oral administration of Ca can fall between treatment and preventative measure. This concept is if the cow's ability to absorb dietary calcium is compromised due to intercellular transport (due to loss of PTH sensitivity), administration of an oral bolus of upwards of 125 g Ca/dose will cause an osmotic difference in the intestines and allow the possibility of passive diffusion of Ca through epithelial tight junctions and into the vascular system.

Recently, there have been numerous studies aimed in identification and prevention of SCH and HC in the transition period (Neves et al., 2016; Oetzel, 2011, Caixeta et al., 2017; Goff, 2006). The most prolific of these strategies in the last 3 decades has been to feed an acidogenic diet in the prepartum phase of the transition period (NRC, 2001; Goff, 2008; Neves, 2016; Horst et al., 1997; Jawor et al., 2012; Overton and Waldron, 2004). This is achieved by feeding the cow an anionic salt supplement to obtain a negative dietary cation-anion difference (DCAD) in the formulation of the diet. The increased concentration of circulating cations (K+, Na+, Ca2+, and Mg2+) prepartum can effectively alkalinize the blood and induce conformational changes in the PTH receptor in target tissues and a loss of sensitivity to the hormone (Littledike and Goff, 1987). In order to achieve the acidogenic diet, the diet will need to be formulated so that the dietary anions (Cl-, S-2, and P-3) are in greater concentration than the dietary cations. Dietary cation-anion difference is commonly measured as milliequivalents per kilogram (mEq/kg) of DM, however, there are various ways to calculate the DCAD in the diet, with one of the earliest equations being from Ender et al. (1971), a four variable equation:

$$DCAD = (Na^+ + K^+) - (Cl^- + S^{-2})$$

It took into consideration the four largest DCAD influencers to be represented equally. Mongin (1981) iteration of this equation assumed dietary sulfur was met (0.2% S DM, 13 mEq of S/100 g DM; NRC (2001):

$$DCAD=(Na^+ + K^+ - Cl^-)$$

Finally, if we truly want to incorporate all the major cations and anions fed in the diet, the equation could be expressed as:

$$DCAD=(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + S^{-2} + P^{-3})$$

However, with this equation, we assume that Ca2+ and Mg2+ are as strong of alkalizing agents as Na+ and K+ and that S-2 and P-3 are as strong of acidifying agents as Cl-. Goff and Horst (1997) and Goff et al. (2004) examined the acidifying/alkalizing capacity of the different anions and cations in various forms and observed differences, and assigned coefficients to these variables based on their acidification/alkalizing capacity:

$$DCAD=(Na^+ + K^+ + 0.15Ca^{2+} + 0.15Mg^{2+}) - (Cl^- + 0.6S^{-2} + 0.5P^{-3})$$

When formulating prepartum diets with a negative DCAD most nutritionists and ration formulation software follow either Goff and Horst (1997) or Ender et al. (1971) iteration of the equation. Mulligan et al. (2006) determined three criteria to be met when formulating a negative DCAD diet: 1) keep DCAD between -100 to -200 mEq/kg DM; 2) dietary Ca concentration should be approximately 1.2% of the diet; and 3) urine pH should stay between 6-6.8. There has been debate surrounding the second criteria in the last couple of two decades, as Oetzel et al. (1991) cautioned against having Ca at 1.16% DM as it increased risk of milk fever. Since then, determination of dietary Ca inclusion with a negative DCAD has not been clearly defined (Chan et al., 2006; Charbonneau et al., 2006; Kronqvist et al., 2009; Esposito et al., 2014; Weich et al., 2013).

The NRC for dairy cattle recommends the required absorbed Ca for a pregnant cow in her third trimester to be represented by the exponential equation:

$$Ca\ (g/day) = 0.02456e^{(0.05581-0.00007t)t} - 0.02456e^{(0.05581-0.00007(t-1)(t-1))}$$

