Old problems, new perspectives: nutritional and behavioral strategies to improve dairy cattle health

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B.S., University of Nebraska - Lincoln, 2014

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2020

Approved by:

Major Professor
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Abstract

The transition period, defined as 3 weeks prepartum to 3 weeks postpartum, is a high-risk time for disease in dairy cattle. There are many strategies to help prevent the onset of disease around calving. Of these, the use of a prepartum diet with a negative dietary cation anion difference (**DCAD**) is widely practiced to increase mobilization of calcium (**Ca**) from bone and reduce the incidence of hypocalcemia. However, the amount of Ca to supplement with a negative DCAD diet to promote optimal health during transition has remained in question. Additionally, technological advancements used to measure feeding behaviors in early lactation have evolved and could be valuable in predicting dry matter intake (**DMI**), lactation outcomes, and herd survival. This is important as greater DMI is associated with lower disease incidence during the transition period, leading to improved performance and reduced culling. An experiment using twenty-one pregnant, nonlactating cows completing at least one lactation was carried out to evaluate the effects of varying levels of Ca carbonate on DMI and metabolic acid-base status in a replicated 3 × 3 Latin square design utilizing 3 treatments and 3 periods. The only effect of treatment in this study was on urine pH, which increased linearly with increasing dietary Ca. However, greater DMI in Period 3 versus Period 1 in this study resulted in a greater degree of acidification during this time, as indicated by a lesser urinary pH. Greater Ca carbonate supplementation with no significant difference in Ca excretion suggests carbonate, not Ca, may be absorbed and converted to HCO_3^-, with the excess HCO_3^- being excreted in urine. Therefore, when feeding high levels of Ca carbonate, urine pH may not accurately reflect acid-base status. In a second experiment, the relationships among feeding behavior variables collected during the first 21 d of lactation from 5 studies at Kansas State University were evaluated. Independent variables included meal frequency, meal length, meal size, and feeding time, along with parity,

calving date, PTA for milk (**PTAM**) and 305-d mature equivalent milk (**305MEM**). Milk PTA, the slope of meal frequency, and mean meal length, specifically meal length during week 2, were predictive of 305MEM, and all were positively associated with 305MEM. For DMI, the quadratic function of feeding time, the intercept of meal frequency, parity and PTAM were predictive of DMI, with the quadratic function of week 2 feeding time and the intercept of meal frequency for week 2 significant when evaluating weekly feeding behaviors. The risk of being removed from the herd was increased by 13% for an additional meal/day in the first 21 d of lactation. Overall, strategies to improve herd health can be implemented both prepartum and postpartum to reduce disease and improve performance during the transition to lactation.

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Acknowledgements

This thesis would not have been possible without the support and guidance of Dr. Barry Bradford, my major professor. Thank you for taking me on as a master's student. You have given me great support and mentorship throughout my master's program, and for that I cannot thank you enough. Even with your busy schedule, you were always willing to block off time to answer questions my questions or talk through something I did not understand. I am forever grateful and feel honored to have worked with you.

In addition, I would like to thank Dr. Laman Mamedova who was there to give me guidance in the laboratory. Your patience and mentorship in the lab were much appreciated. I could not have finished the lab work for my study without you. I will definitely miss your spunky and contagious attitude.

To my committee members, Dr. Jeffrey DeFrain and Dr. Timothy Rozell, I would like to thank you for serving on my committee and for your guidance throughout my master's program.

I would like to thank the Dairy Metabolism group at Kansas State University for the support and friendship over the last few years. Thank you for teaching me the ropes of graduate school when I first arrived. My master's program would not have been the same, nor as fun, without all of you. I know these friendships we have made will last beyond our years at KSU.

To my family, thank you for the constant support in everything I do. And to my future husband, Kyler, you have been so supportive of me through this all. You have dealt with my ups and downs that came along with graduate school without complaining. Thank you for being my rock.

Finally, I need to thank Dr. Chris Canale. He may not know the impact he has had on me, but his passion and excitement for the dairy industry is contagious. It was this passion that

motivated me to pursue a master's degree in the first place. His willingness to talk through the benefits of graduate school when deciding if it was the right fit for me is still appreciated. It has been the best decision I could have made, so thank you Dr. Canale.

Chapter 1 - Literature Review - Monitoring individual feeding behavior of dairy cattle

Monitoring and evaluating feeding behavior during the transition period can be used as a tool for diagnosing disease in dairy cattle. It has been common to use dry matter intake (DMI) as way to quantify nutrient supply to an animal through feed, since greater DMI can lead to improved production outcomes (Llonch et al., 2018) and reduced risk of disease among cows that consume more nutrients (Schirmann et al., 2016). However, quantifying DMI of individual cows in group housing is complex due to environmental, management, health, and social factors that can impede normal behavior patterns (Grant and Albright, 1995). In recent years, there has been an increase in use of precision dairy technologies on commercial farms used to measure behavior of individual cows, which show promise for estimating DMI. Precision dairy management (PDM) technology can be wearable, incorporated into the milking system, stand-alone, or part of the herd management software (Eckelkamp and Bewley, 2020). Even though tracking behavior does not result in precise quantification of nutrient consumption, it is helpful in picking out cows that are not following their normal routine due to some extent of discomfort (whether physical or metabolic) which is usually apparent before any visual signs are present. Applications for use of PDM tools have already been documented (Goldhawk et al, 2009; Gonzalez et al, 2008; Schirmann et al., 2016; Stangaferro et al., 2016) and show distinct differences in activity between healthy and sick animals, with these differences being behavioral mechanisms used as part of a coordinated strategy to help fight disease by sick animals (von Keyserlingk et al, 2009). Therefore, precision dairy management technology can be useful to augment the eyes and ears of farmers to improve herd management and welfare.

Research has focused on better understanding activity patterns and feeding behavior of individual dairy cattle during times of disease, at different physiological stages and in various environmental conditions. Although dairy producers are optimistic about the usefulness of these technologies (Eckelkamp and Bewley, 2020), understanding individual behavioral differences between cows in healthy and impaired states is important for improving these technologies in the future. Additionally, precision dairy management technology may become more important in the future due to ongoing changes in the industry. The topic of animal welfare has spiked research interest, where both subjective and objective aspects of the condition of an animal's life - including health and disease, behavior, husbandry, and management - are being evaluated (Gougoulis et al., 2010). With disease being detected up to 4 days earlier with the use of activity monitoring (Quimby et al., 2001), this could help reduce the extent of animal suffering from early detection of illness and quicker treatment of the animal. The objective of this review is to discuss our current understanding of behavioral patterns associated with disease and intestinal dysfunction on an individual animal basis at different physiological stages when individual activity and feeding behavior patterns are tracked.

Disease impacts

The transition period, defined as 3 weeks before calving to 3 weeks post-calving, is a sensitive time for dairy cattle where they are susceptible to a number of diseases as a result of immune system depression during this time. Early diagnosis of health problems in transition cows is often difficult and can be subjective to individuals on the farm (Gonzalez et al., 2008). The amount of literature associating changes in individual cow activity with disease incidence is increasing. However, if individual activity is going to be used these variables must be easily

monitorable, consistent and reliable for early identification of cows at risk for disease (Gonzalez et al, 2008). Quimby et al. (2001), used radio frequency technology to track total time spent within 50 cm^2 of the feed bunk in newly received feedlot steers and were able to detect morbidity up to 4.5 days sooner than the feedlot personnel. Therefore, there is research that supports the usefulness of PDM technology as an alternative method for detecting sickness. In dairy cattle, cows are diagnosed with one or more health disorders postpartum display changes in feeding behavior, including reduction in feeding time, feeding rate, and frequency of meals, and in some cases rumination time (Schirmann et al., 2016). For example, cows with reduced rumination time prepartum maintain this reduced rumination after calving and suffered from greater frequency of disease (Soriani et al., 2012). In addition, cows without health disorders, or those with only mild health disorders, during the peripartum period show greater rumination time; Cows that ruminated > 550 min/d between 2 and 10 days of lactation were generally in good health (Soriani et al., 2012). Therefore, there is evidence to suggest that behavioral changes specific to certain disease conditions may further aide in appropriate treatments of cows.

Mastitis

Compared to other diseases in dairy cattle, mastitis can cause motivation conflict in the behavioral priorities which may distort behavior commonly associated with sickness (Munksgaard et al., 2005). This may be due to discomfort and pain felt when lying due to a sore udder (Siivonen et al., 2011). In addition, unlike other diseases where changes in behavior may be detected days or even weeks before the diagnosis, cows diagnosed with mastitis show no changes in feeding time or rumination time before the infection, but have significant change in these behavioral variables within 24 hours after infection has occurred (Cyples et al., 2012;

Fogsgaard et al., 2015). Common behavioral changes observed among cows diagnosed with mastitis are a reduction in the time spent ruminating and lying down per day. Cows with mastitis show a distinct dip in rumination within the first 24 hours of the disease (most likely due to high fever) and reduced ruminal contractions (Fogsgaard et al., 2012; Siivonen et al., 2011). Siivonen et al. (2011) challenged cows with acute endotoxin mastitis and reported that cows with mastitis spent less time lying down and more time standing on the day of challenge compared to the 2 prior days of recorded behavior. Likewise, Fogsgaard et al. (2015) and Medrano-Galarza et al. (2012) reported reductions in lying time 2 days after mastitis diagnosis/treatment compared to cows in the control group without mastitis. A reduction in lying time and increased standing observed as soon as 3 hours after the onset of a mastitic infection (Yeiser et al., 2012). Only in one study reviewed did lying time not change with induction of mastitis (Fogsgaard et al., 2012). This suggests that pain experienced in the udder may override the animal's motivation to exhibit normal sickness behaviors, that is lying down to conserve energy (Siivonen et al., 2011).

Reports on the reduction in lying time during mastitis are relatively consistent (Fogsgaard et al., 2015; Siivonen et al. 2011), with lying activity of cows with mastitis being driven by shorter more frequent lying bouts. Fogsgaard et al. (2015) showed that cows reduced lying time, but had a greater number of daily lying bouts for up to 10 days after mastitis treatment compared to their healthy counterparts. The motivational conflict experienced during mastitis may cause cows to become increasingly frustrated and restless, therefore, increasing lying bouts and steps taken per day (Fogsgaard et al., 2015). Siivonen et al. (2011) reported that, while lying time is reduced, lying bouts per day are not altered during mastitis; again, the authors suggest that pain experienced from the infected udder overrides the motivation for a sick animal to lie down.

Changes in lying time and increased standing time have been documented in cows with mastitis (Siivonen et al, 2011; Yeiser et al., 2012), but it is less clear whether a cow's steps per day are increased. The number of steps per day may increase (Siivonen et al., 2011), potentially because of shifts in standing position due to discomfort from the infection. Alternatively, a cow may spend more time standing idle (Fogsgaard et al., 2012) as a way to avoid discomfort from friction between the swollen udder and the legs (Chapinal et al., 2012). Therefore, evaluating standing and lying behavior for diagnosis of mastitis may be most insightful, as the association of steps per day and mastitis are inconsistent.

Metritis

Metritis is defined as inflammation of the uterus caused by bacterial infection following calving. It is characterized by an enlarged uterus and a watery red-brown fluid to viscous off-white uterine discharge, which often has a fetid odor (Sheldon et al., 2006). Links between metritis and behavioral changes in infected cows could help contribute to identification of cows at risk for this disease weeks before observation of clinical signs of infection (DeVries and von Keyserlingk, 2009). Cows diagnosed with metritis after calving are reported to have a reduction in the amount of lying time 2 weeks before calving compared to their counterparts considered to be healthy (Neave et al., 2018). Cows diagnosed with metritis also show increased lying bout duration (min/bout) 3 days before diagnosis of metritis, with no difference in total lying time compared to healthy cows (Neave et al., 2018). Lying down longer throughout the day, with few lying bouts, may be a way for cows to conserve during times of illness. Cows that spend less time lying down, or increase standing time, are exhibiting an energetically exhausting behavior making them more likely to have a suppressed immune system and increased susceptibility to

infection (Neave et al., 2018). However, it has been shown that cows may also display a greater degree of inactive standing time at weeks -2 and -1 relative to calving before being diagnosed with metritis post-calving (Patbandha et al., 2012). This increase in standing time prepartum may be due to the growing fetus putting more pressure on abdominal organs upon lying (Patbandha et al., 2012). Therefore, while it is likely all cows spend more time standing during the close-up period, cows with excessive standing time prepartum are at increased risk of metritis after calving and should be monitored closely.

Monitoring feeding behavior of transition cattle may also prove to be an important tool for diagnosis of metritis. Cows diagnosed with metritis are reported to have reduced feeding time on day -12 through -2 and day 2 through 19 relative to calving compared to those cows found to be healthy (Urton et al, 2005). In a study by Urton et al. (2005), the authors reported a 23.2 and 24.5 min/d decrease in feeding time prepartum and postpartum, respectively, for cows diagnosed with metritis. Similarly, another study found feeding time to be reduced during weeks -2 and -1 relative to calving, with feeding time being reduced by 31.4 and 51.7 min/d, respectively, for metritic cows compared to healthy cows (Patbandha et al, 2012). It has been reported that every 10-min decline in feeding time prepartum is associated with a 2-fold increase in the chance of a cow being diagnosed with metritis (Huzzey et al., 2007). Metritic cows also display a lower frequency of feeding bouts during week -2 and -1, where cows diagnosed with metritis had 2 less bouts/day during both week -2 and -1 than healthy cows (Patbandha et al., 2012). Although using feeding time may be beneficial in diagnosing metritis 2 weeks before calving to 2 weeks post-calving, by 3 weeks in milk the difference in feeding time between metritic and healthy cows may not be useful in diagnosing the infection as no difference in feeding bouts between metritic and healthy cows are seen during this time (Huzzey et al., 2007). There is one report (Schirmann

et al., 2016) where feeding behavior was measured 7 days before parturition to 20 days postpartum, and no differences in precalving feeding time were found between metritic and healthy cows; however, the authors did report an 18% reduction in feeding time for metritic cows relative to healthy cows from day 3 to 14 post calving. In addition, feeding bout frequency was not associated with diagnosis of metritis in this study prepartum or postpartum (Schirmann at al., 2016).

Ketosis

Ketosis is a common disease in dairy cattle in the days after calving and is more often experienced by cows with greater milk yield, greater body condition loss over the transition period, and greater stocking density (Kaufman et al., 2016b). Prepartum behavior variables are likely suitable for early diagnosis of sub-clinical ketosis as behaviors displayed prepartum may interfere with health postpartum (Goldhawk et al., 2009). For example, standing time 6 days before calving was positively correlated with blood ketone concentration 3 days after calving, when blood β-hydroxybutyrate (BHB) reached sub-clinical ketosis levels (Rodriguez-Jimenez et al., 2018). β-hydroxybutyrate is a ketone body synthesized from excess non-esterified fatty acid infiltration in the liver. Measurement of BHB in blood is a reliable method to detect ketosis in dairy cattle (Iwersen et al., 2009). It has also been reported that cows with ketosis postpartum spend more time standing the week before and on the day of calving, with fewer but longer standing bouts than nonketotic cows (Itle et al., 2015). Specifically, cows later diagnosed with ketosis stand 20% longer during the week before calving and 35% longer on the day of calving (Itle et al., 2015). In contrast, Rodriquez-Jimenez et al. (2018) reported standing time to be greatly reduced 6 days prepartum through the first week of lactation for cows later diagnosed

with sub-clinical ketosis, with greater lying time for the ketosis cows in the study. This makes sense, as it is likely that cows with ketosis reduce the amount of time going from the lying to standing position to avoid energetically expensive behaviors, as often seen in ill animals (Susenbeth et al., 2004). However, evaluating some behaviors for diagnosis of ketosis may only be insightful for multiparous cows, as no differences in lying time, bout frequency, or bout length were reported for primiparous cows from week -2 to +4 relative to calving (Kaufman et al., 2016a). Even though increased standing time may seem counterintuitive for cows needing to conserve energy, ketotic cows are known to display subordinance at the feed bunk, which could increase standing time as they wait for feed access (Huzzey et al., 2006). This leads to a reduction in DMI, which can be seen 6 days before calving for cows that are later diagnosed with ketosis (Rodriquez-Jimenez et al., 2018). This reduction in DMI can be up to 26% (Abuajamieh et al., 2016), stemming from decreased visits and time at the feed bunk in the week before and week after calving (Goldhawk et al., 2009; Kaufman et al., 2016a).

Cows with sub-clinical ketosis also show reduced rumination time after calving; a 13% reduction has been reported when ketotic cows were followed for 60 days post-calving (Antanaitis et al., 2019). In another report, multiparous cows diagnosed with subclinical ketosis showed a tendency for reduced rumination during weeks -1 and +1 relative to calving compared to their healthy counterparts. Therefore, using rumination time for diagnosing ketosis may only be useful for multiparous cows, as rumination time of primiparous cows was not associated with sub-clinical ketosis (Kaufmann et al., 2016b).

Additionally, the type of housing facility being utilized on farm should to be taken into consideration when using behavioral changes to predict predisposition to ketosis. Differences may be seen between cows in free-stalls versus tie-stalls. This may be because cows in free-stalls

will spend more time standing (Itle et al., 2015), possibly a consequence of differences in social rank and competition (Itle et al., 2015), while tie-stall housed cows exhibit less standing time and more lying time when diagnosed with ketosis (Rodriguez-Jimenez et al., 2018).

Hypocalcemia

Cows with clinical hypocalcemia experience symptoms including muscle tremors, whih leads to staggering and is most often followed by falling to the lying position. The incidence of clinical hypocalcemia has been reduced through better dry cow nutrition, which includes feeding a diet with negative dietary cation anion difference to support calcium mobilization before the onset of lactation (Santos et al., 2019). Little literature was found on the behavioral patterns associated with this clinical diagnosis of hypocalcemia, and this may be because clinical hypocalcemia is fairly easy to visually diagnose. In cows with clinical hypocalcemia, rumination activity will be almost nondetectable in the hours before and after treatment for hypocalcemia, despite the return of muscle function apparent by the cow's ability to stand and eructate following treatment (Goff et al., 2020). While rumination activity is eventually detectable again after intravenous calcium treatment, rumination will remain lower and show large fluctuations (Goff et al., 2020) for cows diagnosed with clinical hypocalcemia compared to cows with subclinical hypocalcemia or those found to be healthy through day 3 of lactation. While clinical hypocalcemia may be detectible a few hours before the onset of the disease, no literature was found that associated the days or weeks leading up to calving with clinical hypocalcemia, making it difficult to use behavioral cues to identify and proactively treat cows who may be at risk for the disease.

Hypocalcemia can lead to diagnosis of other transition diseases. For example, cows diagnosed with hypocalcemia have 3.57 greater odds of experiencing retained placenta (Rodriguez et al., 2017).While documentation of the behavioral changes associated with clinical hypocalcemia are limited, the behaviors of diseases associated with hypocalcemia, such as retained placenta (Rodriguez et al., 2017), have been studied. Cows that had retained placentas tended to have reduced prepartum activity compared to their counterparts who do not experience retained placenta after calving (Liboreiro et al., 2015).

As discussed previously, a dramatic drop in rumination time is observed in cows with clinical hypocalcemia, but cows that experience subclinical hypocalcemia are not susceptible to this same effect. Cows with subclinical hypocalcemia showed no difference in rumination activity from their healthy counterparts (Goff et al., 2020). In contrast, differences in standing time are evident with subclinical hypocalcemia, as cows diagnosed on the day of calving spent 2.6 hours less time standing on day -1 and tended to stand 2.7 hours less on day 1 relative to calving compared to healthy cows (Jawor et al., 2012). Therefore, standing behavior the day before calving may be the best metric to detect cows at risk for subclinical hypocalcemia, as rumination (Goff et al., 2020) and feeding bout frequency (Jawor et al., 2012) are similar between subclinical hypocalcemic and healthy cows making it difficult to use these behaviors as metrics for diagnosis.

Dystocia

Identifying individuals predisposed to dystocia during the prepartum period is critical, as cows who experience dystocia have increased pain at calving (Huxley and Whay, 2006) along with increased prevalence of stillbirths (Meyer at al., 2000) and health complications. Cows with

dystocia decreased their activity (measured in steps/days) compared to their eutocic counterparts on the day of calving (Swartz et al., 2018). However, this change in activity was only reported in Jersey cows; Holstein cows did not show any differences in activity between dystocic and eutocic cows (Swartz et al., 2018). A decrease in activity by dystocic cows is accompanied by increased lying bouts on the day of calving, where cows who moved from the lying to standing position more frequency were predisposed to dystocia (Swartz et al., 2018). Changes in lying bouts, however, did not change lying bout duration for dystocic cows on the day of calving, but changed lying duration on the first 2 days post-calving where cows with dystocia had fewer lying bouts with greater lying bout duration than cows that had an easy calving (Swartz at al., 2018).

Evaluating rumination time of transition cows may also be helpful in diagnosing cows at risk for dystocia. Rumination time of cows with dystocia was reduced 14 days before calving and on the day of calving relative to their healthy counterparts (Paudyal et al., 2016). Rumination time of cows who delivered stillborn calves was reduced by 25 minutes per day up to 10 days before calving compared to their counterparts who delivered live calves (Liboreiro et al., 2015). Restlessness (measured by the amount of postural transitions) increased 4 hours before calving until birth in cows who needed farm assistance at calving compared to non-assisted cows (Barrier et al., 2012).