Where t = day of gestation

Whereas the recommended requirement for a lactating cow Ca absorbed to be 1.22 g Ca/kg of milk produced. Meeting these necessary absorbed values depends greatly on the bioavailability of Ca in feedstuffs as well as inorganic Ca sources in the diet. While it is difficult to control for Ca available in feedstuff, particularly in fresh cow diets, many producers rely on inorganic sources to meet Ca requirements. Calcium absorption will generally equate to the body requirement if the diet has enough Ca available for absorption. However, depending on the Ca source, the rate of inclusion of the inorganic Ca source may vary (Table 1). There has not been much studies on the effect of a negative DCAD diet on different Ca sources, however, Oetzel et al. (1988) observed that feeding a negative DCAD (-75 mEq/kg DM) when compared to feeding a positive DCAD (189 mEq/kg DM) increased the rate of absorption and increased circulating total and ionized Ca concentrations, improving overall Ca absorption and usage. While not a direct inorganic Ca source, vitamin D is heavily involved in Ca metabolism. When sourced from calcidiol, vitamin D supplementation, when paired with a negative DCAD (-124 ± 11 mEq/kg DM) prepartum diet, increased circulating vitamin D metabolites and improve reproductive performance of transition dairy cows (Rodney et al., 2018; Martinez et al., 2014). It has been observed that feeding a negative DCAD (-100 mEq/kg DM) diet in prepartum diets increases the rate of Ca excretion in urine (Razzaghi et al., 2012), however it also has been observed to increase plasma Ca concentrations in the first day postpartum, when the cow is at most risk of HC or SCH (Razzaghi et al., 2012).

During the process of parturition, the uterus of the cow becomes open to the outside as the cervix, vagina, and vulva open for the passage of the calf. This opening allows for the introduction of pathogens into the uterine environment and could result in infection (Sheldon et al., 2006). While there is evidence of a bacterial symbiotic relationship in the uterus (Sheldon et al., 2006), development of infection occurs in states of uterine dysbiosis in the days following parturition (Sheldon et al., 2006; Rodney et al., 2018). Clinically, incidences of metritis is defined as vaginal discharge with purulent or brown characteristics, and a fetid odor accompanied by a fever (rectal temperature ≥ 39.2°C) (Sheldon et al., 2006; Esposito et al., 2014). Pathologically, metritis is defined as inflammation in the entirety of the uterine wall, whereas endometritis is defined as inflammation limited only to the endometrium lining of the uterus. This inflammation is often accompanied with bacterial contamination; however it is not a prerequisite for diagnosis (Sheldon and Dobson, 2004). Inflammation after parturition is expected, however, if sustained over long periods of time, it can result in decreased fertility and reproductive performance (Sheldon et al., 2006; Sheldon and Dobson, 2004; Ribeiro et al., 2016; Bromfield et al., 2013) and that in the absence

of bacterial infection, inflammation can still have detrimental effects on fertility (Hansen et al., 2004). While it is evident that the first 2 weeks postpartum is when we can expect bacterial contamination, this does not always result in uterine infection (Sheldon and Dobson, 2004). The authors discuss that the process of inflammation of the uterine lining is common and a necessary part of the innate immune system for the clearance and sloughing of tissue and bacterial contaminants.

Various methods exist for evaluation of uterine health in order to classify the severity of bacterial contamination or inflammation. Williams et al. (2005) linked characteristics of vaginal mucus with uterine bacterial contamination, by associating purulent or fetid odor of the mucus with pathogenic bacteria. This evaluation and comparison was achieved by evaluating and scoring the color and content of the vaginal mucus as well an additional binary score of fetid odor. Williams et al. (2005) also associated increased peripheral plasma concentrations of α1-acid glycoprotein (an acute phase protein (APP)) with a fetid mucus score and with increased growth of uterine pathogens cultured from uterine swabs. Reactive oxygen species are vital in some aspects of cell signaling (redox signaling) and cell homeostasis (Fukai et al., 2011). The most prominent of the ROS molecules is the superoxide anion▪$O\_^{2^{\wedge}-}$. However, an accumulation of superoxide can result in oxidative stress by increasing the production of additional ROS such as OH^- , and cause positive feedback of lipid peroxidation in the plasma membrane of cells (Sordillo et al., 2009). There are biological mechanisms that catalyze superoxide and diminish its oxidative potential. Superoxide dismutase (**SOD**) is an antioxidant enzyme found in three different isoforms in the mitochondria (SOD2), cytosol (SOD1), and extracellular plasma membrane (SOD3). All three isoforms of SOD derive from distinct genes and specific localization within the cell, however all three forms catalyze the same reaction of dismutation of superoxide to H2O2 and further reducing it to H_2O via catalase or glutathione peroxidase (**GPX**). Superoxide dismutase and GPX both work in concert to maintain a proper redox environment within the cell (Fukai et al., 2011). Ramos et al. (2015) study in beef cows observed that endometrium tissue of cows with reduced GPX and catalase activity are more prone to lipid peroxidation and associated with smaller follicles and smaller corpus luteum (CL). Bacterial contamination has been associated to induce oxidative stress in tissues, via inflammatory cytokine release in response to PAMPs from bacteria (Lykkesfeldt et al., 2007).

While the underlying reasons to the negative association between milk production and fertility is multifaceted, the physiological changes of various tissues of the dairy cow undergo to support the mammary gland are taxing to the reproductive system of the cow, particularly during the periparturient period (Lucy, 2001). Butler et al. (2003) associated NEB with lower fertility and reduced circulating progesterone at critical points of the ovulation cycle and failure to return to normal cyclicity of the ovaries. After calving, there are two primary objectives that the cow must accomplish to maximize successful breeding: restoring uterine health and a return to normal ovarian cyclicity. As aforementioned, the restoration and involution of the uterus post calving has numerous challenges to overcome in the first 4 weeks postpartum. However, numerous studies have linked ovarian cyclicity with uterine health (Roche et al., 2000; Sheldon et al., 2002). It can be expected that there is a direct relationship between uterine health and ovarian function. Hypocalcemia and SCH at parturition has been strongly associated with decreased odds of pregnancy at first service and that chronically SCH cows had a negative effect of return to ovarian function (Caixeta et al., 2017). Sheldon et al. (2002) observed that uterine bacterial contamination was associated with decreased dominant follicle diameter and circulating estradiol and FSH concentrations. Herath et al. (2007) observed that the granulosa cells of a recruited or dominant ovarian follicle express TLR-4, CD14, MD-2 receptor complex (PAMP receptors) throughout follicular development. Herath et al. (2007) also observed that granulosa cells exposed to LPS *in-vivo* produced less estradiol *in vitro* when cultured in absence of immune cell contamination. The localized immune capability of the follicular granulosa cells perturbs the follicle steroidogenesis. Bromfield and Sheldon (2013) mimicked the LPS concentrations observed in follicular fluid during an active uterine infection by culturing freshly harvested ovarian cortexes in medium with 0.1, 1, and 10 µg/mL of LPS. Bromfield and Sheldon observed that the primordial follicle pool was reduced in the ovarian cortex, and that there was an accumulation (inflammatory response) of inflammatory cytokines with increasing concentrations of LPS in the ovarian cortex.

Association of Prepartum DCAD and CA Concentration on Fertility

Our group, in a recent study, had multiparous Holstein cows were selected to evaluate the effects of feeding an acidogenic diet in the prepartum phase at two different rates of dietary Ca inclusion on the subsequent reproductive performance of the cow. We aimed to compare the effects of feeding a fully-acidified, negative DCAD diet prepartum to multiparous Holstein cows (n = 70) at two concentrations of dietary Ca inclusion versus a non-acidified, positive

DCAD diet prepartum on follicular dynamics and pregnancy postpartum. Treatments began at 28 d before expected calving and were: **CON** (n = 23), a positive DCAD diet (+6 mEq/100g of DM) with low dietary Ca (0.4% DM); **LOW** (n = 22), a fully-acidified, negative DCAD diet (-24 mEq/100g of DM) (urine pH = 5.7) with low dietary Ca (0.4% DM); and **HIGH** (n = 25), a fully-acidified, negative DCAD diet (-24 mEq/100g of DM) (urine pH = 5.7) with high dietary Ca (2.0% DM). Follicular development was monitored via ultrasound every 2 d starting at 7 DIM until ovulation of the first dominant follicle (**DF**). Contrasts included CONT1 (CON vs the average of LOW and HIGH) and CONT2 (LOW vs HIGH). Cows fed CON (18.93± 0.9 d) had increased (*P*= 0.01) days to first ovulation than cows fed LOW (17.93± 0.6 d) and HIGH (16.3 ± 0.4 d) (Figure 1). There was no treatment effect on maximum DF diameter (CON = 17.87mm, LOW = 18.33mm, and HIGH = 17.56mm; SEM 0.44; P = 0.44, CONT1 and *P* = 0.16, CONT2). There was a tendency for a treatment × days relative to ovulation interaction (*P* = 0.11) indicating that cows fed CON had a slower rate of growth in the 4 days prior to ovulation of the first DF than cows fed LOW or HIGH. Cows fed CON (4/19 P/AI) tented to have lower P/AI (*P* = 0.11; 95CI = 1.02 – 16.6) than cows fed HIGH (11/21 P/AI) but not LOW (8/20 P/AI). In conclusion, cows fed HIGH and LOW had improved days to first ovulation than cows fed CON. Cows fed HIGH tended to be more likely to become pregnant than cows fed CON. Overall, cows fed a fully-acidified, negative DCAD diet prepartum had improved reproductive performance postpartum.