Not only can behaviors be used for diagnosis of cows predisposed to dystocia, but they can also be used to predict calving. As parturition approaches, rumination time decreases from baseline in all cows, but rumination time may be up to 19 minutes less between 8 and 4 hours before calving for cows with dystocia than those without dystocia (Kovacs et al., 2017). Similar to hypocalcemia, dystocic cows compared to their healthy counterparts take up to 2.4 days longer to return to their baseline rumination time (Kovacs et al., 2017).

Lameness

The ability to detect lameness in dairy cattle is important due to economic losses as a result of decreased milk production in lame cows (Green et al., 2002) and welfare concerns associated with lameness. In healthy cows, lying time normally decreases over the last 16 days of gestation, whereas it then stabilizes by day 6 postpartum. In moderate and severely lame cows, significantly longer lying times are observed throughout the transition period. Cows diagnosed with higher lameness scores spend more time lying as a result of more lying bouts over the transition period (Calderon and Cook, 2011). In severely lame cows, there may also be a decrease in feeding time with no changes in bout duration, likely a result of faster eating rates to reduce the amount of time spent standing due to increased pain (Norring et al., 2014). However, even though they may have faster feeding rates their body weights are usually lower (Norring et al., 2014). Alternatively, we could look at it from another angle. It is possible submissive cows that eat faster are susceptible to gut acidosis causing systemic inflammation and laminitis as a result. Using feeding time for diagnosis of lameness will be parity specific, as primiparous cows have shorter feeding bout duration with no changes in feeding time compared to multiparous cows (Norring et al., 2014).

Intestinal Dysfunction

There are many nutritional factors that can have an impact on gut health of dairy cattle. Two major factors are the forage to concentrate ratio and the particle size of the constituents of the diet. Diets containing large amounts of grain tend to reduce rumen pH due to their high rate of degradation by rumen microbes. The same is true for feeds that contain smaller particles, such

as those that are further processed have a higher degradation rate. In addition, there are feeding behaviors thought to be associated with a reduction in rumen pH. These include less frequent meals, ingestion of large meals, and faster eating rates which result in a greater amount of substrate at one time for rumen microbes and less saliva production for buffering of the rumen. For example, in a study by Moya et al. (2011), heifers who consumed meals less frequently (mean of 18.5 meals/day) had lower minimum and mean daily pH (4.96 and 5.66, respectively) than their counterparts who consumed an average of 36.7 meals/day (5.44 and 6.25, respectively).

Behavior of cattle may be useful in diagnosing cows who are experiencing ruminal dysfunction, but it may not help distinguish the degree of dysfunction being experienced by these cows. For example, cows with severe acidosis, those fed a diet containing a higher concentration of grain, showed no difference in feeding behaviors such as feeding time, meal frequency, meal duration or meal size compared to cows who were fed a higher forage to concentrate diet and are at less risk for acidosis (Devries et al., 2009). However, these two groups of cows did tend to have different rumination time per day, where cows at risk for severe acidosis ruminated less than low risk cows with no changes in lying or standing time (Devries et al., 2009). This is expected, as acidosis can reduce rumen contractions which would impact a cow's ability to ruminate.

Assessing behavioral deviations of individuals from their baseline activity may prove to be valuable when trying to diagnose digestive dysfunction. Cows at both high and low risk for acidosis show changes in feeding rate, meal size, rumination, lying time, and standing time during induced acidosis compared to baseline means, resulting in 31% , 24%, 18% , and 11% reductions in feeding rate, meal size, rumination, and lying time, respectively, with standing time

being increased by 8% (Devries et al., 2009). Overall, this suggests that a single observation alone, such as rumination time, may not be accurate in detecting intestinal discomfort. Rather a combination of behavioral observations may need to be taken into consideration when assessing digestive health of a herd or individual (DeVries et al., 2009).

Calves

Improving detection of illness in group-housed calves is needed as group-housing poses a challenge when identifying disease. Tracking changes in behaviors may be a beneficial for producers investing in PDM technology for calves. Specifically, milk intake or the pattern of visits to the automated milk feeders can be monitored for changes that can occur days before apparent onset of illness (Borderas et al., 2009). For example, in a study by Sutherland et al. (2018), calves consumed less milk 4 days before diagnosis of disease than calves who remained healthy. However, this behavior is dependent on milk allowance provided to the calves. Calves provided a high allowance of milk had lower milk intake, decreased visits to the feeder and increased visit duration when they were ill. This is in agreement with a study by Swartz et al. (2017) where calves on high milk allowance consumed less milk on the day of diagnosis compared to their healthy counterparts. In contrast, when calves were fed low milk allowance, only an increase in duration of visits with no change in visits to the feeder or milk intake were reported in the 2 days prior to illness compared to healthy calves (Borderas et al., 2009). Therefore, feeding behavior may vary according to feeding motivation where more dramatic changes in behavior are observed if calves are provided a higher allowance of milk (Borderas et al., 2009).

While it is important to take milk intake and milk allowance into consideration when using behavioral data to diagnose illness in calves, evaluating changes in unrewarded visits should be a consideration as part of diagnosing disease. Calves later diagnosed with illness had fewer unrewarded visits (Svensson and Jensen, 2007) up to 2 days before and on the day of clinical signs of the illness (Sutherland et al., 2018). Tracking unrewarded visits may be a more sensitive way to detect sickness in calves as no change in rewarded visits to the feeder were seen between ill and healthy calves (Svensson and Jensen, 2007; Sutherland et al., 2018).
Finally, calves with illness also display more inactive lying time (Borderas et al., 2007; Sutherland et al, 2018) and fewer laying bouts than healthy calves, both before and after diagnosis (Swartz et al., 2017; Sutherland et al, 2018). Specifically, it was reported that inactive lying time increased between 10% and 21% (Borderas et al., 2007; Sutherland et al., 2018) in the days before signs of illness. Sick calves also had 1.6 to 4.3 fewer lying bouts (Swartz et al., 2017; Sutherland et al., 2018) than those calves who remain healthy. A reduction in activity by sick animals is used as an adaptive strategy to conserve energy and fight infection (Hart, 1988).

Heifers

Heifers are an important part of a dairy operation as they are the potential future of the herd. Due to lack of income provided by heifers until the start of their first lactation, minimizing input costs without compromising performance is a key focus for profitable dairy management. Utilizing limit-feeding or the addition of low-quality feedstuffs to heifer rations is common. Limit-feeding using a nutrient-dense diet allows for control of growth rates, while potentially reducing feeding costs and increasing feed efficiency with no significant effect on first-lactation milk production (Hoffman et al., 2007). The addition of low-quality feedstuffs provides the fill

effect to allow for limited nutrient intake without the risk of comprising welfare that can come with limit-feeding, as indicated by increased vocalization (Kitts et al., 2011).

Limit-feeding can cause heifers to spend less time feeding per day (Hoffman et al., 2007; Greter et al., 2008) with more inactive standing time and less lying time (Hoffman et al., 2007; Kitts et al., 2011) resulting in the potential for increased hoof pathologies (Cook et al., 2004). Limit-fed heifers also display patterns of decreased meal frequency, along with increased meal size and decreased meal duration (Greter et al., 2008). Larger meals provide more substrate for rumen fermentation at a faster rate, potentially offsetting the benefits from greater meal frequency. However, feeding low-nutrient feedstuffs, such as straw, in combination with a limit feeding nutritional program may be beneficial in meeting nutrient intake and feed cost targets without negatively affecting feeding behavior (Greter et. al., 2008). Heifers fed 10% and 20% straw with nutrient-dense limit-fed diets had linear increases in feeding time and average meal duration with linear decreases in meal size as straw inclusion rate increases (Greter et al., 2008). Heifers who had low-nutrient feedstuffs as part of their nutrient dense TMR also tended to spend more time ruminating versus those heifers who were on a limit-feeding nutritional program (Kitts et al., 2011). Although advantageous feeding behavior patterns occur in heifers fed low-nutrient feedstuffs in combination with limit feeding, it should also be noted this approach results in poorer feed efficiency due to an increase in DMI with no change in average daily gain (Kitts et al., 2011).

Understanding the effects of feed bunk stocking density on the behavior of heifers is important, as competition can occur and could affect weight gains. Heifers with greater feed bunk space per head spent more time eating per day than heifers with less feed bunk space, with no change in DMI (Keys et al., 1978). This means greater stocking density at the bunk can cause

heifers to increase their feeding rate, and in turn the substrate rapidly available for rumen microbes. Therefore, rumen pH could drop lower in heifers with less feed bunk space compared to heifers with more space and slower feeding rates.

Transition

Moving cows to individual pens just before calving is a common practice in the industry. However, moving cows to the maternity pen at different stages of labor may influence calving behavior and the length of one or more of the 3 calving stages (Proudfoot et al., 2013). Therefore, identifying behaviors to predict calving may be beneficial to reduce disruption of the natural calving process and improve transition success. For example, cows moved at different times early in the calving process display differences in lying time; cows moved before labor versus those moved late in stage I increase lying time with slightly fewer lying bouts in the hour before calving (Proudfoot et al., 2013). It has been suggested that decreased lying time when cows are moved during late stage I may be a response to a more restrictive environment, where cows spend more time exploring to ensure it is a safe place to calve (Proudfoot et al., 2013). That leads to the question: what are behavioral changes that can be used to identify cows expected to calve soon? Rumination time decreases in all cows approaching parturition. This decrease starts 20 hours before calving and continues after calving, where rumination diminishes to less than 10 minutes in the first 4 hours post-calving (Kovacs et al., 2017). Tracking changes in lying time on the day of calving may also be advantageous as cows show reduced lying time during the calving period (Miedema et al., 2011). Compared to day 4 prior to calving, lying time is decreased by an average of 2.55 hours on the day of calving for both primiparous and multiparous cows; however, primiparous cows spend 2.8 fewer hours per day lying than multiparous cows (Dick et

al., 2020). A change in lying time for all cows on the day of calving is likely due to a greater number of brief lying bouts (Miedema et al., 2011). Lying time declines from -22 hours to -12 hours relative to calving by 7.4 minutes per hour with an 80% increase in the number of standing bouts reported (Huzzey et al., 2005), and the decline in lying time further decreases by around 34 minutes per hour just before birth (Kovacs et al., 2017). Increased standing bouts are likely due to restlessness from the discomfort associated with calving (Huzzey et al., 2005). In addition to increased standing time, the number of postural changes (a shift between lying and standing) increase by 29.6 and 45.6% for primiparous and multiparous cows, respectively, when compared to day 4 prior to calving (Dick et al., 2020).

Utility of precision dairy management technology

Wearable PDM technologies designed to track changes in activity for detection of estrus are also being used to detect changes in behaviors that are associated with disease, calving, and production efficiency at different life stages. Benefits include increased efficiency, reduced costs, minimized adverse environmental impacts, and improved animal health and well-being (Bewley, 2010) with larger farms adopting precision dairy management technology at a greater rate than smaller herds (Gargiulo et al., 2018). When deciding whether or not to implement this technology, producers weigh the benefit-to-cost ratio, consider the total investment cost, and review the simplicity and ease of use of the equipment (Borchers and Bewley, 2015) with some influence coming from consultants, nutritionists, or veterinarians (Russel and Bewley, 2013). While more producers are moving toward these technologies, it has been reported that information overload is a reason why they have not been more widely adopted (Russell and Bewley, 2013). In a study by Eckelkamp and Bewley (2020), alerts were generated for individual

cows when a decrease in behavior by >30% was detected compared to the 10-day moving average. It was reported that only 8% of alerts were doubted by producers, but visual assessment of the cows who trigger alerts averaged 21%, with large variations across farms with 2% to 45% of alerts resulting in visual cow assessment. However, producers are more likely to ignore alerts over time and are more likely to visually check cows when < 20 alerts occur per day (Eckelkamp and Bewley, 2020). Cows in early lactation are likely given more attention than other stages of lactation, along with alerts for eating time or a combination of eating time and other activity (Eckelkamp and Bewley, 2020). In terms of usefulness for disease detection, producers have indicated monitoring behavioral changes for detection of mastitis is the most popular way to use the technology at this time (Borchers and Bewley, 2015).

SUMMARY

Precision dairy management technology may be a helpful tool for more than just estrus detection. Evaluating individual changes in rumination, feeding, lying, and standing may give producers an extra set of eyes and ears for early detection of disease at different stages of an animal's life. This technology may be especially helpful during the transition period to track changes in behavior associated with calving and reduce the negative consequences of a difficult calving. Overall, cows who spend more time standing prepartum may be at greater risk of complications post-calving. However, it is difficult to say whether or not cows with disease stand more. As it is not unlikely that increased standing prepartum could cause more energy expenditure and more risk of disease as a result. Continuing to understand the usefulness of PDM technology data will be important as farms consider implementing the technology in their

operations. Managing the quantity and quality of the alerts received by farm staff is important to be able to use the data to its full potential.

REFERENCES

Amundson, L.A., A.D. Rowson, P.M. Crump, A.P. Prichard, A.A. Cheng, C.E. Wimmler, M. Klister, S.R. Weaver, S.S. Bascom, D.E. Nuzback, K.P. Zanzalari, and L.L. Hernandez. 2018. Effect of induced hypocalcemia in nonlactating, nonpregnant Holstein cows fed negative DCAD with low, medium, or high concentrations of calcium. J. Anim. Sci. 96:5010–5023. doi:10.1093/jas/sky371.

Beede, D. K., T. E. Pilbeam, S. M. Puffenbarger, and R.J.T. 2001. Peripartum responses of Holstein cows and heifers fed graded concentrations of calcium (calcium carbonage) and anion (chloride) 3 weeks before calving. J. Dairy Sci. 88(Suppl.:(Abstr.).

Chan, P.S., J.W. West, and J.K. Bernard. 2006. Effect of Prepartum Dietary Calcium on Intake and Serum and Urinary Mineral Concentrations of Cows. J. Dairy Sci. 89:704–713. doi:10.3168/jds.S0022-0302(06)72133-6.

Charbonneau, E., D. Pellerin, and G.R. Oetzel. 2006a. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows : A Meta-Analysis. J. Dairy Sci. 89:537–548. doi:10.3168/jds.S0022-0302(06)72116-6.

Charbonneau, E., D. Pellerin, and G.R. Oetzel. 2006b. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. J. Dairy Sci. 89:537–548. doi:10.3168/jds.S0022-0302(06)72116-6.

Constable, P.D., A.A. Megahed, M.W.H.H. 2019. Measurement of urine pH and net acid excretion and their association with urine calcium excretion in periparturient dairy cows. J. Dairy Sci. 102:11370–11383. doi:10.3168/jds.2019-16805.

Corlett, S.C., and A.D. Care. 1988. The effects of reduced dietary phosphate on plasma osteocalcin levels in sheep. J. Exp. Physiol. 73:443–445.

Diehl, A.L., J.K. Bernard, S. Tao, T.N. Smith, D.J. Kirk, D.J. McLean, and J.D. Chapman. 2018a. Effect of varying prepartum dietary cation-anion difference and calcium concentration on postpartum mineral and metabolite status and milk production of multiparous cows. J. Dairy Sci. 1–11. doi:10.3168/jds.2018-14828.

Diehl, A.L., J.K. Bernard, S. Tao, T.N. Smith, D.J. Kirk, D.J. McLean, and J.D. Chapman. 2018b. Effect of varying prepartum dietary cation-anion difference and calcium concentration on postpartum mineral and metabolite status and milk production of multiparous cows. J. Dairy Sci. 101:9915–9925. doi:10.3168/jds.2018-14828.

Emery, R.S., L.D. Brown, and J.W. Thomas. 1964. Effect of Sodium and Calcium Carbonates on Milk Production and Composition of Milk, Blood, and Rumen Contents of Cows Fed Grain Ad Libitum with Restricted Roughage. J. Dairy Sci. 47:1325–1329. doi:10.3168/jds.S0022-0302(64)88913-X.

Escobosa, A., C.E. Coppock, L.D. Rowe, Jr., W.L. Jenkins, C.E.G. 1984. Effects of Dietary

Sodium Bicarbonate and Calcium Chloride on Physiological Responses of Lactating Dairy Cows in Hot Weather. J. Dairy Sci. 67:574–584. doi:10.3168/jds.S0022-0302(84)81341-7.

G.R. Oetzel, J.D. Olson, C.R. Curtis, M.J.F. 1988. Ammonium Chloride and Ammonium Sulfate for Prevention of Parturient Paresis in Dairy Cows. J. Dairy Sci. 71:3302–3309. doi:10.3168/jds.S0022-0302(88)79935-X.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. J. Dairy Sci. 101:2763–2813. doi:10.3168/jds.2017-13112.

Goff, J.P., and R.L. Horst. 1993. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. J. Dairy Sci. 76:101–108. doi:10.3168/jds.S0022-0302(93)77328-2.

Goff, J.P., and R.L. Horst. 1997. Effects of the Addition of Potassium or Sodium, but Not Calcium, to Prepartum Rations on Milk Fever in Dairy Cows. J. Dairy Sci. 80:176–186. doi:10.3168/jds.S0022-0302(97)75925-3.

Goff, J.P., R. Ruiz, and R.L. Horst. 2004. Relative Acidifying Activity of Anionic Salts Commonly Used to Prevent Milk Fever. J. Dairy Sci. 87:1245–1255. doi:10.3168/jds.S0022-0302(04)73275-0.

Grünberg, W., S.S. Donkin, and P.D. Constable. 2011. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base , calcium , and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. J. Dairy Sci. 94:727–745. doi:10.3168/jds.2010-3230.

Horst, R.L. and J.P.G. 1997. Milk fever and dietary potassium. Pages 181–189 in Proc. Cornell Nutr. Conf. Feed Manuf., Rochester, NY,Cornell Univ., Ithaca, NY.

Hu, W., L. Kung, and M.R. Murphy. 2007. Relationships between dry matter intake and acid – base status of lactating dairy cows as manipulated by dietary cation – anion difference. Anim. Feed Sci. Technol. 136:216–225. doi:10.1016/j.anifeedsci.2006.09.013.

Hu, W., and M.R. Murphy. 2004. Dietary Cation-Anion Difference Effects on Performance and Acid-Base Status of Lactating Dairy Cows: A Meta-Analysis. J. Dairy Sci. 87:2222–2229. doi:10.3168/jds.S0022-0302(04)70042-9.

Kamiya, Y., M. Kamiya, M. Tanaka, and S. Shioya. 2005. Effects of calcium intake and parity on plasma minerals and bone turnover around parturition. Anim. Sci. J. 76:325–330.

Kronqvist, C., U. Emanuelson, R. Spörndly, and K. Holtenius. 2011. Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. J. Dairy Sci. 94:1365–1373. doi:10.3168/jds.2009-3025.

Martinez, N., L.D.P. Sinedino, R.S. Bisinotto, R. Daetz, C. Lopera, C.A. Risco, K.N. Galvão, W.W. Thatcher, and J.E.P. Santos. 2016a. Effects of oral calcium supplementation on

mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. J. Dairy Sci. 99:8397–8416. doi:10.3168/jds.2015-10527.

Martinez, N., L.D.P. Sinedino, R.S. Bisinotto, R. Daetz, C.A. Risco, and K.N. Galvão. 2016b. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. J. Dairy Sci. 99:8417–8430. doi:10.3168/jds.2015-10529.

McKinnon, J.J, D.A. Christensen, and B.L. 1990. The influence of bicarbonate buffers on milk production and acid-base balance in lactating dairy cows. Can. J. Anim. Sci. 70:875–886.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th re. ed. Washington, D.C.

Oba, M., A.E. Oakley, and G.F. Tremblay. 2011. Dietary Ca concentration to minimize the risk of hypocalcaemia in dairy cows is affected by the dietary cation-anion difference. Anim. Feed Sci. Technol. 164:147–153. doi:10.1016/j.anifeedsci.2011.01.002.

Oetzel, G.R., and J.A. Barmore. 2015. Intake of a Concentrate Mixture Containing Various Anionic Salts Fed to Pregnant, Nonlactating Dairy Cows. J. Dairy Sci. 76:1617–1623. doi:10.3168/jds.S0022-0302(93)77495-0.

Pitkin, R. 1983. Endocrine regulation of calcium homeostasis during pregnancy.. Clin. Perinatol. 10:575–592.

Rérat, M., A. Philipp, H.D. Hess, and A. Liesegang. 2009. Effect of different potassium levels in hay on acid – base status and mineral balance in periparturient dairy cows. J. Dairy Sci. 92:6123–6133. doi:10.3168/jds.2009-2449.

Rodriguez-Suarez, L.A. 1998. Periparturient responses of cows fed varying dietary cation-anion differences and calcium contents prepartum. Michigan State University,.

Santos, J.E.P., I.J. Lean, H. Golder, and E. Block. 2019. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. J. Dairy Sci. 1–21. doi:10.3168/JDS.2018-14628.

Seifi, H.A., and S. Kia. 2018. Subclinical Hypocalcemia in Dairy Cows: Pathophysiology, Consequences and Monitoring 9:1–15. doi:10.22067/veterinary.v9i2.69198.

Takagi, H., and E. Block. 1991. Effects of Manipulating Dietary Cation-Anion Balance on Macromineral Balance in Sheep. J. Dairy Sci. 74:4202–4214. doi:10.3168/jds.S0022-0302(91)78616-5.

Vagnoni, D.B., and G.R. Oetzel. 1998. Effects of Dietary Cation-Anion Difference on the Acid-Base Status of Dry Cows. J. Dairy Sci. 81:1643–1652. doi:10.3168/jds.S0022-0302(98)75732-7.

Valadares, R.F.D, G.A. Broderick, S.C.V.F. and M.K.C. 1999. Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of

Total Purine Derivatives 1. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.S0022-0302(99)75525-6.

Wu, W.X., Y.M. Wu, and J.X. Liu. 2007. Blood profiles and health in Holstein cows fed diets with varying cation-anion difference and calcium. J. Anim. Feed Sci. 16:376–381.

Chapter 2 - Effects of calcium carbonate supplementation rate on metabolic acid-base status and feed intake of cows with compensated metabolic acidosis

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ABSTRACT

The benefits of feeding prepartum dairy cattle diets with negative dietary cation-anion difference (**DCAD**) to prevent postpartum hypocalcemia have been studied extensively. However, controversy still exists regarding the appropriate amount of calcium (**Ca**) to feed with these anionic diets, because of the potential negative impacts on dry matter intake (**DMI**) and reversal of induced acidosis. In this study, twenty-one pregnant, nonlactating cows (57.4 $\pm$ 13.9 days prepartum) completing at least one lactation were used in a replicated 3 × 3 Latin square design. Treatments included a) no supplemental Ca carbonate (**LOW**; 0.6% DM Ca), b) a moderate level of Ca carbonate supplementation (**MOD;** 1.2% DM Ca), and c) a high level of Ca carbonate supplementation (**HIGH**; 1.8% DM Ca). Each of the 3 periods were divided into an acclimation phase (days 1-3) and a collection phase (days 4-7). All cows were fed a diet formulated to provide a DCAD of -6.0 mEq/ 100 g of DM, using an anionic salt supplement to achieve the desired DCAD level. Urinary pH was measured once daily. Urine and blood samples were collected approximately 6 hours after the morning feeding during the collection phase. Urine samples were analyzed for concentrations of creatinine, ionized calcium (**iCa)**, and deoxypyridinoline (**DPD**). Whole blood samples were analyzed for metabolic indicators of acid-base status, including pH, partial pressure of CO_2 (**pCO_2**), oxygen saturation (**sO_2**) and concentrations of total carbon dioxide (**TCO_2**), bicarbonate (**HCO_3^-**), base excess (**BE**), iCa, sodium (**Na**), and potassium (**K**) using a handheld biochemical analyzer. There was no effect of treatment on DMI. Urine pH increased linearly with increasing dietary Ca, and urine pH was significantly greater for HIGH than LOW (6.73, 6.62, and 6.41 $\pm$ 0.12 for LOW, MOD and HIGH, respectively). There was no significant difference among treatments for urinary Ca excretion or DPD concentration or excretion. The level of Ca supplementation had no effect on

measures of acid-base status in blood. However, DMI was greater in Period 3 versus Period 1, associated with a greater degree of acidification as indicated by a lesser urine pH in Period 3 versus Period 1 (6.05 and 6.75 $\pm$ 0.12, respectively). When mildly acidifying cows (urine pH of 6-7) during the prepartum period, Ca carbonate concentrations between 0.6 and 1.8% of diet DM do not affect DMI, water intake, urinary Ca or DPD excretion, or blood parameters related to acid-base status. Urine pH was significantly greater for cows on the HIGH treatment with no significant difference in Ca excretion, suggesting carbonate, not Ca, may be absorbed and converted to HCO_3^-, with the excess HCO_3^- being excreted in urine. Therefore, when feeding high levels of Ca carbonate urine pH may not accurately reflect internal acid-base status.

INTRODUCTION

During the transition period, cows are at risk for hypocalcemia due to the high demand for Ca by the mammary system at the onset of lactation. Hypocalcemia occurs when calcium ingestion from the diet does not meet calcium demand by the mammary gland. Additionally, there is a delayed reaction to secretion of parathyroid hormone, and therefore an inadequate rate of bone resorption to replenish blood Ca reserves being depleted for mammary use. It has been established that incorporating anionic salts into prepartum dairy cattle diets reduces the prevalence of hypocalcemia as concluded in a review by Santos et al., 2019. Diets with a negative dietary cation-anion difference (**DCAD**) create a state of internal mild metabolic acidosis, resulting in a need for blood and urine buffering due to excess anions. Therefore, release of Ca phosphate from bone is needed to offset this acidic state, and the spilling of Ca into urine. The spilling of Ca into urine triggers release of parathyroid hormone (PTH), increased intestinal Ca absorption, and tissue sensitivity to PTH (Seifi and Kia, 2018). This homeostatic mechanism jump starts the ability of a cow to utilize Ca from bone to improve Ca status at calving.

Although the practice of feeding anionic prepartum diets is widely adopted, controversy exists regarding the role that dietary Ca plays when utilizing the DCAD strategy. This controversy has led to a wide range of field recommendations for Ca supplementation, from no supplemental Ca up to 200 grams of dietary Ca per day. While no clear advantage has been established for feeding low or high dietary Ca in prepartum diets, high supplemental Ca, much of which is Ca carbonate, has been reported to decrease dry matter intake (**DMI**) of cows prepartum (Diehl et al., 2018). Additionally, dietary Ca carbonate has the potential to alter acid-base balance and the induction of metabolic acidosis due to the cation properties of Ca. This could

offset the degree to which anionic salt supplementation is able to trigger Ca homeostatic mechanisms. This is evident with some DCAD equations developed to predict the degree of metabolic acidosis, where Ca is included as a strong cation (Horst and Goff, 1997; National Research Council, 2001).

Furthermore, dietary carbonate (from supplementing Ca carbonate in prepartum diets) has the potential to affect metabolic acid-base status through manipulation of the systemic carbonate/bicarbonate buffer system. Our hypothesis is that the dissociation of Ca carbonate in an acidic environment leads to production of bicarbonate, and therefore works as a buffer to offset the nutritionally induced metabolic acidosis needed to maintain Ca homeostasis during the transition period. The objectives of this study were to determine if increasing the amount of Ca carbonate in the diet affects DMI, metabolic acid-base status of cows fed a negative DCAD diet, and to assess impacts of dietary Ca carbonate on markers of Ca metabolism.

MATERIALS AND METHODS

Experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol #3754).

Cows and treatments

Twenty-one pregnant, nonlactating cows (57.4 $\pm$ 13.9 days prepartum) completing at least one lactation (range: 1-3 lactations) were used in a replicated 3 × 3 Latin square design, with 6 of 7 squares balanced for carry over effects. The treatments included a) the basal diet with no supplemental Ca carbonate (**LOW**; 0.60% DM Ca), b) the basal diet supplemented with a moderate level of Ca carbonate (**MOD;** 1.2% DM Ca), and c) the basal diet supplemented with a

high level of Ca carbonate (**HIGH;** 1.8% DM Ca). Cows were rotated through the 3 treatments during the 3 experimental periods, with each period lasting 7 days. Each period was divided into an acclimation phase (days 1-3) and a collection phase (days 4-7).Initially, a 5-day equilibrium period was implemented in which all cows were fed the basal diet formulated to provide a DCAD of -6.0 mEq/ 100 g, using an anionic salt supplement (SoyChlor; Landus Cooperative, Ames, IA) as the anion source to accomplish the desired degree of metabolic acidosis. To confirm that the targeted degree of metabolic acidosis was being achieved, urine samples were collected once per day during the equilibrium period and pH was measured using pH strips (Hydrion pH strips, Micro Essential Laboratory, Brooklyn, NY) until a urinary pH range of 6-7 was achieved for all cows. The basal diet was formulated to meet NRC (2001) requirements for all nutrients and was fed as a total mixed ration (**TMR**) twice daily (0600 and 1800 h). All treatments were formulated to have similar nutrient composition, including DCAD, other than the amount of Ca. However, due to greater than anticipated urine pH values during Period 1, additional anionic salt supplement was added to the basal diet at the beginning of Period 2 and continued through Period 3, as shown in Table 1. Supplemental Ca carbonate for the MOD and HIGH treatments was top-dressed twice per day and mixed thoroughly into the basal diet for each individual cow. The total grams of supplemental Ca carbonate top dressed for each treatment were adjusted for the quantity of TMR delivered during each feeding throughout the experiment. Cows were housed and fed in individual tie-stalls.

Data collection and sampling procedure

The as-fed feed intake for each cow was recorded daily by taking the difference between the weight of the feed offered and the refusals for that same day. As-fed intakes were adjusted by

TMR DM for determination of daily DMI. Samples of the TMR were collected twice per week during both the acclimation and collection periods. Samples were composited by period for nutrient analysis by Dairy One Forage Laboratory (Ithaca, NY; Table 1). Additionally, cows had access to individual automated water dispenser systems, which included meters to track total water intake. Urine pH was measured once daily using pH strips (Hydrion pH strips, Micro Essential Laboratory, Brooklyn, NY) to ensure the target level of acidification (pH range 6-7) was being met throughout the experiment. During the 4-day collection phase, midstream urine samples were collected into specimen cups following manual stimulation of the perineal region. Urine samples were acidified using 1 part urine and 1 part 0.15 N H_2SO_4, aliquoted into 10-mL tubes, and stored at -20˚C until analysis for concentrations of creatinine (Creatinine Colorimetric Assay Kit, Cayman Chemical, Ann Arbor, MI), ionized calcium (**iCa;** Calcium Assay Kit, BioVision, Milpitas, CA), and total deoxypyridinoline (**DPD**; MicroVue DPD; Quidel Corporation, Athens, OH) according to manufacture instructions. Urinary creatinine concentrations and expected excretion of creatinine at 29 mg/kg of BW/d (Valadares et al., 1999) were used to estimate total daily urine volume. Daily urine volume and sample concentrations of Ca and DPD were used to estimate total Ca and DPD excretion each day of the collection period. Urinary DPD, a product formed specifically during degradation of bone collagen during resorption, was analyzed and used as an indicator of treatment impacts on bone resorption. Validation of the MicroVue DPD assay was performed by spiking in 15 µL of sample and measuring the recovery. Samples were diluted 1:10 for DPD analysis to fit within the standard curves. This assay was performed as a single run on a single plate. The inter- and intra-assay coefficients of variation for urinary creatinine were 3.9% and 3.8%, respectively, and 5.2% and 5.5%, respectively, for urinary Ca.

Additionally, blood samples were collected via the coccygeal vein into 10-mL lithium heparin evacuated tubes (Vacuette, Greiner Bio-One, Monroe, NC). Within 5 minutes of collection, the whole blood samples were analyzed for determination of the concentration of metabolic indicators of acid-base status, including pH, partial pressure of CO_2 (**pCO_2**), oxygen saturation (**sO_2**) and concentrations of total carbon dioxide (**TCO_2**), bicarbonate (**HCO_3^-**), base excess (**BE**), iCa, sodium (**Na**), and potassium (**K**) using a handheld biochemical analyzer (VetScan i-STAT, Abaxis, Union City, CA) and CG8+ cartridges (Abbott, Princeton, NJ). Urine and blood samples were collected approximately 6 hours after the morning feeding each day of the collection period.

Statistical analysis

Before statistical analysis was performed, data were averaged across the 4-day collection for each variable of interest, yielding one data point per cow for each period. Statistical analysis was performed in JMP Pro (version 14.0, SAS Institute Inc., Cary, NC) using a mixed model with fixed effects of treatment and period, and the random effect of cow. The interaction of treatment × period was tested and removed from the model when $P > 0.20$. Outliers were excluded when the Studentized residual exceeded an absolute value of 4. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$).

RESULTS

Calcium supplementation for MOD and HIGH was designed to double and triple the amount of Ca in the common basal diet. The basal diet was formulated to supply only the Ca endogenous to the forages and other macro-ingredients in the TMR. Formulated Ca intake was

71, 142, and 213 g/d for LOW, MOD and HIGH treatments, respectively. Small deviations in basal Ca were observed by period when the chemical composition of the TMR was analyzed for each period (Table 1) Actual Ca intake was 89.2 ± 3.2, 156.3 ± 3.4, and 225.2 ± 2.7 g/d for LOW, MOD and HIGH, respectively.

The DCAD for the common basal diet was formulated for -6.0 mEq/ 100 g DM using the equation: DCAD = (Na + K) – (S + Cl). Chemical analysis revealed DCAD variation in the basal diet by period (Table 1), where DCAD was more negative in Period 3 than in either Period 1 or Period 2. Additional anionic salt was added after the conclusion of Period 1 due to higher than anticipated urinary pH values. However, upon chemical analysis the variation in DCAD can be attributed to less potassium in the TMR in Period 3 (1.17% DM) compared to Period 1 and Period 2 (1.25% and 1.33% DM, respectively). Potassium is a strong cation that has the potential to alter Ca homeostasis (Goff and Horst, 1997) and acid-base balance (Rérat et al., 2009). Under most circumstance, potassium is the hardest ion to control in the ration as application of manure and fertilizers rich in potassium can result in high potassium content of forages (Goff, 2018).

Dry matter and water intake

There was no difference ($P = 0.21$) in DMI for cows fed the LOW, MOD or HIGH treatment diets (Figure 2.1). A significant period effect was found for DMI, where cows consumed less feed in Period 1 versus Period 2 ($P = 0.003$) and Period 3 ($P < 0.001$) at 10.68, 11.70 and 12.31 ± 0.38 kg DM/d, respectively. Water intake was not affected by treatment (Figure 2.2), but a significant decrease in water intake was observed in Period 1 versus Period 3 ($P = 0.009$) at 44.58 and 49.21 ± 1.94 L/d, respectively, with Period 2 intermediate at 46.46 ± 1.94 L/d.

Urinary pH

Effect of treatment on urinary pH is summarized in Figure 2.3. Cows on the HIGH treatment had significantly greater urine pH than cows on the LOW treatment ($P = 0.03$), with MOD intermediate to LOW and HIGH. There was a period effect where a lesser urine pH was observed in Period 3 versus Period 1 ($P < 0.001$) and Period 2 ($P < 0.001$) at 6.75, 6.95 and 6.06 $\pm$ 0.12 for Period 1, 2 and 3, respectively. This corresponds with the previously mentioned change in DCAD from dietary potassium in Period 3 and indicates a greater degree of acidification was experienced by cows during this time.

Calcium excretion

There was no significant difference between treatments for urinary Ca excretion ($P = 0.67$; Figure 2.4). There was an effect of period, where urinary Ca excretion was significantly reduced in Period 1 ($P < 0.001$) and Period 2 ($P = 0.02$) compared to Period 3 at 4.78, 3.89 and 6.51 $\pm$ 0.63 g/d for Period 1, 2 and 3, respectively.

Deoxypyridinoline

Concentration ($P = 0.45$; Figure 2.5) and excretion of DPD ($P = 0.43$; Figure 2.6) did not differ by treatment. There was a period effect for urinary DPD concentration, where urinary DPD concentration was lower in Period 1 ($P < 0.0005$) and Period 2 ($P = 0.0001$) than in Period 3 at 6.42, 5.97, and 7.87 $\pm$ 0.26 nmol DPD/mmol creatinine, respectively. A similar period effect was seen for urinary DPD excretion, where DPD excretion was lesser in Period 1 ($P = 0.0006$) and

Period 2 ($P < 0.0001$) than in Period 3 (1,157.60, 1,076.41, and 1424.40 $\pm$ 47.14 nmol/d, respectively).

Acid-base status

Treatment had no effect on blood parameters indicative of acid-base balance (pH, pCO_2, sO_2, TCO_2, HCO_3^-, and BE), or on strong cation concentrations (Na^+ and K^+). These results are summarized in Figure 2.7. Period effects were found to be significant for pH ($P = 0.004$), and concentrations of TCO_2 ($P < 0.001$), HCO_3^- ($P < 0.001$), BE ($P < 0.001$), iCa^{2+} ($P = 0.03$) and Na^+ ($P < 0.001$). Blood pH linearly decreased ($P = 0.001$) from Period 1 to Period 3 at 7.44, 7.43, and 7.42 $\pm$ 0.01, respectively. Concentration of TCO_2 in blood followed a similar pattern to pH, decreasing across periods (27.73, 26.55, and 26.17 $\pm$ 0.33 mmol/L for Period 1, 2 and 3, respectively; $P < 0.0001$). Blood HCO_3^- concentration was greater in Period 1 than Period 2 ($P = 0.0002$) or Period 3 ($P < 0.0001$) at 26.48, 25.43 and 25.06 $\pm$ 0.31 mmol/L, respectively. Similarly, BE was significantly greater in Period 1 versus Period 2 ($P = 0.0001$) and Period 3 ($P < 0.0001$) at 2.12, 1.02, and 0.52 $\pm$ 0.29 mmol/L, respectively. Na^+ was significantly decreased in Period 1 versus Period 2 ($P = 0.0004$) and Period 3 ($P < 0.0001$) with blood Na^+ concentrations at 140.58, 141.18 and 141.27 $\pm$ 0.16 mmol/L for Period 1, 2 and 3, respectively.

Blood ionized calcium

Treatment did not affect blood iCa ($P = 0.57$; Figure 2.8). However, there was a significant decrease in blood iCa for Period 2 versus Period 3 ($P = 0.04$) at 1.179 and 1.192 $\pm$ 0.01 mmol/L, respectively.

As a secondary objective, a linear regression was performed to determine which DCAD equation (of the four mentioned prior) can be used to best predict indicators of acid-base balance (blood pH, pCO_2, sO_2, TCO_2, HCO_3^-, BE, iCa^{2+}, Na^+, and K^+). Results from this analysis are listed in Table 2. DCAD1 was best able to predict most indicators of acid-base balance, including blood pH ($P = 0.01$), TCO_2 ($P = 0.008$), HCO_3^- ($P = 0.01$), BE ($P = 0.001$), and Na ($P = 0.02$). DCAD4 successfully predicted BE ($P = 0.02$) and tended to be associated with TCO_2 ($P = 0.07$), HCO_3^- ($P = 0.06$), and Na ($P = 0.09$). Neither DCAD2 nor DCAD3 were associated with any of the variables used for indication of acid-base status. The longer DCAD equations, which incorporated other minerals besides Na, K, Cl and S, had no advantage over the shorter equations.

DISCUSSION

The effects of calcium carbonate supplementation on DMI, acid-base status, and urinary excretion of Ca and DPD were examined in this study. No difference in DMI was observed in this study when cows were fed varying levels of dietary Ca, sourced primarily from Ca carbonate. Inconsistencies in DMI responses to dietary Ca are reported in previous research. When Ca was included at either 0.60% or 1.20% of DM in an anionic prepartum diet, no difference in DMI was found (Oetzel et al.; 1988). Additionally, Chan et al. (2006) fed 0.99% or 1.50% total dietary Ca with an anionic diet (average DCAD of -5.8 mEq/ 100 g DM), and did not see differences in DMI during the prepartum period. In contrast, when cows were fed a more anionic prepartum diet (DCAD = -22 mEq/100 g DM; target urinary pH of 5.5 to 6) with either 1.30% or 1.80% dietary Ca, a 2.2 kg/d reduction in DMI was observed for cows that were fed more Ca (Diehl et al., 2018). Although no difference in DMI was observed in this study, it is

thought the addition of dietary minerals, such as Ca, are deleterious to DMI due to lack of palatability (Beede et al., 2001). In addition to unpalatability of dietary minerals, another factor that has the potential to reduce DMI is a more aggressive anion feeding strategy as seen when the inclusion of anions is greater than 30 mEq / 100 g DM in the diet during the transition period (Charbonneau et al., 2006a). Theories proposed to explain this reduction in DMI with a lower DCAD are poor palatability of the anion source (Oetzel and Barmore, 2015) and possible discomfort from metabolic acidosis (Vagnoni and Oetzel, 1998). For example, Diehl et al. (2018) used an anionic supplement with a DCAD concentration of 706.4 mEq / 100 g DM of DCAD at 4.17% DM in their prepartum diet to provide an additional 29.5 mEq of anions/ 100 g of DM (30% greater than the anion addition in this study) and observed a reduction in DMI with increased levels of dietary Ca. In this study, 7.12% of an anion supplement with a DCAD of 252.6 mEq/ 100 g was utilized to provide 20.5 mEq of anions/ 100 g of DM with no reduction in DMI with increased dietary Ca. Therefore, reduced DMI of cows fed anionic diets during the prepartum period could be the result of a combination of decreased palatability from large amounts of Ca supplementation, in addition to anion supplementation greater than 30 mEq / 100 g DM (Charbonneau et al., 2006a).