Additionally, endometrial tissue samples were collected at 30 DIM and analyzed for GPX and SOD activity, and glandular morphology. Cows fed HIGH had greater (P = 0.02) epithelial height (22.47± 1.08mm) than cows fed LOW (18.67 ± 1.08mm) and cows fed CON (18.01 ± 1.08 mm) tended (*P* = 0.06) to have shorter epithelial height than the average of cows fed LOW and cows fed HIGH. Cows fed HIGH had a greater (*P* = 0.05) number of epithelial cells per gland (25.93 ± 1.07) than cows fed LOW (22.93 ± 1.07). Anti-oxidative enzymes SOD and GPX relieve oxidative stress in cells. Cows fed HIGH had increased (*P* = 0.05) activity of SOD (73.50 ± 2.83%) and decreased (P < 0.001) activity of GPX (32.89 ± 5.05 %) than cows fed LOW (69.49 ± 2.83 % and 68.31 ± 2.83 %, respectively). In conclusion, cows fed HIGH had improved glandular epithelial cells, greater SOD activity, and lower GPX activity than cows fed LOW indicating an improved redox environment in the uterine tissue, which may lead to improved post-partum fertility.

Post-partum gland development and function are essential to uterine reproductive function. The tendency of effect of a negative DCAD (comparing cows fed CON vs cows fed LOW and cows fed HIGH) on the glandular epithelial height suggests that the negative DCAD alleviated tissue damage to the glandular epithelial cells. A negative DCAD coupled with supplementation of high Ca (2.0% DM) amplified this alleviative effect. To our current knowledge, this effect of a negative DCAD and high Ca supplementation on glandular development has not been previously studied, however Martinez et al. (2014, 2018) suggest that an improved Ca status around parturition led to lower incidences of endometritis, thus, less tissue damage. Our study also indicates differences in glandular epithelial cell numbers increased with increasing Ca supplementation while on a negative DCAD diet. These findings could indicate that a negative DCAD diet may alleviate uterine tissue damage and is amplified by increasing Ca supplementation.

Superoxide dismutase activity in cows fed HIGH was increased in comparison to cows fed LOW, while GPX activity was decreased in the same comparison. Superoxide dismutase and GPX work in concert to maintain a proper redox environment in tissue by reducing reactive oxygen species (ROS) that cause oxidative stress in high concentrations (Fukai et al., 2011). Ramos et al. (2015) study on beef cows, indicated an increase in GPX activity with larger follicles and larger CL, which does not agree with the results from our current study. The loss or decrease of SOD activity has been linked with controlling tissue damage signaling by perturbing reactive oxygen species (ROS) signaling by lowering H_2O_2 concentrations (Carreira, 2018). Brigelius-Flohé (1999) review of GPX function and activity in different tissues indicates that cytosolic GPX reduces cytosolic H_2O_2 concentrations to H_2O. In eukaryotic species, H_2O_2 is not only a mild ROS, but it is an important signaling molecule in immune cell activation (Veal, 2007). Glutathione peroxidase, under oxidative stress conditions, is activated via nuclear factor-κB (NFκB), a key regulator in immune response to infection and inflammation (Stoytcheva and Berry, 2009). The inverse relationship of the enzyme activities of SOD and GPX observed from cows fed HIGH in the current study is a part of the controlling mechanism of the redox environment within cells, and is an intrinsic controller to immune response activation.

Development of the dominant follicle of the first follicular wave postpartum is highly correlated with conception rates (Butler, 2001; Royal et al., 2000; Butler, 2003). Butler (2003) described a positive association of the early commencement of the ovulatory cycle and increased conception rates for the cow is able to have multiple ovulatory cycles before the first timed artificial insemination. In the current study, the average days to first ovulation, postpartum, of cows

fed HIGH and cows fed LOW was less than cows fed CON. Caixeta et al. (2017) observed cows that had abnormal Ca levels at the first three days after calving tended to take longer before returning to normal cyclicity. Martinez et al. (2018) indicated that there were no differences in likelihood of pregnancy at first TAI between a positive DCAD (145 ± 11 mEq/kg DM) and a negative DCAD (-129 ± 11 mEq/kg DM) diet. However, in this study we observed a tendency for increased likelihood of cows fed HIGH to be pregnant after the first TAI in comparison to CON.