While the addition of anionic supplements more than 30 mEq / 100 g DM may seem to be a valid explanation for a reduction in DMI during the prepartum period, Chan et al. (2006) and Oetzel et al. (1988) supplemented anions well above 30 mEq/ 100 g DM, at 39.2 and 38.8 mEq / 100 g of DM, respectively, to deliver diets with DCAD of -5.2 and -7.5, respectively, and did not see a reduction in DMI. Therefore, this theory of reduced DMI with anionic supplementation greater than 30 mEq / 100 g of DM does not always hold true. Thus, it is possible that acid-base status (and the degree to which cows are acidified) may be more

detrimental to prepartum DMI than the rate of anion supplementation. In a study by Hu et al. (2007), DMI increased quadratically with increased DCAD. It was concluded that DMI is closely associate with acid-base status as DMI also increased as blood HCO_3^- concentrations, blood pH, and urinary pH increased. Similar conclusions were reported by Hu and Murphy (2004), as feed intake, blood pH and HCO_3^- decreased with decreasing DCAD indicating less optimal acid-base status (Hu and Murphy, 2004). Utilizing a less aggressive dietary DCAD (mildly acidified to a urinary pH of closer to 7) over full acidification strategies (indicated by urinary pH range of 5.5-6) may help increase DMI prepartum, regardless of total dietary Ca. Mild acidification may be beneficial when using on farm tools for estimation of net acid excretion through measurement of urinary pH when feeding anionic salt supplementation, as urinary pH of 6.1 or less poorly reflects blood pH and the magnitude of nutritionally induced systemic acidosis (Constable et al., 2019). In addition, mild acidification (urinary pH below 7) is sufficient to reasonably prevent clinical milk fever (Charbonneau et al., 2006a).

In this study, it was hypothesized that Ca carbonate supplementation could reduce the metabolic acidosis induced when feeding anionic supplements, and a change in acid-base status would be reflected by measuring urinary pH when feeding varying levels of Ca carbonate. Urinary pH increased linearly (P = 0.009; Figure 2.1) with increasing dietary Ca. Amundson et al. (2018) also reported that urinary pH was significantly decreased for cows fed 0.4% versus 1.0% and 1.6% dietary Ca during the 21-day feeding period prior to a hypocalcemia challenge. However, no effect of dietary Ca inclusion was seen on urinary pH in two previous studies which supplemented Ca carbonate with anionic diets prepartum (Chan et al., 2006; Diehl et al., 2018a). However, because Ca is a strong cation increased dietary Ca could result in greater urinary pH offsetting the compensated metabolic acidosis induced from feeding anion supplements during

the prepartum period. When Ca is absorbed from the diet, in order to maintain electroneutrality, the blood will undergo a reduction in the positively charged hydrogen ions (H^+) and increase in negatively charged hydroxyl ions. Since pH is based on the concentration of H^+ an increase in pH would be observed, which could explain the increase in urinary pH in this study when greater amounts of dietary Ca were fed.

Large quantities of dietary Ca can be directed to 3 places after ingestion: urine, bone, and feces. Urinary Ca excretion was not affected by treatment in this study and was similar to that reported by Rodriguez-Suarez (1998). Therefore, if excess Ca was being absorbed from the diet when greater amounts of dietary Ca were supplemented it was not being excreted in the urine subsequent to absorption from feeding. However, cows in this study did not have a high demand for Ca given that they were in late gestation and were not lactating. It is possible to speculate that much of the Ca from the diet was not being absorbed and would not have impacted systemic acid/base balance and urinary pH as a result.

Similar to urinary Ca excretion, no changes in urinary DPD, a marker of bone degradation, were observed in this study. It has been suggested increased levels of plasma Ca are linked to changes in bone metabolism (Corlett and Care, 1988). If Ca was being absorbed from the diets with greater Ca supplementation, it is likely that a change in DPD would have been observed in this study. Additionally, if Ca was being deposited into bone as a result of greater Ca absorption, then decreased urinary DPD concentration and excretion with greater dietary Ca concentration would have been expected. A lack of changes in DPD concentration and excretion by treatment suggests it is unlikely that enough Ca was being absorbed to affect plasma Ca levels, uptake of Ca by bone, and as a result acid-base status and urinary pH.

It would be advantageous to measure fecal calcium in future studies to ascertain Ca absorption from each of the dietary treatments; it is likely that dietary Ca fed in excess of requirements in this study ended up in the feces. This is speculated for two reasons. First, as previously discussed, neither urinary Ca nor urinary DPD changed with varying levels of dietary Ca, so Ca was most likely not directed to urine or bone. Secondly, feeding nonlactating cows an anion supplement with either 0.48% or 2.0% Ca carbonate resulted in an average apparent absorption of 13% and 23%, respectively, of the available dietary Ca, with most of the Ca being excreted in feces (Rodriguez-Suarez, 1998). Therefore, significantly greater dietary Ca provided to cows in this study would have only had a minor effect on apparent absorption of Ca allowing little chance of Ca affecting acid-base status and urinary pH.

With these observations, an alternative explanation to greater urinary pH with increased Ca carbonate supplementation may be that carbonate, and not Ca, from Ca carbonate is the driver of changes in urinary pH with increasing Ca carbonate supplementation in this study, with dissociation of Ca carbonate likely occurs in the acidic abomasum as calcium carbonate did not decrease rumen lactic acid and volatile fatty acid concentrations, in contrast to sodium bicarbonate, when fed with a high grain diet (Emery et al., 1964), suggesting limited ruminal buffering effect. Ca carbonate supplementation instead allows carbonate to contribute to the blood bicarbonate buffer system, as carbonate dissociates from Ca in the presence of acid, which then reacts with hydrogen ions to produce carbonic acid and eventually bicarbonate.

One may expect mild shifts in acid-base status with additional systemic buffering, but no indication of changes in acid-base status were detected with different levels of dietary Ca carbonate as measured by blood pH, pCO_2, sO_2, TCO_2, HCO_3^-, BE in this study. However, this is not surprising as sodium bicarbonate fed as a feed supplement to lactating dairy cows showed no

compensation to acid-base status, specifically HCO_3^-, pCO_2, or BE, although blood pH was increased with the addition of sodium bicarbonate (McKinnon et al., 1990). Escobosa et al. (1984) also reported cows fed either control or sodium bicarbonate treatments had similar acid-base status, with increased urinary pH for cows on the sodium bicarbonate diet. It is likely that the kidneys maintained acid-base homeostasis in the current study by excreting excess HCO_3^- (from the Ca carbonate), thereby maintaining blood acid-base homeostasis in the face of increasing Ca carbonate supplementation. However, it is uncertain if the concentration of HCO_3^- in the urine of these cows was altered.

Increased Ca flux, measured by increased urine Ca excretion, is positively associated with net acid excretion through feeding anionic diets (Grünberg et al., 2011). Mean urine Ca excretion in late-gestation cows fed a non-acidogenic ration is typically less than 1 g/d but increases to $\geq$ 4 g/d with acidogenic diets. Mean Ca excretion in this study was 5.1 $\pm$ 0.4 g/d, indicating the effects of the acidogenic diet in this study to increase Ca flux. However, Ca excretion did not differ when varying levels of dietary Ca were fed in this study indicating a negligible increase in Ca absorption or altered bone Ca flux in response to greater dietary Ca. In sheep, Takagi and Block (1991) reported Ca absorption was increased with increasing Ca intake, but no difference in urinary Ca excretion was reported. Likewise, Diehl et al. (2018) fed an anionic diet prepartum with 1.3% or 1.8% dietary Ca and did not see a difference in fractional excretion of Ca. A lack of difference between treatments for urinary Ca excretion, along with no effect of treatment on DPD concentration and excretion in this study, suggests that even if more Ca was absorbed when feeding high levels of Ca it is not enough to alter bone Ca flux as a result.

Urinary DPD is commonly used marker for bone resorption as it is excreted into urine unmetabolized when released. No differences in urinary DPD concentration or excretion were

observed in this study. In agreeance with this study, Kamiya et al. (2005) fed 0.46% or 0.86% Ca to cows 3 weeks prepartum to 3 days post-partum and found that there was no treatment effect on urinary DPD at 13 days prepartum or 3 days postpartum. The authors did, however, report a 111% increase in urinary DPD excretion 3 days after calving compared to 13 days prepartum for multiparous cows, and not primiparous cows, on a low Ca diet. Amundson et al. (2018) saw similar results when nonpregnant Holstein cows were fed either 0.4%, 1.0% or 1.6% dietary Ca for a 21-d period prior to a hypocalcemia challenge as no difference in urinary DPD was observed by treatment. Therefore, the dietary Ca level seems to have no impact on bone resorption prepartum.

In the present study, target levels of 0.60%, 1.20% and 1.80% dietary Ca concentrations were fed with no significant difference in blood concentrations of iCa^{2+}, Na^{+}, or K^{+}. Negative DCAD diets fed prepartum are known to be effective in increasing postpartum Ca concentrations in blood (Santos et al., 2019), but increased dietary Ca has not been found effective in altering blood Ca (Beede et al., 2001; Chan et al., 2006; Diehl et al., 2018; Kronqvist et al., 2011; Goff and Horst, 1997), K^{+} (Diehl et al., 2018a), or Na^{+} (Diehl et al., 2018a) concentrations prepartum. The same results have been reported in sheep, when Takagi and Block (1991) fed diets containing either 0.47% or 0.82% dietary Ca with no treatment effects on blood Ca, K^{+} or Na^{+} concentrations. However, Amundson et al. (2018) reported increased blood iCa^{2+} for cows fed 1.6% dietary Ca versus 0.4% and 1.0% Ca diets during the 21-d feeding period prior to a hypocalcemia challenge. The cows utilized in the study by Amundson et al. (2018) were dry and nonpregnant, which means cows were in a slightly different physiological state, in terms of calcium metabolism, than cows in this study which were dry, but pregnant. During pregnancy, an increase in parathyroid hormone helps maintain serum calcium levels within the physiological

range as extracellular fluid volume expands, urinary excretion increases, and calcium is transferred to the fetus for growth (Pitkin, 1983). It was not unexpected to find that increased dietary Ca had no effect on blood Ca in the previous mentioned studies, as circulating Ca concentrations are kept within a very narrow range between 2.25-2.5 m*M*. Excess Ca which may have been absorbed from the diet would trigger calcitonin secretion, resulting in bone deposition and/or excretion of excess Ca into urine (Goff, 2018).

Dietary Ca does not appear to play a role in acid-base regulation at up to 1.8% in the diet as evident by the results of this study where no treatment effect was observed for blood indicators of acid-base status. Similarly, Martinez et al. (2016a) administered one dose of oral Ca supplementation at 0, 43, or 86 g to cows on the day of calving and did not find that Ca impacted blood pH. However, the authors did report reductions in HCO_3^-, BE and pCO_2 (Martinez et al., 2016a), which was not observed in this study. An increase in blood Ca with no subsequent change in pH could be offset by buffering with HCO_3^- as a result of the dissociation of water and carbon dioxide. Additionally, Ca absorption can occur by passive diffusion in the digestive tract when a concentration of 6 m*M* is achieved, which is rapidly reached when cows are given oral Ca boluses (Goff and Horst, 1993). It has been suggested that a portion of the strong anions can be neutralized by the adsorbed Ca, therefore reducing the retention of hydrogen and the consequent drop in pH (Martinez et al., 2016a). Blood pH is dependent on the dietary balance of cations and anions, and therefore an upset in this relationship might be expected with large amounts of Ca ingestion. We did not see any significant changes in acid-base blood parameters for cows fed target levels of 0.60%, 1.20% or 1.80% dietary Ca in this study, and therefore it is likely that even 1.80% dietary Ca is not enough to cause paracellular absorption and affect acid-base status.

There was a period effect for DMI in this study, where cows consumed more feed in Period 3 (12.31 kg/d) versus Period 1 (10.68 kg/d) and Period 2 (11.70 kg/d). There was also a numerical difference in DCAD by period (Table 1), where DCAD was slightly more negative in Period 3 than in either Period 1 or Period 2. Therefore, cows had greater dry matter and anion intake in Period 3, which induced a greater degree of metabolic acidosis as indicated by urinary pH of 6.06 during this period. A greater degree of acidosis was also evident when evaluating blood parameters, with blood pH, TCO_2, HCO_3^-, BE, iCa , and Na, as well as urinary Ca excretion different between Period 1 and Period 3. Lower blood pH (Oba et al., 2011) and greater blood Ca (Wu et al., 2007) can result from feeding a greater amount of anionic salts prepartum, consistent with our observations across periods. It has been suggested that the optimal dietary Ca concentration to minimize hypocalcemia is likely dependent on the level of DCAD (Oba et al., 2011) as Ca status changes with varying levels of DCAD as opposed to dietary Ca concentration.

CONCLUSION

The amount of dietary Ca, in the form of Ca carbonate, had no effect on DMI, water intake, acid-base status, or urinary Ca and DPD excretion in prepartum cows. However, urine pH was significantly increased when supplementing greater amounts Ca carbonate. This is likely due to the effects of carbonate, not Ca, from the Ca carbonate supplementation. With no apparent change to blood acid-base balance, feeding high levels of supplemental Ca may justify allowing a slightly greater target urinary pH on-farm when tracking urinary pH for indication of internal acid-base status and successful prepartum acidification programs.

ACKNOWLEDGEMENTS

The authors would like to thank Landus Cooperative (Ames, IA) for financial support of this research. Additionally, for assistance in animal care, feeding, and sample collection authors would like to thank Mike Scheffel (Manhattan, KS), the staff at the Kansas State University Dairy Cattle Teaching and Research Center, the graduate students of the Dairy Metabolism group at Kansas State University (Manhattan, KS), and the undergraduate students who aided in this study.

REFERENCES

Amundson, L.A., A.D. Rowson, P.M. Crump, A.P. Prichard, A.A. Cheng, C.E. Wimmler, M. Klister, S.R. Weaver, S.S. Bascom, D.E. Nuzback, K.P. Zanzalari, and L.L. Hernandez. 2018. Effect of induced hypocalcemia in nonlactating, nonpregnant Holstein cows fed negative DCAD with low, medium, or high concentrations of calcium. J. Anim. Sci. 96:5010–5023. doi:10.1093/jas/sky371.

Beede, D. K., T. E. Pilbeam, S. M. Puffenbarger, and R.J.T. 2001. Peripartum responses of Holstein cows and heifers fed graded concentrations of calcium (calcium carbonage) and anion (chloride) 3 weeks before calving. J. Dairy Sci. 88(Suppl.:(Abstr.).

Chan, P.S., J.W. West, and J.K. Bernard. 2006. Effect of Prepartum Dietary Calcium on Intake and Serum and Urinary Mineral Concentrations of Cows. J. Dairy Sci. 89:704–713. doi:10.3168/jds.S0022-0302(06)72133-6.

Charbonneau, E., D. Pellerin, and G.R. Oetzel. 2006a. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows : A Meta-Analysis. J. Dairy Sci. 89:537–548. doi:10.3168/jds.S0022-0302(06)72116-6.

Charbonneau, E., D. Pellerin, and G.R. Oetzel. 2006b. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. J. Dairy Sci. 89:537–548. doi:10.3168/jds.S0022-0302(06)72116-6.

Constable, P.D., A.A. Megahed, M.W.H.H. 2019. Measurement of urine pH and net acid excretion and their association with urine calcium excretion in periparturient dairy cows. J. Dairy Sci. 102:11370–11383. doi:10.3168/jds.2019-16805.

Corlett, S.C., and A.D. Care. 1988. The effects of reduced dietary phosphate on plasma osteocalcin levels in sheep. J. Exp. Physiol. 73:443–445.

Diehl, A.L., J.K. Bernard, S. Tao, T.N. Smith, D.J. Kirk, D.J. McLean, and J.D. Chapman. 2018a. Effect of varying prepartum dietary cation-anion difference and calcium concentration on postpartum mineral and metabolite status and milk production of multiparous cows. J. Dairy Sci. 1–11. doi:10.3168/jds.2018-14828.

Diehl, A.L., J.K. Bernard, S. Tao, T.N. Smith, D.J. Kirk, D.J. McLean, and J.D. Chapman. 2018b. Effect of varying prepartum dietary cation-anion difference and calcium concentration on postpartum mineral and metabolite status and milk production of multiparous cows. J. Dairy Sci. 101:9915–9925. doi:10.3168/jds.2018-14828.

Emery, R.S., L.D. Brown, and J.W. Thomas. 1964. Effect of Sodium and Calcium Carbonates on Milk Production and Composition of Milk, Blood, and Rumen Contents of Cows Fed Grain Ad Libitum with Restricted Roughage. J. Dairy Sci. 47:1325–1329. doi:10.3168/jds.S0022-0302(64)88913-X.

Escobosa, A., C.E. Coppock, L.D. Rowe, Jr., W.L. Jenkins, C.E.G. 1984. Effects of Dietary

Sodium Bicarbonate and Calcium Chloride on Physiological Responses of Lactating Dairy Cows in Hot Weather. J. Dairy Sci. 67:574–584. doi:10.3168/jds.S0022-0302(84)81341-7.

G.R. Oetzel, J.D. Olson, C.R. Curtis, M.J.F. 1988. Ammonium Chloride and Ammonium Sulfate for Prevention of Parturient Paresis in Dairy Cows. J. Dairy Sci. 71:3302–3309. doi:10.3168/jds.S0022-0302(88)79935-X.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. J. Dairy Sci. 101:2763–2813. doi:10.3168/jds.2017-13112.

Goff, J.P., and R.L. Horst. 1993. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. J. Dairy Sci. 76:101–108. doi:10.3168/jds.S0022-0302(93)77328-2.

Goff, J.P., and R.L. Horst. 1997. Effects of the Addition of Potassium or Sodium, but Not Calcium, to Prepartum Rations on Milk Fever in Dairy Cows. J. Dairy Sci. 80:176–186. doi:10.3168/jds.S0022-0302(97)75925-3.

Goff, J.P., R. Ruiz, and R.L. Horst. 2004. Relative Acidifying Activity of Anionic Salts Commonly Used to Prevent Milk Fever. J. Dairy Sci. 87:1245–1255. doi:10.3168/jds.S0022-0302(04)73275-0.

Grünberg, W., S.S. Donkin, and P.D. Constable. 2011. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base , calcium , and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. J. Dairy Sci. 94:727–745. doi:10.3168/jds.2010-3230.

Horst, R.L. and J.P.G. 1997. Milk fever and dietary potassium. Pages 181–189 in Proc. Cornell Nutr. Conf. Feed Manuf., Rochester, NY,Cornell Univ., Ithaca, NY.

Hu, W., L. Kung, and M.R. Murphy. 2007. Relationships between dry matter intake and acid – base status of lactating dairy cows as manipulated by dietary cation – anion difference. Anim. Feed Sci. Technol. 136:216–225. doi:10.1016/j.anifeedsci.2006.09.013.

Hu, W., and M.R. Murphy. 2004. Dietary Cation-Anion Difference Effects on Performance and Acid-Base Status of Lactating Dairy Cows: A Meta-Analysis. J. Dairy Sci. 87:2222–2229. doi:10.3168/jds.S0022-0302(04)70042-9.

Kamiya, Y., M. Kamiya, M. Tanaka, and S. Shioya. 2005. Effects of calcium intake and parity on plasma minerals and bone turnover around parturition. Anim. Sci. J. 76:325–330.

Kronqvist, C., U. Emanuelson, R. Spörndly, and K. Holtenius. 2011. Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. J. Dairy Sci. 94:1365–1373. doi:10.3168/jds.2009-3025.

Martinez, N., L.D.P. Sinedino, R.S. Bisinotto, R. Daetz, C. Lopera, C.A. Risco, K.N. Galvão, W.W. Thatcher, and J.E.P. Santos. 2016a. Effects of oral calcium supplementation on

mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. J. Dairy Sci. 99:8397–8416. doi:10.3168/jds.2015-10527.

Martinez, N., L.D.P. Sinedino, R.S. Bisinotto, R. Daetz, C.A. Risco, and K.N. Galvão. 2016b. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. J. Dairy Sci. 99:8417–8430. doi:10.3168/jds.2015-10529.

McKinnon, J.J, D.A. Christensen, and B.L. 1990. The influence of bicarbonate buffers on milk production and acid-base balance in lactating dairy cows. Can. J. Anim. Sci. 70:875–886.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th re. ed. Washington, D.C.

Oba, M., A.E. Oakley, and G.F. Tremblay. 2011. Dietary Ca concentration to minimize the risk of hypocalcaemia in dairy cows is affected by the dietary cation-anion difference. Anim. Feed Sci. Technol. 164:147–153. doi:10.1016/j.anifeedsci.2011.01.002.

Oetzel, G.R., and J.A. Barmore. 2015. Intake of a Concentrate Mixture Containing Various Anionic Salts Fed to Pregnant, Nonlactating Dairy Cows. J. Dairy Sci. 76:1617–1623. doi:10.3168/jds.S0022-0302(93)77495-0.

Pitkin, R. 1983. Endocrine regulation of calcium homeostasis during pregnancy.. Clin. Perinatol. 10:575–592.

Rérat, M., A. Philipp, H.D. Hess, and A. Liesegang. 2009. Effect of different potassium levels in hay on acid – base status and mineral balance in periparturient dairy cows. J. Dairy Sci. 92:6123–6133. doi:10.3168/jds.2009-2449.

Rodriguez-Suarez, L.A. 1998. Periparturient responses of cows fed varying dietary cation-anion differences and calcium contents prepartum. Michigan State University,.

Santos, J.E.P., I.J. Lean, H. Golder, and E. Block. 2019. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. J. Dairy Sci. 1–21. doi:10.3168/JDS.2018-14628.

Seifi, H.A., and S. Kia. 2018. Subclinical Hypocalcemia in Dairy Cows: Pathophysiology, Consequences and Monitoring 9:1–15. doi:10.22067/veterinary.v9i2.69198.

Takagi, H., and E. Block. 1991. Effects of Manipulating Dietary Cation-Anion Balance on Macromineral Balance in Sheep. J. Dairy Sci. 74:4202–4214. doi:10.3168/jds.S0022-0302(91)78616-5.

Vagnoni, D.B., and G.R. Oetzel. 1998. Effects of Dietary Cation-Anion Difference on the Acid-Base Status of Dry Cows. J. Dairy Sci. 81:1643–1652. doi:10.3168/jds.S0022-0302(98)75732-7.

Valadares, R.F.D, G.A. Broderick, S.C.V.F. and M.K.C. 1999. Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of

Total Purine Derivatives 1. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.S0022-0302(99)75525-6.

Wu, W.X., Y.M. Wu, and J.X. Liu. 2007. Blood profiles and health in Holstein cows fed diets with varying cation-anion difference and calcium. J. Anim. Feed Sci. 16:376–381.

TABLES AND FIGURES

Table 2.1: Ingredient and nutritional composition of the basal diet.

	Value		
	Period 1	Period 2	Period 3
Ingredient, % of DM			
Corn silage	20.4	20.3	20.3
Wheat straw	33.0	32.8	32.8
Wet corn gluten feed[1]	23.4	23.3	23.3
Micronutrient premix[2]	16.0	15.9	15.9
Anionic feed supplement[3]	7.2	7.8	7.8
Nutrient, % of DM (unless otherwise specified)			
DM	56.4	54.1	54.3
CP	13.4	14.4	14.1
ADF	27.7	23.9	25.3
aNDF	46.8	44.3	44.4
Starch	13.8	15.1	13.0
Ca	0.56	0.60	0.65
P	0.52	0.55	0.56
Mg	0.44	0.45	0.49
K	1.25	1.33	1.17
Na	0.20	0.22	0.23
S	0.25	0.27	0.27
Cl	0.99	1.08	1.09
DCAD, mEq/100g	-2.77	-3.63	-7.58

[1]Sweet Bran (Cargill Inc., Blair, NE)

[2]Premix consisted of 55.9% ground corn grain, 30.7% expeller soybean meal (SoyPlus, Landus Cooperative, Ames, IA), 5.05% XP Yeast (Diamond V, Cedar Rapids, IA), 2.53% ReaShure (Balchem Corp., New Hampton, NY), 2.02% vitamin E premix (20 kIU/lb), 1.21% stock salt, 0.97% NiaShure (Balchem Corp., New Hampton, NY), 0.51% magnesium oxide, 0.32% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.20% vitamin A premix (30 kIU/g), 0.18% selenium premix

(0.06%), 0.14% Zinpro 120 (Zinpro Corp., Eden Prairie, MN), 0.09% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.09% Biotin (ADM Alliance Nutrition, Quincy IL), 0.06% vitamin D premix (30 kIU/g), 0.02% ethylenediamine dihydriodide premix (3.65% I).
[3]SoyChlor (Landus Cooperative, Ames, IA)

Table 2.2: Linear regression using different DCAD equations for predicting indicators of acid-base balance and calcium homeostasis across cows.

	R^2				*P*-values			
	DCAD1[1]	**DCAD2[2]**	**DCAD3[3]**	**DCAD4[4]**	**DCAD1[1]**	**DCAD2[2]**	**DCAD3[3]**	**DCAD4[4]**
Urine								
pH	0.28	0.05	0.14	0.15	< 0.001	0.06	0.002	0.002
Ca excretion	-0.10	0.0	-0.01	-0.02	0.01	0.86	0.34	0.30
Blood								
pH	0.09	0.01	0.03	0.04	0.01	0.48	0.20	0.11
pCO_2	0.01	0.00	0.00	0.00	0.43	0.87	0.73	0.64
TCO_2	0.11	0.01	0.03	0.05	0.008	0.40	0.15	0.07
sO_2	0.01	0.00	0.00	0.00	0.55	0.78	0.69	0.62
Bicarbonate	0.11	0.02	0.04	0.06	0.01	0.33	0.12	0.06
Base excess	0.16	0.02	0.05	0.08	0.001	0.27	0.07	0.02
Ionized calcium	0.01	0.00	0.00	0.00	0.44	0.86	0.88	0.95
Sodium	0.08	0.01	0.03	0.05	0.02	0.40	0.19	0.09
Potassium	0.01	0.00	0.00	0.01	0.45	0.67	0.58	0.48

[1]DCAD = (Na + K) – (Cl + S)
[2]DCAD = (Na + K + 0.38 Ca + 0.3 Mg) – (Cl + 0.6 S + 0.5 P)
[3]DCAD = (Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.2 S + 0.3 P)
[4]DCAD = (Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.6 S + 0.5 P)

Figure 2.1: Dry matter intake of cows fed a negative DCAD diet supplemented with varying levels of calcium carbonate.

Dry matter intake of pregnant nonlactating cows supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate was measured. Dry matter intake was not different for cows fed the NC, MOD or HIGH treatment ($P = 0.21$). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different ($P < 0.05$).

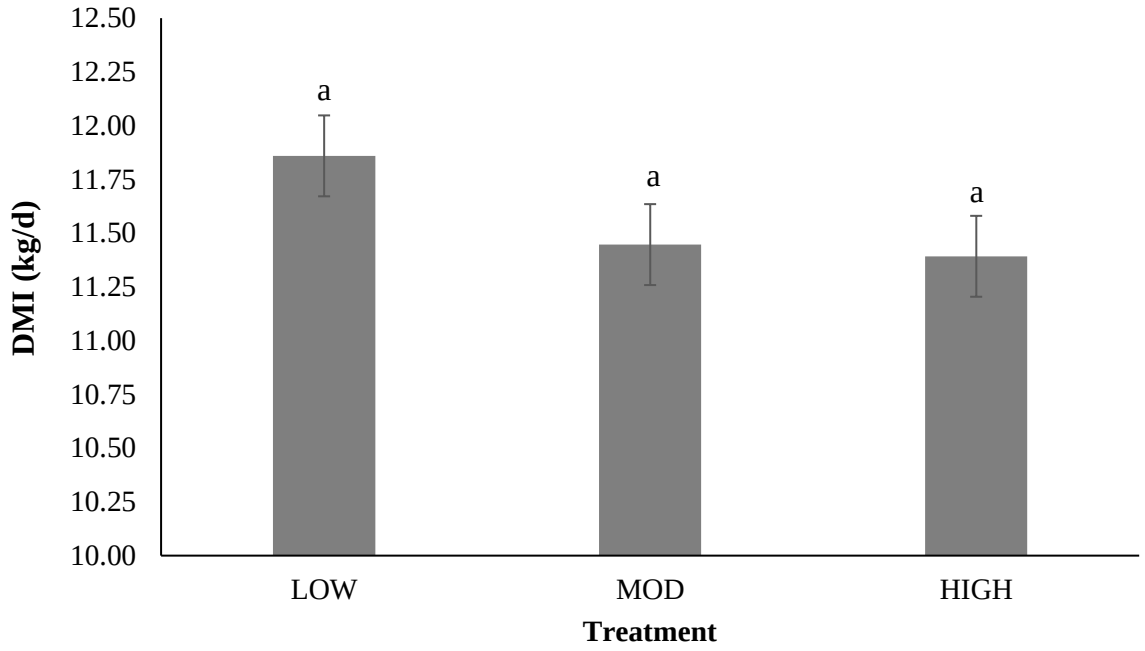


Figure 2.2: Water intake for cows supplemented with low, moderate and high levels of calcium carbonate.

Water intake of pregnant nonlactating cows supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate was measured. There was no effect of treatment on water intake ($P = 0.28$). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different ($P < 0.05$).

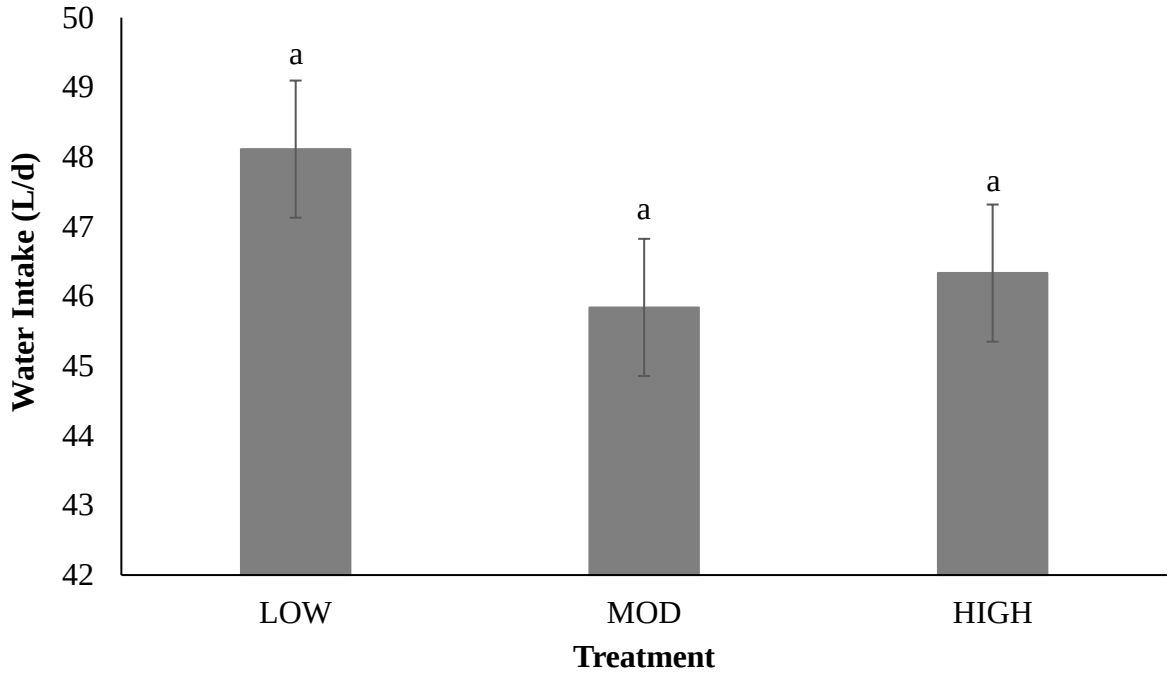


Figure 2.3: Urinary pH in response to supplemental calcium carbonate.

Urinary pH of pregnant nonlactating cows supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate was measured. Urinary pH was greater for cows that received the HIGH diet than the NC diet ($P = 0.03$) and increased linearly from NC to HIGH ($P = 0.009$). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different ($P < 0.05$).

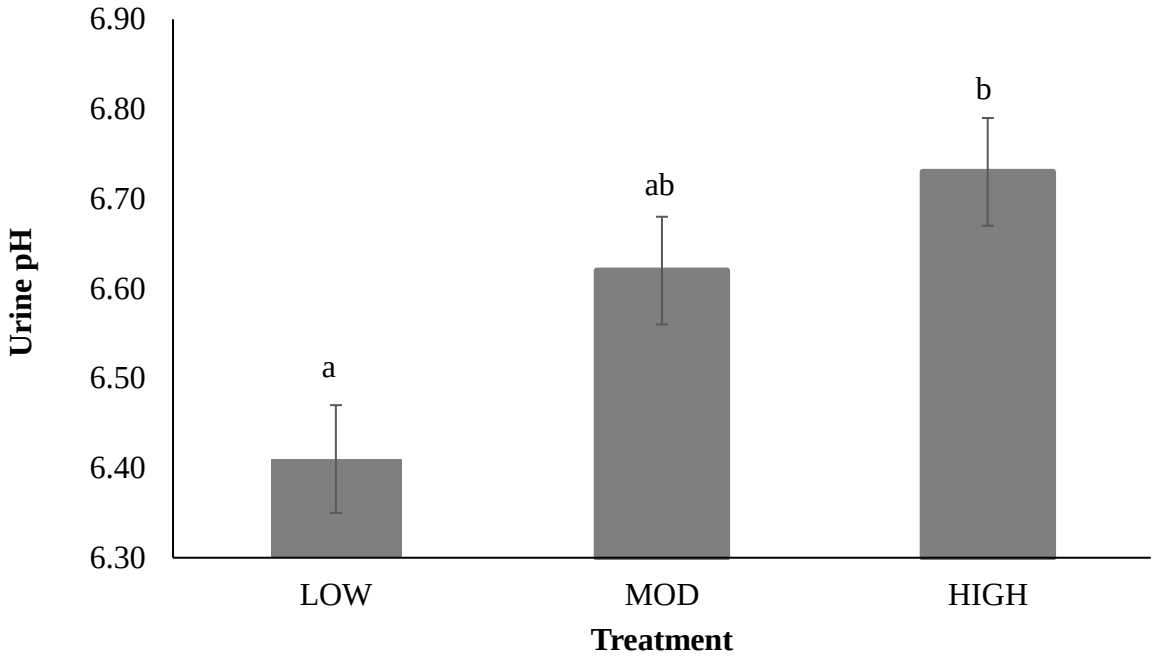


Figure 2.4: Urinary calcium excretion for cows supplemented with low, moderate, and high levels of calcium carbonate.

Urinary calcium of pregnant nonlactating cows supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate was measured. Urinary calcium excretion did not differ between treatments ($P = 0.67$). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different ($P < 0.05$).

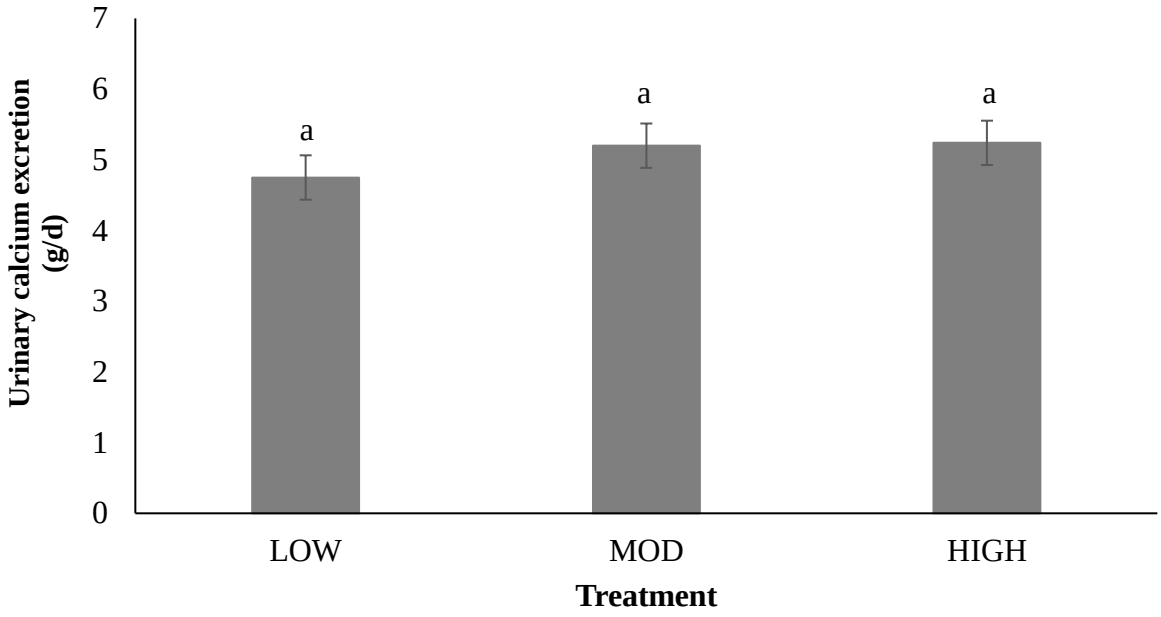


Figure 2.5: Urinary deoxypyridinoline (DPD) concentration for cows supplemented with low, moderate and high levels of calcium carbonate.

Urinary DPD concentration of pregnant nonlactating cows supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate was measured. Urinary DPD concentration did not differ between treatments ($P = 0.46$). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different ($P < 0.05$).

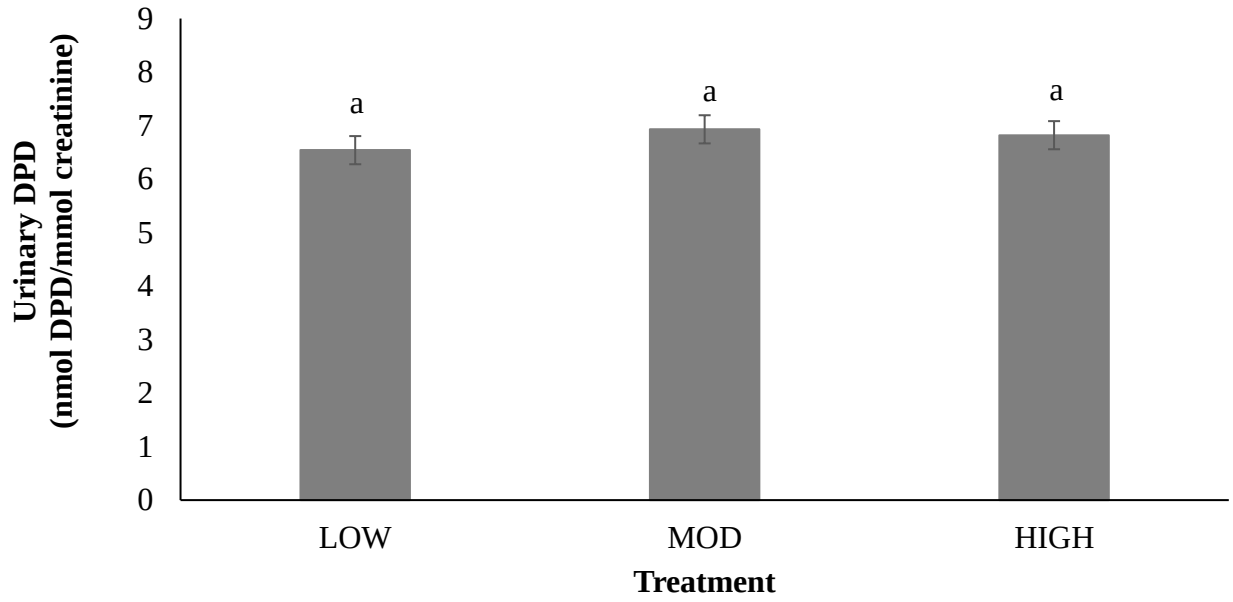


Figure 2.6: Total urinary deoxypyridinoline (DPD) excretion for cows supplemented with low, moderate and high levels of calcium carbonate.

Urinary DPD excretion of pregnant nonlactating cows supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate was measured. Total urinary DPD excretion did not differ between treatments ($P = 0.43$). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different ($P < 0.05$).