Conclusions

In conclusion, cows that received a negative DCAD diet prepartum had decreased days to ovulation of the dominant follicle of the first follicular wave after calving. Cows in HIGH had improved uterine immune response than cows fed LOW. Additionally, cows that received DCAD diets had improved uterine glandular epithelial cell morphology than cows that received CON; moreover, cows fed HIGH had increased uterine glandular epithelial cells than cows fed LOW. Cows fed HIGH had greater SOD activity and lower GPX activity than cows fed LOW indicating an improved redox environment in the uterine tissue. Overall, providing a DCAD diet prepartum with increased calcium concentration (HIGH) enhanced the immune response in the days following parturition

References

Allen AJ. Parturient paresis in cows. MSD Vet Man 2016:16–20.

Bell AW. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J Anim Sci 1995;73:2804–19. doi:10.2527/1995.7392804x.

Brigelius-Flohe R. Two automatic mine hoists for potash mining in Canada, (Zwei automatische Schachtfoerdermaschinen fuer den Kalibergbau in Kanada). Free Radic Med 1999;27:951–65. doi:10.1016/S0891-5849(99)00173-2.

Bromfield JJ, Sheldon IM. Lipopolysaccharide Reduces the Primordial Follicle Pool in the Bovine Ovarian Cortex Ex Vivo and in the Murine Ovary In Vivo1. Biol Reprod 2013;88:1–9,1–98,98.

Butler WR. Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. BSAP Occas Publ 2001;26:133–45. doi:10.1017/s0263967x00033644.

Butler WR. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci 2003;83:211–8. doi:10.1016/S0301-6226(03)00112-X.

Chan PS, West JW, Bernard JK. Effect of Prepartum Dietary Calcium on Intake and Serum and Urinary Mineral Concentrations of Cows. J Dairy Sci 2006;89:704–13. doi:10.3168/JDS.S0022-0302(06)72133-6.

Chapinal N, LeBlanc SJ, Carson ME, Leslie KE, Godden S, Capel M, et al. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. J Dairy Sci 2012;95:5676–82. doi:10.3168/jds.2011-5132.

Charbonneau E, Pellerin D, Oetzel GR. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. J Dairy Sci 2006;89:537–48. doi:10.3168/JDS.S0022-0302(06)72116-6.

Caixeta LS, Ospina PA, Capel MB, Nydam DV. Association between subclinical hypocalcemia in the first 3 days of lactation and reproductive performance of dairy cows. Theriogenology 2017;94:1–7. doi:10.1016/J.THERIOGENOLOGY.2017.01.039.

Carreira RP, Reproduction A. CECAV-UTAD Publications. 2018.

DeGaris PJ, Lean IJ. Milk fever in dairy cows: A review of pathophysiology and control principles. Vet J 2008;176:58–69. doi:10.1016/J.TVJL.2007.12.029.

Drackley JK. Biology of Dairy Cows During the Transition Period: the Final Frontier? J Dairy Sci 1999;82:2259–73. doi:10.3168/JDS.S0022-0302(99)75474-3.

Ender F, Dishington IW, Helgebostad A. Calcium Balance Studies in Dairy Cows under Experimental Induction and Prevention of Hypocalcaemic Paresis Puerperalis: The solution of the aetiology and the prevention of milk fever by dietary means. Zeitschrift Für Tierphysiologie Tierernährung Und Futtermittelkd 1971;28:233–56. doi:10.1111/j.1439-0396.1971.tb01573.x.

Esposito G, Irons PC, Webb EC, Chapwanya A. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim Reprod Sci 2014;144:60–71. doi:10.1016/J.ANIREPROSCI.2013.11.007.

Fukai T, Ushio-Fukai M. Superoxide Dismutases: Role in Redox Signaling, Vascular Function, and Diseases. Antioxid Redox Signal 2011;15:1583–606. doi:10.1089/ars.2011.3999.

Goff JP. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. Anim Feed Sci Technol 2006;126:237–57. doi:10.1016/J.ANIFEEDSCI.2005.08.005.

Goff JP. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet J 2008;176:50–7. doi:10.1016/J.TVJL.2007.12.020.

Goff JP, Horst RL. Effects of the Addition of Potassium or Sodium, but Not Calcium, to Prepartum