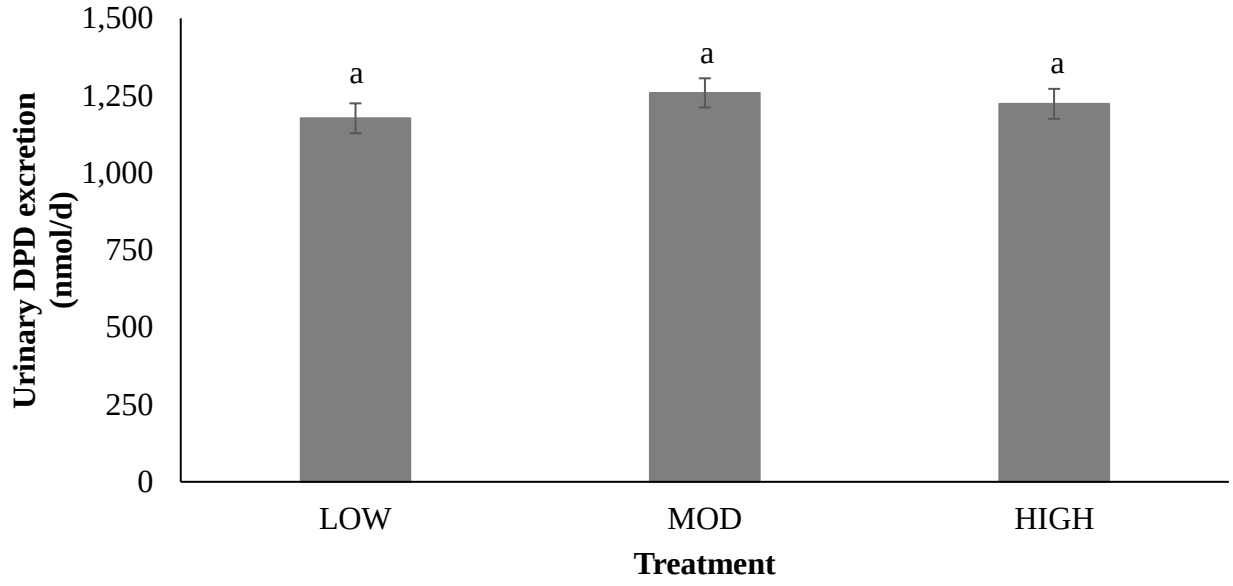
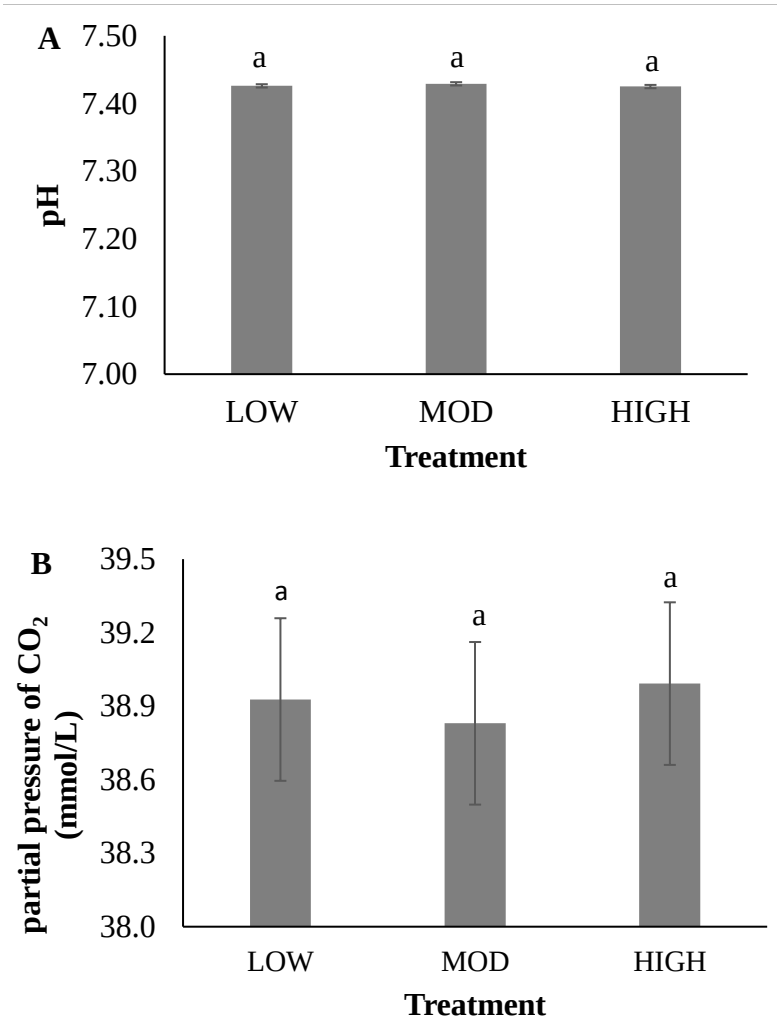
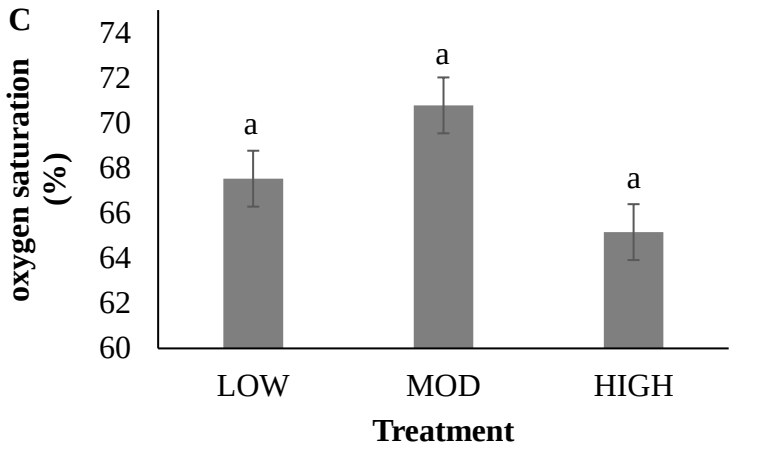
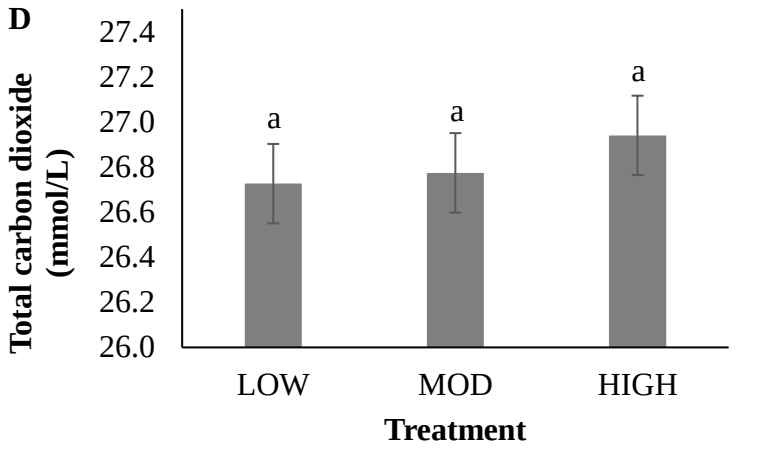


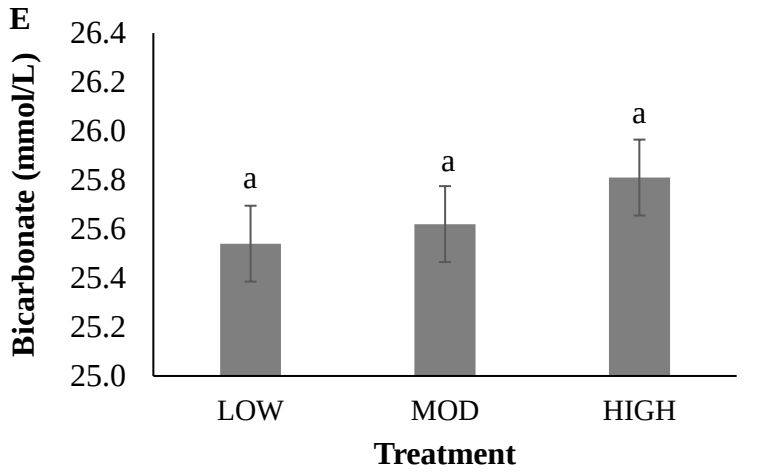
Figure 2.7: The response of pH (A), partial pressure of carbon dioxide (B), oxygen saturation (C), total carbon dioxide (D) bicarbonate (E), base excess (F), sodium (G), and potassium (H) blood concentrations in cows supplemented with low, moderate, and high levels of calcium carbonate.

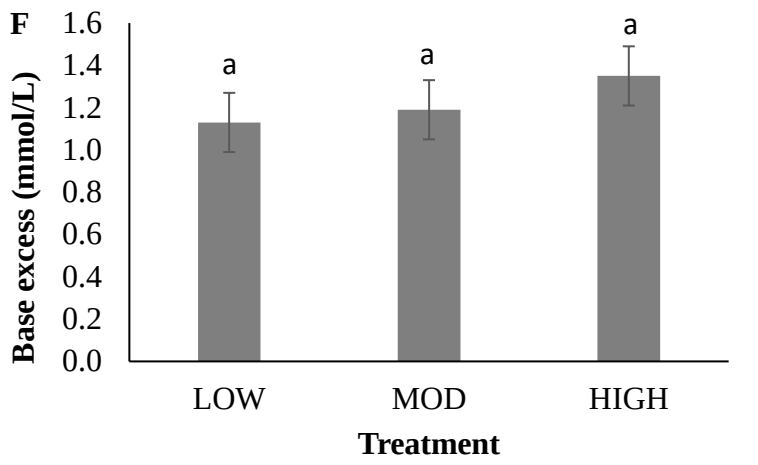
Pregnant nonlactating cows supplemented were supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate. Blood indictors of metabolic acid-base status were measured. Treatment had no effect when whole blood samples were analyzed for A) pH (P = 0.79), B) partial pressure of carbon dioxide (P = 0.98), C) oxygen saturation (P = 0.56), D) total carbon dioxide (P = 0.64), E) bicarbonate (P = 0.64), F) base excess (P = 0.65), G) sodium (P = 0.84), and H) potassium (P = 0.68). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different (P < 0.05).

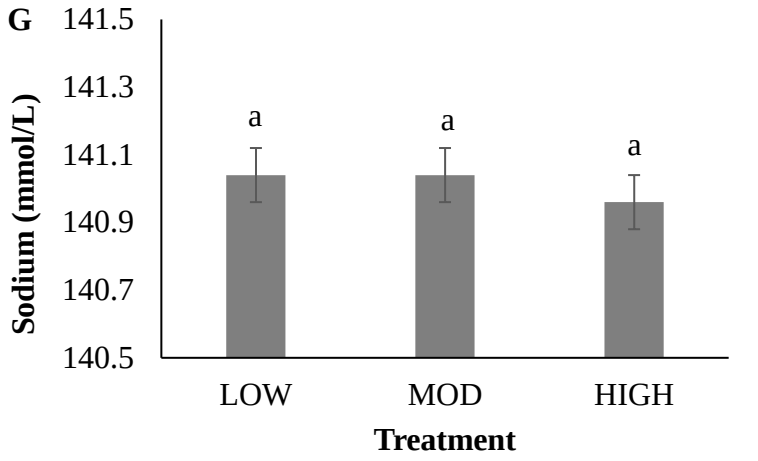



C
oxygen saturation (%)
74
72
70
68
66
64
62
60
a
a
a
LOW
MOD
HIGH
Treatment


D
Total carbon dioxide (mmol/L)
27.4
27.2
27.0
26.8
26.6
26.4
26.2
26.0
a
a
a
LOW
MOD
HIGH
Treatment


E
Bicarbonate (mmol/L)
26.4
26.2
26.0
25.8
25.6
25.4
25.2
25.0
a
a
a
LOW
MOD
HIGH
Treatment


F
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Base excess (mmol/L)
a
a
a
LOW
MOD
HIGH
Treatment


G
141.5
141.3
141.1
140.9
140.7
140.5
Sodium (mmol/L)
a
a
a
LOW
MOD
HIGH
Treatment

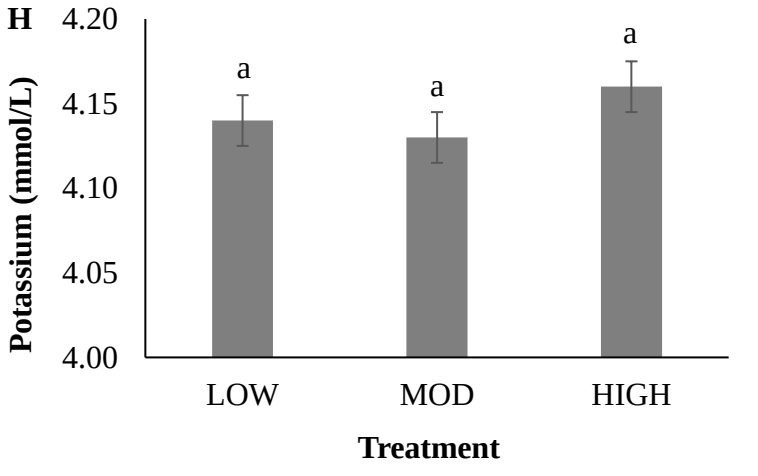
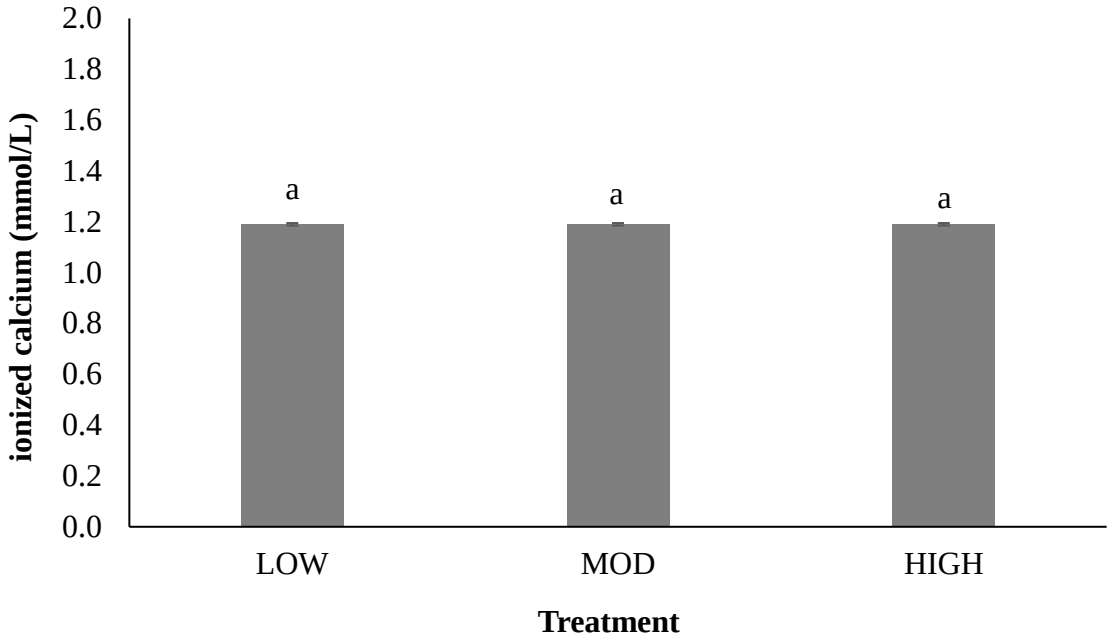

H
4.20
4.15
4.10
4.05
4.00
Potassium (mmol/L)
a
a
a
LOW
MOD
HIGH
Treatment

Figure 2.8: Concentration of blood ionized calcium of cows supplemented with low, moderate, and high levels of calcium carbonate.

Pregnant nonlactating cows supplemented were supplemented with LOW (no calcium supplementation), MOD (1.20% supplemental calcium carbonate) and HIGH (1.80% supplemental calcium carbonate) levels of calcium carbonate. Blood ionized calcium was measured but did not differ by treatment ($P = 0.57$). Values are least square means; error bars represent standard errors. Treatment means that do not share a common letter are significantly different ($P < 0.05$).



Chapter 3 - Early lactation feeding behavior as a predictor lactation success

ABSTRACT

Feed intake is frequently associated with lactation performance. However, it is difficult to assess individual dry matter intake (**DMI**) in group-housed cows. Therefore, technological advancements measuring feeding behaviors in early lactation could be valuable in predicting DMI, lactation outcomes, and herd survival. To investigate relationships among these variables, feeding behavior collected during the first 21-d of lactation from five studies at Kansas State University were compiled for analysis. Cows (n = 260) were housed the same tie-stall facility where feeding behaviors and DMI were recorded using individual feed bunks continuously measuring feed weight. Independent variables included meal frequency (13.9 $\pm$ 2.4 meals/d; mean ± SD), meal length (18.6 $\pm$ 7.0 min/meal), meal size (1.5 $\pm$ 0.5 kg/meal) and feeding time (244.5 $\pm$ 79.0 min/d). Additional data collected were parity, calving date, PTA for milk (186$\pm$ 223; **PTAM**), and 305-d mature equivalent milk (**305MEM**). Feeding behaviors were evaluated as either means or slopes (change/d) over the 21-day period. Potential linear and quadratic predictor variables were then included in a mixed stepwise elimination model, along with fixed effects of parity and PTAM and the random effect of treatment nested within study, using a *P*-value threshold of 0.05 as the criterion for model selection. Explanatory variables found to be predictive of DMI and 305MEM were further evaluated by week using the same procedure described for the 21-d means. Finally, a proportional hazards model was used to assess early lactation feeding behavior for prediction of herd retention. Retention in the herd was assessed with censoring at 365 d or the next calving, with 192 cows enrolled for analysis. Milk PTA ($P < 0.0001$), the slope of meal frequency ($P < 0.01$), and mean meal length ($P = 0.02$) were predictive of 305MEM ($R^2 = 0.49$), and both positively associated with 305MEM. Weekly variables predictive of 305MEM were the positive association of the slope of week 2 meal length

($P = 0.05$) and PTAM ($P < 0.001$). For DMI, the quadratic function of feeding time ($P = 0.0006$) and the intercept of meal frequency ($P = 0.003$), parity ($P < 0.001$) and PTAM ($P < 0.01$) were retained in the final model ($R^2 = 0.40$), with the quadratic function of week 2 feeding time ($P < 0.001$) and the intercept of meal frequency for week 2 ($P = 0.01$) significant when evaluating weekly feeding behaviors ($R^2 = 0.37$). The variables retained in the proportional hazards model were the fixed effects of study ($P < 0.001$) and PTAM ($P = 0.001$), as well as mean meal frequency ($P = 0.06$; risk ration of 1.13). The risk of being removed from the herd was increased by 13% for each addition al meal in the first 21-d of lactation.

INTRODUCTION

Optimizing nutrient uptake of dairy cattle, largely driven by dry matter intake (DMI), is important for maximizing lactation performance. However, it is difficult to assess individual dry matter intake (**DMI**) in group-housed cows, as commonly seen on commercial farms today. Specifically, physical and chemical characteristics of the diet, including fiber content, ease of starch hydrolysis, and particle size (Allen, 2000), as well as competition at the feed bunk and feed barrier systems (Huzzey et al, 2006), can influence intake patterns making equations predictive of individual intake complex and extremely variable across farms with group housed cows. Additionally, the stage of lactation, such as the transition period, can drive changes in intake where a decrease in DMI is seen shortly before and after calving (Schirmann et al, 2013), as well as parity where lower DMI is consumed by primiparous than multiparous cows (Proudfoot et al., 2009).

Precision dairy management technology allows for continuous tracking of behavior, biometrics, and productivity by automated methods that require minimal human interaction. Behaviors such as rumination, feeding, activity, such as lying and standing time, and resting are easily tracked using various forms of this technology. The usefulness of precision dairy management technology has been validated (Grinter et al., 2019) and studied for practical application on farms (Eckelkamp and Bewley, 2020). Therefore, when trying to predict intake and performance success it may be more practical to monitor feeding behavior patterns than intake, as intake can be described as a function of these feeding behavior terms. This includes the number of meals consumed per day, the length of those meals, and the rate of eating during meals (Dado and Allen, 1994), as well as changes in meal size and daily feeding time as lactation progresses (Nielsen, 1999). Feeding behavior patterns change when cows experience

physiological changes, such as during the transition period, which is associated with decreased meal frequency and time spent feeding pre-calving (Huzzey et al., 2005).

The evaluation of feeding behavior of dairy cattle continues to be a topic of interest for prediction of production and disease incidence with increased precision dairy management technology. While it is becoming easier to find literature correlating feeding behavior with disease (Fogsgaard et al., 2015; Neave et al., 2017; Hoeij et al., 2019) and milk production (Johnston and DeVries, 2018), less information has been reported on the association of early lactation feeding behavior, defined in this case as the first 3 weeks postpartum, with whole lactation milk production. The question also remains as to whether feeding behavior patterns can be predictive of herd retention and surviving to the next lactation. It is also worth revisiting how to best predict DMI with measurable feeding behavior patterns. Therefore, the objectives of this study were to identify associations between measures of feeding behavior and whole lactation milk production, and to understand if feeding behavior patterns in early lactation can be helpful in predicting DMI and survival in the herd.

MATERIALS AND METHODS

Data collection

Feeding behaviors were recorded during five studies previously performed at the Kansas State University Teaching and Research Facility (Table 3.1). In all studies, cows were housed in individual tie-stalls following calving and fed a TMR composed of corn silage, wet corn gluten feed, alfalfa hay, cottonseed, ground corn, and a micronutrient premix. All cows were milked 3 times per day, except for the study by Olagaray et al. (2019) where cows were milked 2 times per day. A summary of the TMR nutrient composition is provided in Table 3.1.

Individual cow feeding activity was collected using individual feed bunks suspended from load cells, with feed weight measured continuously by computer. Feed weights and times were stored before and immediately after any deviation in feed bunk weight. Feeding behavior variables were calculated from the logged data that included start and end weights, as well as start and end times of meals. Therefore, DMI and feeding patterns, including meal frequency, meal duration, and meal size, were recorded electronically. To generate meaningful meal pattern data, feeding bouts were grouped into meals as described by Yuan et. al (2015), where feeding bouts less than 12 minutes apart were considered part of the same meal and were therefore combined. Additionally, stand-alone meals less than 0.2 kg of DM were not considered biologically meaningful and were removed. Final variables of interest produced from the dataset included the dependent variable DMI (kg/d), and the explanatory variables meal frequency (no./d), meal duration (min/meal), and meal size (kg/meal) for each day of the individual study. Feeding time (min/day) for the first 3 weeks of lactation was also calculated as the product of meal frequency and meal duration. For this analysis, individual cow feeding behavior for the first 3 weeks of lactation (d 2 – d 21) was extracted from each study. Feeding behavior for d 1 of lactation was excluded from the dataset, as many times there was not a full day of information due to variation in calving times. The average, slope, and intercepts over the 21-d collection period were calculated for each explanatory variable producing 3 values for each cow in the final model from each feeding behavior variable. In addition to the average, slope, and intercept over the 21-d period, feeding behavior variables for week 1, 2 and 3 of lactation were also calculated for each cow for analysis in a separate model to check for weekly significance that may have been diluted when averaging the feeding behavior over the full 21 d of collection.

In addition to obtaining feeding behavior from prior studies, data for the associated lactation was extracted from PCDART (Dairy Records Management Systems, Raleigh, NC). The whole-lactation production was evaluated using 305-day mature equivalent milk (**305MEM**), generated by DHIA monthly testing. In addition, predicted transmitting ability for milk (**PTAM**), parity, calving date at the time of the study, calving date following completion of the study, and cull date (if applicable) were gathered for each individual cow for use in this study. For exploration of herd retention, time to removal from the herd (either culling or death) was determined, with data right-censored at 365 d or at the next calving date, whichever came first. The objective of censoring data at the end of the lactation was to avoid the increase in risk of culling associated with the next calving event, which is less related to transition success in the previous lactation.

Statistical analysis

A total of 260 cows with the first 21 d of recorded feeding behavior were included from the 5 independent studies as summarized in Table 3.1. Due to failures in recording, deviations from feeding management protocol, and early removal of cows from studies, feeding behavior was not collected at all times. Furthermore, the loss of even an hour of behavior within a day would have made daily behavior summaries biased, so the entire day was excluded when short-term disruptions occurred. Therefore, before performing any statistical analysis, the data were screened so that any cow with less than 67% of the 21 d of feeding behavior data (< 14 d) was eliminated from the dataset. This resulted in a total of 199 cows included in the final dataset for analysis (Table 3.1).

The 21 d of individual cow feeding behavior data were averaged to produce one data point per cow for the dependent variable DMI (kg/d) and each explanatory variable: meal frequency (no./d), meal duration (min/meal), meal size (kg/meal), and feeding time (min/d). To further explore feeding behavior patterns predictive of the outcome variables, SAS (Version 9.3, SAS Institute Inc., Cary, NC) was used to calculate the linear slope and intercept for meal frequency (no./d), meal duration (min/meal), meal size (kg/meal), and feeding time (min/d) over the 21-day period. Descriptive statistics for outcome and explanatory variables are summarized in Table 3.2. Following calculation of the linear slopes and intercepts, JMP Pro (version 14.0, SAS Institute Inc., Cary, NC) was used to perform all remaining statistical analyses. Initially, all explanatory variables were screened for normality and outliers. Bivariate regression analysis for the average, slope, and intercept of meal frequency, meal duration, meal size, and feeding time over the 21-d collection period were used as a preliminary screen for predictors of 305MEM (kg) and DMI using $P \leq 0.20$ for inclusion in the stepwise regression model.

For 305MEM, potential feeding behavior variables predictive of a more productive lactation, their quadratic functions, parity, and PTAM were included in a mixed stepwise regression and ran using *P*-value thresholds as the criteria for model selection. All predictor variables were entered into the model when $P \leq 0.20$ and were retained if $P \leq 0.05$. The random effect of treatment nested within study was included in the final model before interpretation of the results.

For DMI, a mixed stepwise regression was utilized and modeled identically to 305MEM as previously outlined. However, the objective of this study was to use monitorable feeding behavior variables that could potentially be produced from existing technology to predict DMI. Therefore, the average, slope, and intercept of meal size were excluded as potential predictor

variables of DMI, as meal size is not known to be estimable with any technologies currently available in the industry.

The results for both 305MEM and DMI were examined for outliers a second time by plotting Studentized residuals. A data point with a Studentized residual exceeding an absolute value of 4 was removed from the dataset. Explanatory variables found to be predictive of the outcome variables were further analyzed by week, producing feeding behavior (which included the average, slopes and intercepts) for weeks 1, 2 and 3 of lactation using the same procedure previously described. The objective of this analysis was to assess whether a particular week postpartum was most associated with the dependent variable of interest. The weekly data for each significant 21-d feeding behavior variable for 305MEM and DMI were placed in separate models and followed the same mixed stepwise regression procedure previously described.

Finally, a proportional hazards model was used to examine the associations between feeding behaviors and survival in the herd using 21-d explanatory variables. The time variable for this model was calving to herd removal, with cows being censored at 365 d or at the next calving. Fixed effects in the proportional hazards model included explanatory variables found in Table 3.2 and their quadratic functions. Parity, calving year, calving month, study, and treatment within each study were also included in the model to help explain additional variation. Only variables with $P \leq 0.10$ were retained in the final proportional hazards model used for interpretation of herd retention. The overall influence of each variable on survival was assessed using unit risk ratios.

RESULTS

305-d mature equivalent milk

A total of 182 observations were used in the model for analysis of 305MEM. The potential predictor variables analyzed as part of the stepwise regression model included meal frequency ($P < 0.01$), meal duration ($P < 0.001$), meal size ($P = 0.001$), and feeding time ($P < 0.001$), as well as the slope of meal size ($P < 0.001$) and the intercepts of meal frequency ($P < 0.01$), meal duration ($P < 0.001$), meal size ($P < 0.001$), and feeding time ($P < 0.001$). The variables retained in the model by the mixed stepwise regression and used as part of the prediction equation for 305MEM are summarized in Table 3.3. The explanatory variables predictive of 305MEM included PTAM ($P < 0.001$), the slope of meal frequency ($P < 0.01$) and mean meal length ($P = 0.02$). These three explanatory variables and the random effect of treatment nested within study were retained in the 305MEM model and explained 49% of the variation in 305MEM ($P < 0.001$) with a root mean square error (RMSE) of 1464 kg (Figure 3.1).

When controlling for genetic effects using PTAM, 305MEM was positively associated with the slope of meal frequency and mean meal length. Specifically, for every 0.10 increase in the slope of meal frequency over the first 21 d of lactation, 305MEM was predicted to increase by 296 $\pm$ 105 kg, which equates to a 343 kg expected difference between cows at the 75th percentile for meal frequency (slope of meal frequency = -0.039 meals/d) versus those at the 25th percentile (slope of meal frequency = -0.155 meals/d) for the slope of meal frequency when all other variables are held constant. Additionally, an increase in mean meal length by 1 min over the first 21-d of lactation was associated with a 55 $\pm$ 23 kg increase in 305MEM, where cows at the 75th percentile (mean meal length = 23.6 min/meal) would be predicted to have a 594 kg

advantage in 305MEM over cows at the 25th percentile (mean meal length = 12.8 min/meal) in this dataset.

The mixed stepwise regression to evaluate weekly feeding behavior predictors for 305MEM included PTAM, the slope of week 1, 2 and 3 meal frequency, average meal length of week 1, 2 and 3, and the associated quadratic functions. The variables retained in the final model for prediction of 305MEM were PTAM ($P < 0.001$) and week 2 meal length ($P = 0.05$), both being positively associated with 305MEM (Figure 3.2). Increasing meal length by 1-min during week 2 was associated with a 62 $\pm$ 24 kg increase in 305MEM. Therefore, a 645 kg advantage in 305MEM would be expected for cows at the 75th percentile (week 2 meal length = 23.5 min/meal) compared to those at the 25th percentile (week 2 meal length = 13.1 min/meal) in this dataset.

Dry matter intake

A total of 185 observations were used for analysis. The explanatory variables of meal duration ($P < 0.01$) and feeding time ($P < 0.001$), as well as the slope of meal frequency ($P = 0.16$) and the intercept of meal frequency ($P = 0.17$), meal duration ($P = 0.01$), and feeding time ($P < 0.001$) were included in the mixed stepwise regression for DMI. The explanatory variables retained in the final model and used for prediction of DMI are summarized in Table 3.3. The quadratic functions of feeding time ($P < 0.001$; Figure 3.5) and the intercept of meal frequency ($P < 0.01$; Figure 3.6) were retained in the final model, along with parity ($P < 0.001$) and PTAM ($P < 0.01$). These explanatory variables were able to explain 40% of the variation in DMI ($P < 0.001$) with a RMSE of 2.7755 (Figure 3.3).

When controlling for parity and genetic effects using PTAM, DMI was predicted to increase for each additional minute of feeding time per day when feeding time was $\leq$ 300 min/d Therefore, DMI decreased with an additional minute of feeding time when feeding time exceeded 300 min/d. This equates to a 1.8 kg/d difference in DMI between cows at the 75th percentile (feeding time = 307.1 min/d) and those at the 25th percentile (feeding time = 184.2 min/d) for average feeding time over the 21-d collection period in this dataset. The quadratic function of the intercept of meal frequency was expected to decrease DMI with each additional meal on the first day of lactation until reaching a threshold of 14.9 meals/d, which then had a positive effect on DMI with each additional meal. This resulted in a 0.22 kg/d increase in DMI for cows at the 75th percentile (intercept of meal frequency = 16.1 meals/d) versus those cows at the 25th percentile (intercept of meal frequency = 13.5 meals/d) in this study.

The mixed stepwise regression to evaluate weekly feeding behavior predictors included parity, PTAM, average feeding time for week 1, 2, and 3, and the intercept of meal frequency for week 1, 2, and 3, along with their quadratic functions. Variables retained in the final prediction equation for DMI were parity ($P < 0.001$), the quadratic function of week 2 feeding time ($P < 0.001$), the intercept for meal frequency for week 2 ($P = 0.01$), and PTAM ($P = 0.01$; Figure 3.4).

When controlling for parity and genetic effects using PTAM, every increase in week 2 feeding time by 1 minute was predicted to increase DMI when week 2 feeding time is $\leq$ 250 min/d, where DMI is then negatively by week 2 feeding time. This equates to a 2 kg/d difference in average DMI for cows in the upper 75th percentile (week 2 feeding time = 166.7 min/d) versus those cows in the bottom 25th percentile (week 2 feeding time = 321.2 min/d). Additionally, the intercept of meal frequency for week 2 was positively associated with DMI. Therefore, for every increase in the intercept of meal frequency by an increment of 1 meal, DMI is predicted to

increase by 0.23 kg/d. This would predict an increased DMI for cows in the upper 75th percentile (intercept of meal frequency for week 2 = 13.9 meals/d) by 0.12 kg/d, compared to cows in the bottom 25th percentile (intercept of meal frequency for week 2 = 13.4 meals/d) in this dataset.

Herd survival

A total of 189 observations were included for analysis of survival in the herd using feeding behavior patterns and descriptive variables. A complete summary of the data censoring and events by study is listed in Table 3.4. The variables retained in the proportional hazards model were the fixed effects of study ($P < 0.001$) and PTAM ($P < 0.01$), as well as mean meal frequency ($P = 0.06$). The risk ratios corresponding to the explanatory variables are summarized in Table 3.5. A risk ratio of 1.13 was reported for mean meal frequency. Therefore, in the first 21 d of lactation the risk of being removed from the herd increased by 13% for cows who ate an additional meal in early lactation. This equates to a 36% greater chance of being removed from the herd for cows in the upper 75th percentile (mean meal frequency = 15.7 meals/d) versus those cows in the 25th percentile (mean meal frequency = 12.9 meals/d) of mean meal frequency in this data set.

DISCUSSION

In this study, feeding behavior patterns as described by the slope of meal frequency and mean meal length were significant factors in predicting 305MEM. The positive associations of the slope of meal frequency with 305MEM was not unexpected. Feeding behavior patterns have the potential to affect rumen pH (Allen, 1997) and milk production as a result (Antanaitis et al., 2019). Greater meal frequency has been associated with a reduction in ruminal pH variation

(Lunn et al., 2005). A decrease in meal frequency, when accompanied by increased meal size, resulted in a greater production, without synchronized elimination, of fermentation end products between meals and a larger fluctuation in ruminal pH (Pitt and Pell, 1997); thus greater meal frequency creates a more stable rumen environment by reducing the potential for sub-acute ruminal acidosis. In this study, the slope of meal frequency over the first 21 d of lactation was positively associated with 305MEM. In a previous study, meal frequency tended to be positively associated with milk production in early lactation (Johnston and DeVries, 2018), where an extra meal per day was associated with an additional 0.3 kg/d of milk yield. In a metanalysis which reviewed the effects of feeding frequency on milk production, the author reported significant increases in milk yield, fat concentration, and fat yield in response to increased feeding frequency (Gibson, 1984), although the average increases in milk and fat concentration were fairly small at 7.3% and 2.7%, respectively. Dado and Allen (1994) examined the relationship between feeding behavior and milk production in noncompeting cows (in tie-stalls) and found that the association between meal frequency and milk yield was parity dependent, where production tended to be positively related to meal frequency for primiparous cows, but was unrelated for multiparous cows.

While greater meal frequency has the potential to boost lactation performance, the association of the slope of meal frequency and 305MEM has not been reported to our knowledge and deserve more interpretation. It is well known that an increase in DMI is associated with greater milk production and less incidence of disease. Additionally, DMI has been found to be a function of meal frequency and meal size (Nielsen, 1999). Severe inflammation around parturition was associated with a slower increase of rumination time after calving (Calamari et al., 2014). Cows with short ruminating time in the first 10 d of lactation experienced a greater

frequency of disease, and cows with subclinical diseases or health disorders have decreased rumination time during the first few days of lactation (Soriani et al., 2012). While it has been shown that clinically ill cattle spend more time lying per day with longer duration of lying bouts than healthy cows (Sepúlveda-Varas et al., 2016), in the first week of lactation it may be advantageous for cows to spend more time lying and ruminating, up to a certain degree. This would more than likely result in fewer but larger meals with less time spent going to and from the feed bunk. Indeed, cows in the first 28 d after calving had a greater number of lying bouts, and lying bouts were positively associated with milk production (Steensels et al., 2012). Therefore, the positive relationship between the slope of meal frequency, not simply mean meal frequency, and 305MEM may be more indicative of the positive effect of lying for longer periods of time between meals immediately after calving before ramping up DMI through increased meal frequency.

Meal length was positively associated with 305MEM in this study. Few studies have reported performance outcomes associated with meal length. It is thought achieving higher DMI through increased meal frequency, not meal length, produces a healthier rumen environment, because eating greater meals per day better distributes the quantity of substrate to the rumen at one time, therefore, decreasing the time the rumen is in an acidotic state (Allen, 1997). Instead, increased meal length is often associated with disease. Milk-fed calves diagnosed with illness had increased meal length in the days preceding and on the day that the illness was detected (Borderas et al., 2009). However, increased buffering capacity from greater saliva production could be one benefit to greater meal length. Cows with greater meal length, usually associated with high roughage diets, secreted more saliva per meal (Beauchemin et al., 2008). In addition, transition from gestation to lactation was associated with a dramatic decrease in DMI, between

20% - 40% (Bertics et al., 1992; Hayirli et al., 2002), with DMI slowly increasing after calving. This drop in DMI may result in negative energy balance for transition cows not meeting nutrient requirements, and therefore challenging the ability of the cow to adapt to physiological changes (Gummer, 1995). When considering meal length in combination with the slope of meal frequency, it may be possible that cows who increase DMI through increased meal frequency, while keeping meal length (and thus meal size) consistent, have greater milk production throughout lactation. And those cows that are able to consume feed for longer, and thus have increased meal size, are more successful throughout lactation due to the postive assocation of meal length and meal size ($P < 0.001$; Beauchemin et al., 2002).

The quadratic function of feeding time and the intercept of meal frequency were significant predictors for average DMI over the first 21 d of lactation. Feeding time (Johnston and DeVries, 2018; Huzzey et al, 2007; Azizi et al., 2009) and meal frequency (tendency; Johnston and DeVries, 2018) have previously been associated with DMI. While Johnston and DeVries (2018) found a positive association between meal frequency and DMI as in our study, Azizi et al. (2009) reported a significant negative association between meal frequency and DMI.

The association of the quadratic function of feeding time and DMI found in this study was not completely unexpected in that cows are only able to consume a certain amount of feed each day. Feeding time per day would increase DMI only if feeding rate is unchanged. If instead cows spend more time feeding but eat more slowly than there may be no impact on DMI. When looking at the graph for the quadratic function of feeding time, it appears that as cows increase feeding time from around 100 to 225 min/d DMI becomes substantially greater. However, after reaching 225 min/d of feeding time DMI seems to plateau with minimal DMI gained from greater feeding time. While cows who spend $\geq$ 225 min/d feeding benefit from greater DMI,

feeding for > 400 min/d can potentially hindering DMI. Therefore, there is little difference in DMI for cows who spend between 225 and 400 min/d feeding.

The intercept of meal frequency had a quadratic relationship with DMI in this study. Meal frequency was negatively associated with DMI when meal frequency was less than 14.9 meals/d, but positively associated with DMI when cows consumed greater than 14.9 meals/d at the onset of lactation. Azizi et al. (2009) reported an average meal size of 2.3 and 3.3 kg/meal for primiparous and multiparous cows, respectively. Johnson and DeVries (2018), Beauchemin et al. (2002), and Dado and Allen (1994) reported average meal sizes of 3.2, 2.4 and 1.8 kg/meal, respectively. While there is variation in meal size, the rumen of a particular cow may not have the capacity to accommodate meals larger than previously mentioned. In this dataset, a negative relationship existed between meal size and meal frequency on both d 1 of lactation (intercept) and over the 21-d collection period (mean). As meal frequency decreased there was an increase in meal size, but mean meal size only increased up to a maximum of 3.4 kg/d. Therefore, it is unlikely that cows consuming meals less frequently at the onset of lactation would be able to make up for this difference in intake with greater meal size. Furthermore, animals have a maximal rate at which they can utilize nutrients. Mechanisms exist that help balance an animal's supply and demand for these nutrients, such as satiety and hunger (Allen, 2000). Consuming larger meals would increase production of fermentation end productions without the capacity to eliminate them fast enough resulting in acidosis from a reduction in rumen pH with the possibility of compromising milk production (Antanaitis et al., 2019).

To our knowledge, few studies have been conducted to assess feeding behavior of large numbers of cows during the transition period as in this study. More commonly, studies reported feeding behavior measures in early to peak lactation. Meal frequency was found to be negatively

associated with DMI when cows were fed a TMR (Beauchemin et al., 2002; Azizi et al., 2009). Meal frequency averaged 10.3 and 8.0 meals/d, respectively, in these studies. However, Johnston and DeVries (2018) and Dado and Allen (1994) reported a mean meal frequency of 9 and 11.3 meals/d, respectively, and showed that meal frequency was positively associated with DMI.

When comparing the data reported by the four studies mentioned previously it is important to mention two differences which could help explain variations in results. First, meal data reported in this study, and those by Beauchemin et al. (2002) and Dado and Allen (1994), used minimum meal criteria of 0.20, 0.30 and 0.05 kg DM of feed consumed and 12, 20, and 7.5 min between meals, respectively. In contrast, Johnston and DeVries used meal criteria which were individually calculated for each cow as described by DeVries et al. (2003). Secondly, the results reported in this study, and by Beauchemin et al. (2002), Azizi et al. (2009), and Dado and Allen (1994) were conducted with cows housed in individual tie-stalls, where Johnston and DeVries (2018) used free-stalls. It is known that competition can change feeding behavior. Therefore, inconsistencies of the association between meal frequency and DMI may simply be explained by differences in analysis of the raw feeding behavior data (defining meal criteria), environmental factors, and diet conformation associated with each study.

Parity was retained in the model for DMI. A 3.2 kg/d advantage in DMI was found for multiparous compared to primiparous cows in this study based on the prediction equation in Figure 3.3. This was not unexpected as primiparous cows throughout the transition period have been found to have reduced DMI (Proudfoot et al., 2009), with more time spent feeding, slower feeding rate, and greater meal frequency (Neave et al, 2017) than multiparous cows. Lower DMI with greater meal frequency by primiparous cows has been explain by competition, with primiparous cows being replaced at the feed bunk more frequently than multiparous cows. In our

study mean meal frequency was 14.7 ± 3.0 and 13.7 ± 2.3 meals/d for primiparous and multiparous cows, respectively, but did not result in a significant difference ($P = 0.83$; 42 primiparous and 97 multiparous included in the analysis). This may be due to an abnormally low number of primiparous cows compared to multiparous cows in this study resulting in a potential power analysis problem to detect a significant difference. So, even though all cows were housed in tie-stalls with no competition a numeric difference was observed between parities in our study. Beauchemin et al. (2002) and Dado and Allen (1994) reported lower intakes for primiparous versus that of multiparous cows, where DMI was found to be highly correlated to body weight (Dado and Allen, 1994). Additionally, Neave et al. (2017) reported that primiparous cows had greater meal frequency than multiparous cows, but this disappeared after controlling for body weight and production. Therefore, variation in body weight and production between primiparous and multiparous cows is a better explanation for numerical difference in meal frequency by parity in this study.

While data on time spent lying, ruminating, and standing was not recorded in any of our studies, and therefore not included as an explanatory variable in this study, it may be advantageous to include these variables in future models for prediction of production outcomes. It has been reported that cows in the top 10% for milk production in the herd versus the average of the herd spend the same time eating but more time resting and less time standing (Grant, 2006). Additionally, Johnston and DeVries (2018) found an association between rumination time and milk yield in early lactation. In free stall housed cows, using rumination time may be a more measurable trait than some variables of feeding behavior used in this study when predicting DMI. Rumination time estimates have shown to be significant in DMI prediction models (Clement et al., 2014) as it has been stated that feeding without ruminating may not provide

benefit to a cow (Llonch et al., 2018). However, rumination time for prediction of DMI may only be useful postpartum as no relationship was found between rumination time and DMI during the dry period (Schirmann et al., 2012).

Herd survival in this study had a tendency to be negatively associated with mean meal frequency, when accounting for study and PTAM. This was surprising as many studies associate greater meal frequency with improved health and production. In a study by Hoeij et al. (2019), cows with good metabolic status 4 weeks postpartum had greater meal frequency than those cows with poor metabolic status. Additionally, it has been reported that cows diagnosed with one or more transition diseases after calving had fewer frequent meals than their healthy counterparts 1-3 d before onset of the disease (Neave et al., 2018). Impaired health, reduced production and reproductive performance led to higher likelihood of being culled (Duffield, 2006). For example, cows diagnosed with clinical ketosis (Grohn et al., 1989) and metritis (Wittrock et al., 2011) have an increased risk of culling in early lactation. One explanation for the 13% increased risk of removal from the herd with greater meal frequency is the reduction in rumination and lying time. High producing cows who have adapted to larger, less frequent meals would have more time to spend ruminating. However, this requires more investigation as to our knowledge no studies have been done that associate rumination with herd survival.