Rations on Milk Fever in Dairy Cows. J Dairy Sci 1997;80:176–86. doi:10.3168/JDS.S0022-0302(97)75925-3.
Goff JP, Ruiz R, Horst RL. Relative Acidifying Activity of Anionic Salts Commonly Used to Prevent Milk Fever. J Dairy Sci 2004;87:1245–55. doi:10.3168/JDS.S0022-0302(04)73275-0.
Griffin JFT, Hartigan PJ, Nunn WR. Non-specific uterine infection and bovine fertility: I. Infection patterns and endometritis during the first seven weeks post-partum. Theriogenology 1974;1:91–106. doi:10.1016/0093-691X(74)90052-1.
Grummer RR, Mashek DG, Hayirli A. Dry matter intake and energy balance in the transition period. Vet Clin North Am Food Anim Pract 2004;20:447–70.
Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol 2006;113:21–9. doi:10.1016/j.vetimm.2006.03.022.
Hansen PJ, Soto P, Natzke RP. Mastitis and Fertility in Cattle - Possible Involvement of Inflammation or Immune Activation in Embryonic Mortality*. Am J Reprod Immunol 2004;51:294–301. doi:10.1111/j.1600-0897.2004.00160.x.
Herath S., Williams EJ, Lilly ST, Gilbert RO, Dobson H, Bryant CE, et al. Ovarian follicular cells have innate immune capabilities that modulate their endocrine function. Reproduction 2007;134:683–93. doi:10.1530/REP-07-0229.
Horst RL, Goff JP, Reinhardt TA, Buxton DR. Strategies for Preventing Milk Fever in Dairy Cattle,. J Dairy Sci 1997;80:1269–80. doi:10.3168/JDS.S0022-0302(97)76056-9.
Horst RL. Regulation of Calcium and Phosphorus Homeostasis in the Dairy Cow. J Dairy Sci 1986;69:604–16. doi:10.3168/JDS.S0022-0302(86)80445-3.
Horst RL, Goff JP, Reinhardt TA. Calcium and Vitamin D Metabolism in the Dairy Cow. J Dairy Sci 1994;77:1936–51. doi:10.3168/JDS.S0022-0302(94)77140-X.
Jawor PE, Huzzey JM, LeBlanc SJ, von Keyserlingk MAG. Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. J Dairy Sci 2012;95:1240–8. doi:10.3168/JDS.2011-4586.
Kamgarpour R, Daniel RCW, Fenwick DC, McGuigan K, Murphy G. Post partum Subclinical Hypocalcaemia and Effects on Ovarian Function and Uterine Involution in a Dairy Herd. Vet J 1999;158:59–67. doi:10.1053/TVJL.1999.0348.
Kimura K, Reinhardt TA, Goff JP. Parturition and Hypocalcemia Blunts Calcium Signals in Immune Cells of Dairy Cattle. J Dairy Sci 2006;89:2588–95. doi:10.3168/JDS.S0022-0302(06)72335-9.
Kronqvist C, Emanuelson U, Spörndly R, Holtenius K. Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. J Dairy Sci 2011;94:1365–73. doi:10.3168/JDS.2009-3025.
Liang D, Arnold LM, Stowe CJ, Harmon RJ, Bewley JM. Estimating US dairy clinical disease costs with a stochastic simulation model. J Dairy Sci 2017;100:1472–86. doi:10.3168/JDS.2016-11565.
Littledike ET, Goff J. Interactions of calcium, phosphorus, magnesium and vitamin D that influence their status in domestic meat animals. J Anim Sci 1987;65:1727–43. doi:10.2527/jas1987.6561727x.
Lucy M.C. Reproductive Loss in High-Producing Dairy Cattle: Where Will It End? J Dairy Sci 2001;84:1277–93. doi:10.3168/JDS.S0022-0302(01)70158-0.
Lykkesfeldt J, Svendsen O. Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet J 2007;173:502–11. doi:10.1016/J.TVJL.2006.06.005.
Martinez N, Sinedino LDP, Bisinotto RS, Ribeiro ES, Gomes GC, Lima FS, et al. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. J Dairy Sci 2014;97. doi:10.3168/jds.2013-7408.
Martinez N, Rodney RM, Block E, Hernandez LL, Nelson CD, Lean IJ, et al. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Health and reproductive responses. J Dairy Sci 2018;101:2563–78. doi:10.3168/JDS.2017-13740.
Mongin P. Recent advances in dietary anion-cation balance: applications in poultry. Proc Nutr Soc 1981;40:285–94. doi:10.1079/PNS19810045.
Mulligan FJ, O'Grady L, Rice DA, Doherty ML. A herd health approach to dairy cow nutrition and production diseases of the transition cow. Anim Reprod Sci 2006;96:331–53. doi:10.1016/J.ANIREPROSCI.2006.08.011.
Neves RC, Leno BM, Stokol T, Overton TR, McArt JAA. Risk factors associated with postpartum subclinical hypocalcemia in dairy cows. J Dairy Sci 2017;100:3796–804. doi:10.3168/JDS.2016-11970.
NRC. Nutrient Requirements of Dairy Cattle. 2001. doi:10.17226/9825.
Oetzel GR. Diseases of Dairy Animals | Non-Infectious Diseases: Milk Fever. Encycl Dairy Sci 2011:239–45. doi:10.1016/B978-0-12-374407-4.00145-X.
Oetzel GR, Fettman MJ, Hamar DW, Olson JD. Screening of Anionic Salts for Palatability, Effects on Acid-Base Status, and Urinary Calcium Excre-

tion in Dairy Cows. J Dairy Sci 1991;74:965–71. doi:10.3168/JDS.S0022-0302(91)78245-3.
Oetzel GR, Olson JD, Curtis CR, Fettman MJ. Ammonium Chloride and Ammonium Sulfate for Prevention of Parturient Paresis in Dairy Cows. J Dairy Sci 1988;71:3302–9. doi:10.3168/JDS.S0022-0302(88)79935-X.
Overton TR, Waldron MR. Nutritional Management of Transition Dairy Cows: Strategies to Optimize Metabolic Health. J Dairy Sci 2004;87:E105–19. doi:10.3168/JDS.S0022-0302(04)70066-1.
Ramos RS, Oliveira ML, Izaguirry AP, Vargas LM, Soares MB, Mesquita FS, et al. The periovulatory endocrine milieu affects the uterine redox environment in beef cows. Reprod Biol Endocrinol 2015;13:1–10. doi:10.1186/s12958-015-0036-x.
Razzaghi A, Aliarabi H, Tabatabaei MM, Saki AA, Valizadeh R, Zamani P. Effect of Dietary Cation-Anion Difference during Prepartum and Postpartum Periods on Performance, Blood and Urine Minerals Status of Holstein Dairy Cow. Asian-Australasian J Anim Sci 2012;25:486–95. doi:10.5713/ajas.2011.11325.
Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP, Horst RL. Prevalence of subclinical hypocalcemia in dairy herds. Vet J 2011;188:122–4. doi:10.1016/J.TVJL.2010.03.025.
Ribeiro ES, Gomes G, Greco LF, Cerri RLA, Vieira-Neto A, Monteiro PLJ, et al. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J Dairy Sci 2016;99:2201–20. doi:10.3168/JDS.2015-10337.
Roche J., Mackey D., Diskin M. Reproductive management of postpartum cows. Anim Reprod Sci 2000;60–61:703–12. doi:10.1016/S0378-4320(00)00107-X.
Rodney RM, Martinez N, Block E, Hernandez LL, Nelson CD, Lean IJ, et al. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Health and reproductive responses. J Dairy Sci 2018;101:2563–78. doi:10.3168/jds.2017-13740.
Royal MD, Darwash AO, Flint APF, Webb R, Woolliams JA, Lamming GE. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 2000;70:487–501. doi:10.1017/S1357729800051845.
Santos JEP, Lean IJ, Golder H, Block E. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. J Dairy Sci. 102:2134-2154.
Sheldon IM, Dobson H. Postpartum uterine health in cattle. Anim Reprod Sci 2004;82–83:295–306. doi:10.1016/j.anireprosci.2004.04.006.
Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU, Dobson H. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 2002a;123:837–45. doi:10.1530/rep.0.1230837.
Sheldon IM, Noakes DE, Dobson H. Effect of the Regressing Corpus Luteum of Pregnancy on Ovarian Folliculogenesis after Parturition in Cattle. Biol Reprod 2002b;66:266–71. doi:10.1095/biolreprod66.2.266.
Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. Defining postpartum uterine disease in cattle. Theriogenology 2006. doi:10.1016/j.theriogenology.2005.08.021.
Sordillo LM, Contreras GA, Aitken SL. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Anim Heal Res Rev 2009;10:53–63. doi:10.1017/S1466252309990016.
Stoytcheva ZR, Berry MJ. Transcriptional regulation of mammalian selenoprotein expression. Biochim Biophys Acta 2009;1790:1429–40. doi:10.1016/j.bbagen.2009.05.012.
Veal EA, Day AM, Morgan BA. Hydrogen Peroxide Sensing and Signaling. Mol Cell 2007;26:1–14. doi:10.1016/J.MOLCEL.2007.03.016.
Vernon RG. Lipid metabolism during lactation: A review of adipose tissue-liver interactions and the development of fatty liver. J Dairy Res 2005;72:460–9. doi:10.1017/S0022029905001299.
Weich W, Block E, Litherland NB. Extended negative dietary cation-anion difference feeding does not negatively affect postpartum performance of multiparous dairy cows. J Dairy Sci 2013;96:5780–92. doi:10.3168/JDS.2012-6479.
Williams EJ, Fischer DP, Pfeiffer DU, England GCW, Noakes DE, Dobson H, et al. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 2005;63:102–17. doi:10.1016/J.THERIOGENOLOGY.2004.03.017.

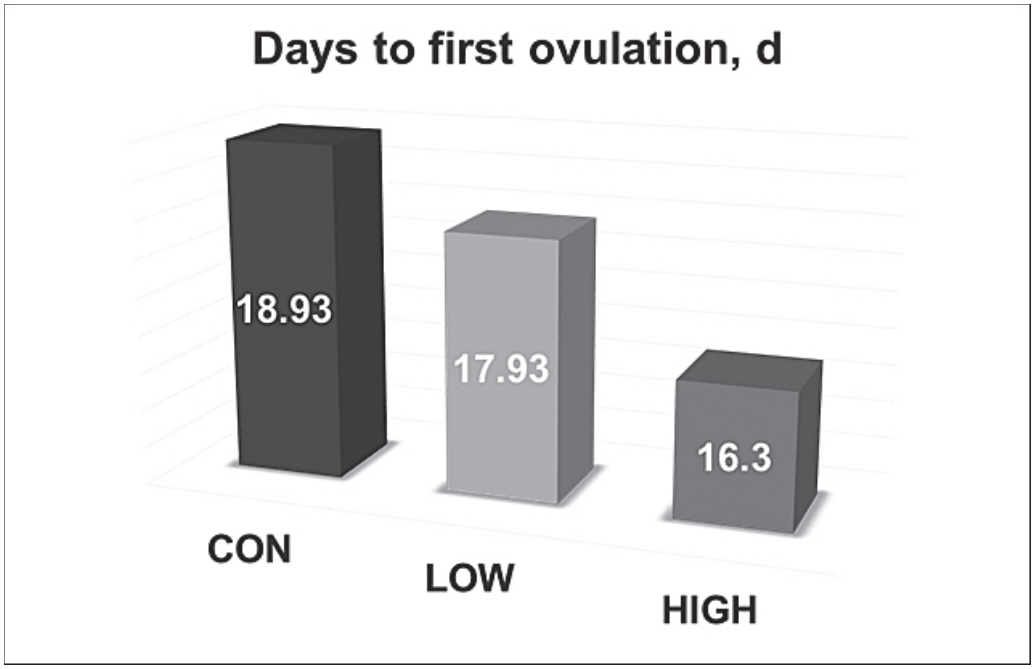

Figure 1. Cows fed CON (18.93± 0.9 d) had increased (*P*= 0.01) days to first ovulation than cows fed LOW (17.93± 0.6 d) and HIGH (16.3 ± 0.4 d). CON: Positive DCAD (+6 mEq/100g of DM; urine pH > 8.0) with low dietary Ca (0.40% of DM; 46.2 ±15.2 g Ca/d; n = 23); LOW: Negative DCAD (-24 mEq/100g of DM; urine pH = 5.8) with low dietary Ca (0.40% of DM; 44.1 ± 16.1 Ca/d; n = 22); HIGH: Negative DCAD (-24 mEq/100g of DM; urine pH = 5.8) with high dietary Ca (2.0% of DM; 226.6 ± 96.0 g Ca/d; n = 25).

Table 1. Composition of inorganic mineral sources and absorption coefficient of Calcium

Calcium Sources	Dry Matter (%)	Crude Protein	Calcium Content (%)	Absorption Coefficient
Bone meal, steamed, fg[2]	97	13.2	30.71	0.95
Calcium carbonate, fg	100	-[4]	39.39	0.75
Calcium chloride anhydrous, cp[3]	100	-	36.11	0.95
Calcium chloride dehydrate, cp	100	-	27.53	0.95
Calcium propionate, fg	94	-	21.50	0.90
Calcium hydroxide, cp	100	-	54.09	0.55
Calcium oxide, fg	100	-	71.47	0.50
Calcium phosphate, fg	97	-	16.40	0.95
Calcium sulfate dihydrate, cp	97	-	23.28	0.70
Curacao, phosphate, fg	99	-	34.34	0.71
Dicalcium phosphate, fg	97	-	22.00	0.94
Dolomitic limestone, fg	99	-	22.30	0.60
Limestone, ground, fg	100	-	34.00	0.70
Magnesium oxide, fg	98	-	3.07	0.70

[1]Table adapted from NRC ([2001]; Table 15-4)
[2]Fg = feed grade
[3]Cp = chemically pure
[4]Not present