CONCLUSION

Feeding behavior was useful in predicting whole lactation milk production and DMI. Mean meal length and the slope of meal frequency were positively associated with 305MEM. The quadratic effects of feeding time and the intercept of meal frequency were retained in the model for DMI. Dry matter intake increased when feeding time was between 225 and 400 min/d

and the intercept of meal frequency was ≥ 14.9 meals/d. As expected, parity was significant in predicting DMI with multiparous cows consuming more feed than primiparous cows. Week 2 feeding behavior was most predictive of DMI, as the quadratic function of week 2 feeding time and the intercept of meal frequency were retained in the model when feeding behavior variables were broken down by week 1, 2 and 3. Mean meal frequency was the only feeding behavior variable retained in the model for analysis of herd survival and increased the risk of removal from the herd by 13% with each additional meal.

ACKNOWLEDGEMENTS

First and foremost, this study would not have been possible without the work and dedication of the graduate students, undergraduate students, and collaborators of the publications from which data was extracted for this study. The authors would also like to thank Mikaela Wood and Gail Carpenter for initial consolidation of the dataset.

REFERENCES

Amundson, L.A., A.D. Rowson, P.M. Crump, A.P. Prichard, A.A. Cheng, C.E. Wimmler, M. Klister, S.R. Weaver, S.S. Bascom, D.E. Nuzback, K.P. Zanzalari, and L.L. Hernandez. 2018. Effect of induced hypocalcemia in nonlactating, nonpregnant Holstein cows fed negative DCAD with low, medium, or high concentrations of calcium. J. Anim. Sci. 96:5010–5023. doi:10.1093/jas/sky371.

Beede, D. K., T. E. Pilbeam, S. M. Puffenbarger, and R.J.T. 2001. Peripartum responses of Holstein cows and heifers fed graded concentrations of calcium (calcium carbonage) and anion (chloride) 3 weeks before calving. J. Dairy Sci. 88(Suppl.:(Abstr.).

Chan, P.S., J.W. West, and J.K. Bernard. 2006. Effect of Prepartum Dietary Calcium on Intake and Serum and Urinary Mineral Concentrations of Cows. J. Dairy Sci. 89:704–713. doi:10.3168/jds.S0022-0302(06)72133-6.

Charbonneau, E., D. Pellerin, and G.R. Oetzel. 2006a. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows : A Meta-Analysis. J. Dairy Sci. 89:537–548. doi:10.3168/jds.S0022-0302(06)72116-6.

Charbonneau, E., D. Pellerin, and G.R. Oetzel. 2006b. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. J. Dairy Sci. 89:537–548. doi:10.3168/jds.S0022-0302(06)72116-6.

Constable, P.D., A.A. Megahed, M.W.H.H. 2019. Measurement of urine pH and net acid excretion and their association with urine calcium excretion in periparturient dairy cows. J. Dairy Sci. 102:11370–11383. doi:10.3168/jds.2019-16805.

Corlett, S.C., and A.D. Care. 1988. The effects of reduced dietary phosphate on plasma osteocalcin levels in sheep. J. Exp. Physiol. 73:443–445.

Diehl, A.L., J.K. Bernard, S. Tao, T.N. Smith, D.J. Kirk, D.J. McLean, and J.D. Chapman. 2018a. Effect of varying prepartum dietary cation-anion difference and calcium concentration on postpartum mineral and metabolite status and milk production of multiparous cows. J. Dairy Sci. 1–11. doi:10.3168/jds.2018-14828.

Diehl, A.L., J.K. Bernard, S. Tao, T.N. Smith, D.J. Kirk, D.J. McLean, and J.D. Chapman. 2018b. Effect of varying prepartum dietary cation-anion difference and calcium concentration on postpartum mineral and metabolite status and milk production of multiparous cows. J. Dairy Sci. 101:9915–9925. doi:10.3168/jds.2018-14828.

Emery, R.S., L.D. Brown, and J.W. Thomas. 1964. Effect of Sodium and Calcium Carbonates on Milk Production and Composition of Milk, Blood, and Rumen Contents of Cows Fed Grain Ad Libitum with Restricted Roughage. J. Dairy Sci. 47:1325–1329. doi:10.3168/jds.S0022-0302(64)88913-X.

Escobosa, A., C.E. Coppock, L.D. Rowe, Jr., W.L. Jenkins, C.E.G. 1984. Effects of Dietary

Sodium Bicarbonate and Calcium Chloride on Physiological Responses of Lactating Dairy Cows in Hot Weather. J. Dairy Sci. 67:574–584. doi:10.3168/jds.S0022-0302(84)81341-7.

G.R. Oetzel, J.D. Olson, C.R. Curtis, M.J.F. 1988. Ammonium Chloride and Ammonium Sulfate for Prevention of Parturient Paresis in Dairy Cows. J. Dairy Sci. 71:3302–3309. doi:10.3168/jds.S0022-0302(88)79935-X.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. J. Dairy Sci. 101:2763–2813. doi:10.3168/jds.2017-13112.

Goff, J.P., and R.L. Horst. 1993. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. J. Dairy Sci. 76:101–108. doi:10.3168/jds.S0022-0302(93)77328-2.

Goff, J.P., and R.L. Horst. 1997. Effects of the Addition of Potassium or Sodium, but Not Calcium, to Prepartum Rations on Milk Fever in Dairy Cows. J. Dairy Sci. 80:176–186. doi:10.3168/jds.S0022-0302(97)75925-3.

Goff, J.P., R. Ruiz, and R.L. Horst. 2004. Relative Acidifying Activity of Anionic Salts Commonly Used to Prevent Milk Fever. J. Dairy Sci. 87:1245–1255. doi:10.3168/jds.S0022-0302(04)73275-0.

Grünberg, W., S.S. Donkin, and P.D. Constable. 2011. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base , calcium , and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. J. Dairy Sci. 94:727–745. doi:10.3168/jds.2010-3230.

Horst, R.L. and J.P.G. 1997. Milk fever and dietary potassium. Pages 181–189 in Proc. Cornell Nutr. Conf. Feed Manuf., Rochester, NY,Cornell Univ., Ithaca, NY.

Hu, W., L. Kung, and M.R. Murphy. 2007. Relationships between dry matter intake and acid – base status of lactating dairy cows as manipulated by dietary cation – anion difference. Anim. Feed Sci. Technol. 136:216–225. doi:10.1016/j.anifeedsci.2006.09.013.

Hu, W., and M.R. Murphy. 2004. Dietary Cation-Anion Difference Effects on Performance and Acid-Base Status of Lactating Dairy Cows: A Meta-Analysis. J. Dairy Sci. 87:2222–2229. doi:10.3168/jds.S0022-0302(04)70042-9.

Kamiya, Y., M. Kamiya, M. Tanaka, and S. Shioya. 2005. Effects of calcium intake and parity on plasma minerals and bone turnover around parturition. Anim. Sci. J. 76:325–330.

Kronqvist, C., U. Emanuelson, R. Spörndly, and K. Holtenius. 2011. Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. J. Dairy Sci. 94:1365–1373. doi:10.3168/jds.2009-3025.

Martinez, N., L.D.P. Sinedino, R.S. Bisinotto, R. Daetz, C. Lopera, C.A. Risco, K.N. Galvão, W.W. Thatcher, and J.E.P. Santos. 2016a. Effects of oral calcium supplementation on

mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. J. Dairy Sci. 99:8397–8416. doi:10.3168/jds.2015-10527.

Martinez, N., L.D.P. Sinedino, R.S. Bisinotto, R. Daetz, C.A. Risco, and K.N. Galvão. 2016b. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. J. Dairy Sci. 99:8417–8430. doi:10.3168/jds.2015-10529.

McKinnon, J.J, D.A. Christensen, and B.L. 1990. The influence of bicarbonate buffers on milk production and acid-base balance in lactating dairy cows. Can. J. Anim. Sci. 70:875–886.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th re. ed. Washington, D.C.

Oba, M., A.E. Oakley, and G.F. Tremblay. 2011. Dietary Ca concentration to minimize the risk of hypocalcaemia in dairy cows is affected by the dietary cation-anion difference. Anim. Feed Sci. Technol. 164:147–153. doi:10.1016/j.anifeedsci.2011.01.002.

Oetzel, G.R., and J.A. Barmore. 2015. Intake of a Concentrate Mixture Containing Various Anionic Salts Fed to Pregnant, Nonlactating Dairy Cows. J. Dairy Sci. 76:1617–1623. doi:10.3168/jds.S0022-0302(93)77495-0.

Pitkin, R. 1983. Endocrine regulation of calcium homeostasis during pregnancy.. Clin. Perinatol. 10:575–592.

Rérat, M., A. Philipp, H.D. Hess, and A. Liesegang. 2009. Effect of different potassium levels in hay on acid – base status and mineral balance in periparturient dairy cows. J. Dairy Sci. 92:6123–6133. doi:10.3168/jds.2009-2449.

Rodriguez-Suarez, L.A. 1998. Periparturient responses of cows fed varying dietary cation-anion differences and calcium contents prepartum. Michigan State University,.

Santos, J.E.P., I.J. Lean, H. Golder, and E. Block. 2019. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. J. Dairy Sci. 1–21. doi:10.3168/JDS.2018-14628.

Seifi, H.A., and S. Kia. 2018. Subclinical Hypocalcemia in Dairy Cows: Pathophysiology, Consequences and Monitoring 9:1–15. doi:10.22067/veterinary.v9i2.69198.

Takagi, H., and E. Block. 1991. Effects of Manipulating Dietary Cation-Anion Balance on Macromineral Balance in Sheep. J. Dairy Sci. 74:4202–4214. doi:10.3168/jds.S0022-0302(91)78616-5.

Vagnoni, D.B., and G.R. Oetzel. 1998. Effects of Dietary Cation-Anion Difference on the Acid-Base Status of Dry Cows. J. Dairy Sci. 81:1643–1652. doi:10.3168/jds.S0022-0302(98)75732-7.

Valadares, R.F.D, G.A. Broderick, S.C.V.F. and M.K.C. 1999. Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of

Total Purine Derivatives 1. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.S0022-0302(99)75525-6.

Wu, W.X., Y.M. Wu, and J.X. Liu. 2007. Blood profiles and health in Holstein cows fed diets with varying cation-anion difference and calcium. J. Anim. Feed Sci. 16:376–381.

TABLES AND FIGURE

Table 3.1: Cows and TMR nutrient composition from the 5 studies from which the dataset was extracted.

	Initial dataset[3]		Screened dataset[4]		TMR nutrient composition			
Study[1]	Cow no.	Parity[2]	Cow no.	Parity[2]	DM (%)	CP (%)	NDF (%)	NE_L (Mcal/kg)
1	29	0 PP; 29 MP	22	0 PP; 22 MP	54.2	17.3	36.0	1.68
2	78	39 PP; 39 MP	63	31 PP; 32 MP	53.2	18.6	30.5	1.72
3	39	3 PP; 36 MP	39	3 PP; 36 MP	51.1	17.7	31.0	1.60
4	55	0 PP; 55 MP	30	0 PP; 30 MP	51.2	17.8	33.2	1.67
5	59	14 PP; 45 MP	45	11 PP; 34 MP	59.7	17.0	31.3	1.66
Total	260	56 PP; 204 MP	199	45 PP; 154 MP				

[1]Studies are as follows: (1) Mullins et al., 2012; (2) Farney et al., 2013; (3) Yuan et al., 2015; (4) Carpenter et al., 2018; (5) Olagaray et al., 2019.
[2]PP = primiparous; MP = multiparous.
[3]Initial dataset = all cows from 5 studies where 21-day feeding behavior was collected
[4]Screened dataset = cows having greater than 65% feeding behavior data

Table 3.2: Descriptive statistics, including mean, standard deviation, minimum, and maximum of explanatory and outcome variables.

	Mean	SD	Min	Max
Outcome variables				
305MEM, kg	13892	2001	9106	19692
Dry matter intake[1], kg/d	19.06	3.51	7.44	26.74
Explanatory variables				
Meal frequency[1], no./d	13.86	2.42	5.74	26.19
Slope of meal frequency[2]	-0.10	0.11	-0.44	0.26
Intercept of meal frequency[3]	14.91	2.38	6.33	23.70
Meal duration[1], min/meal	18.60	6.97	5.38	44.07
Slope of meal duration[2]	0.19	0.23	-0.91	1.68
Intercept of meal duration[3]	17.12	3.88	10.63	39.88
Meal size[1], kg/meal	1.53	0.48	0.61	3.39
Slope of meal size[2]	0.031	0.014	0.003	0.099
Intercept of meal size[3]	1.19	0.30	0.60	2.39
Feeding time[1], min/d	244.54	79.04	71.00	473.06
Slope of feeding time[2]	0.22	2.46	-10.88	7.09
Intercept of feeding time[3]	242.13	84.56	68.29	491.59
PTAM, kg[4]	187	224	-371	852

[1]Average of feeding behavior from 2 - 21 DIM
[2]Slope of feeding behavior from 2 - 21 DIM
[3]Intercept of feeding behavior from 2 - 21 DIM
[4]PTAM = Predicted transmitting ability for milk

Table 3.3: Final multivariate linear regressions for variables predictive of 305-d mature equivalent milk and dry matter intake.

	305MEM (kg)			DMI (kg/d)		
Explanatory variable	β[1]	*P*-value	SE	β[1]	*P*-value	SE
Intercept	12,345	< 0.001	809	13.288	< 0.001	1.535
Slope of meal frequency	2,960	0.005	1049			
Meal length	55	0.02	23			
Feeding time				0.013	0.006	0.004
(Feeding time – 245.5)[2]				-0.00013	< 0.001	0.00003
Intercept of meal frequency				0.092	0.40	0.107
(Intercept of meal frequency – 14.9)[2]				0.060	0.030	0.020
PTAM	2	< 0.001	0.23	0.0013	0.007	0.0005
Parity[2]				-1.585	< 0.001	0.304

[1]β = estimated regression coefficient
[2]Parity = primiparous versus multiparous; primiparous = 1, multiparous = -1

Table 3.4: Summary of cows at risk, the number of events and censored cows by study.

Study[1]	No.[2]	Events[3]	Censored[4]	% Censored[5]
1	21	9	12	57.1
2	61	10	51	82.3
3	38	4	34	89.7
4	30	14	16	53.3
5	39	17	22	56.4
Total	189	54	135	71.2

[1]Studies are as follows: (1) Mullins et al., 2012; (2) Farney et al., 2013; (3) Yuan et al., 2015; (4) Carpenter et al., 2018; (5) Olagaray et al., 2019.
[2]No. = total number of animals enrolled per study.
[3]Events = number of events (culled or death) per study.
[4]Censored = number of cows that were censored per study (reached 365 d postcalving or the next calving).
[5]% Censored = percentage of cows censored per study.

Table 3.5: Proportional hazards analysis outcome table for survival in the herd.

Variable	df	Parameter Estimate	SE	Risk ratio	*P*-value
PTA for milk (kg)	1	-0.223	0.068	0.63	< 0.01
Mean meal frequency (no./d)	1	0.124	0.066	1.13	0.06

Figure 3.1: Actual versus predicted 305-d mature equivalent milk (305MEM) from multivariate linear regression equation for significant feeding behavior pattern variables.

Prediction equation: 305MEM = 12345.5 + 54.6*meal length + 2959.7*slope of meal frequency + 2.10*PTAM.

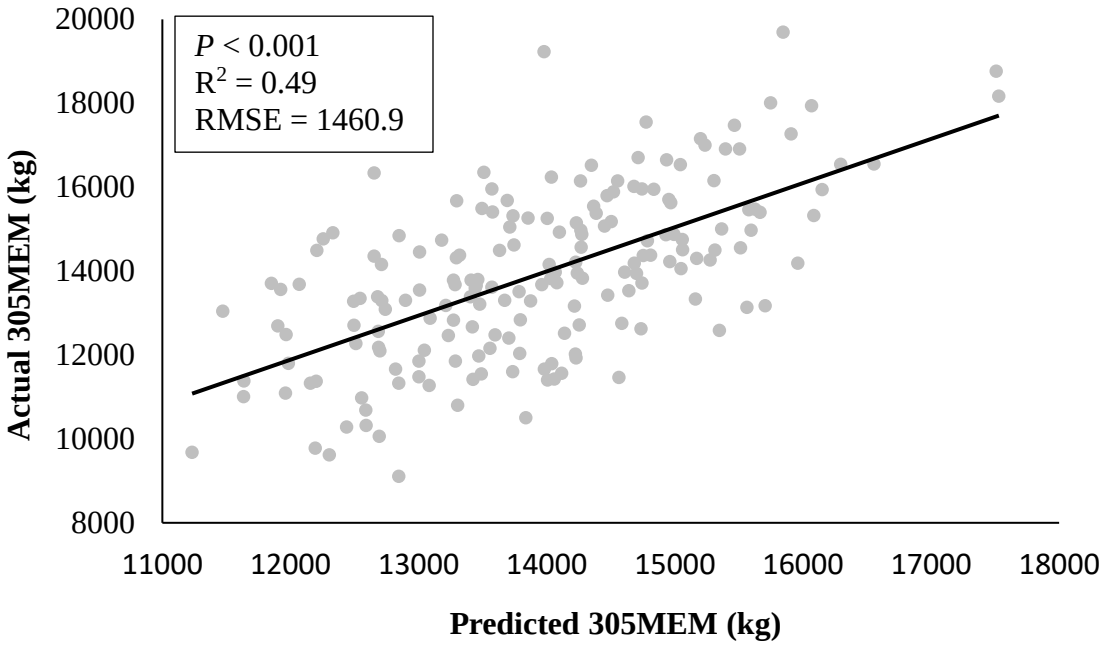


Figure 3.2: Actual versus predicted 305-d mature equivalent milk (305MEM) from multivariate linear regression equation for significant weekly feeding behavior pattern variables.

Prediction equation: 305MEM = 12382.1 + 40.03*week 2 meal length + 2.00*PTAM.

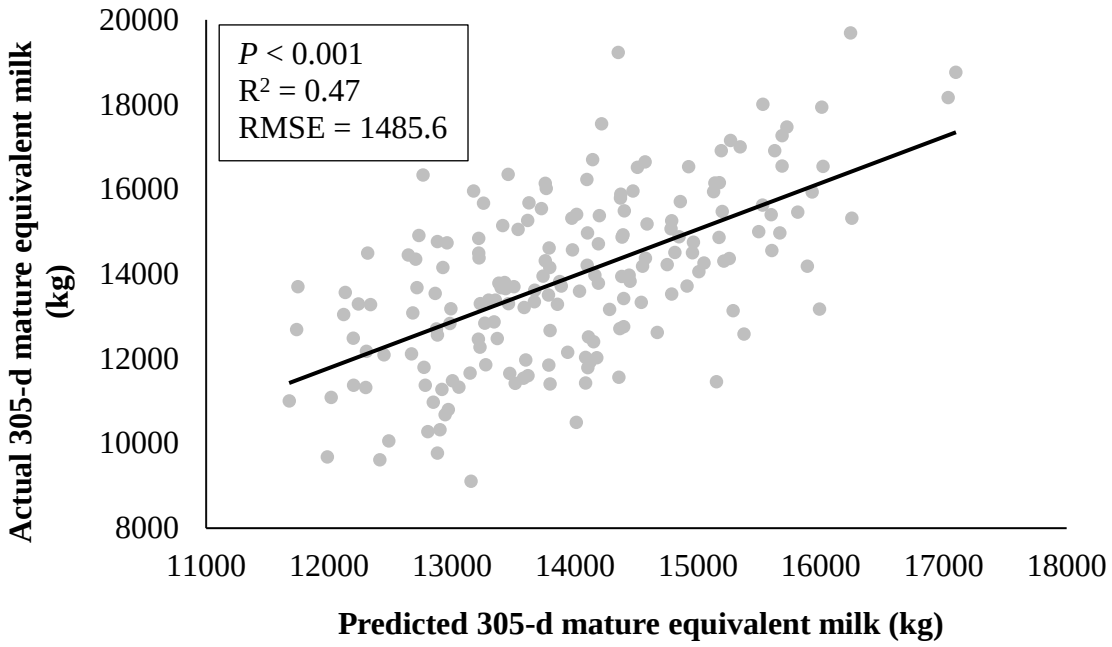


Figure 3.3: Actual versus predicted dry matter intake (DMI) from multivariate linear regression equation for significant feeding behavior pattern variables.

Prediction equation: DMI = 13.27 + 0.015*Feeding time + 0.088*Intercept of meal frequency + 0.0013*PTAM + (feeding time – 244.7)*((feeding time – 244.7)*-0.000133) + (Intercept of meal frequency – 14.88)*((intercept of meal frequency – 14.88)*0.060)) – 1.581*Parity (where parity 1 = 1; 2 = -1; 3 = -1; 4 = -1).

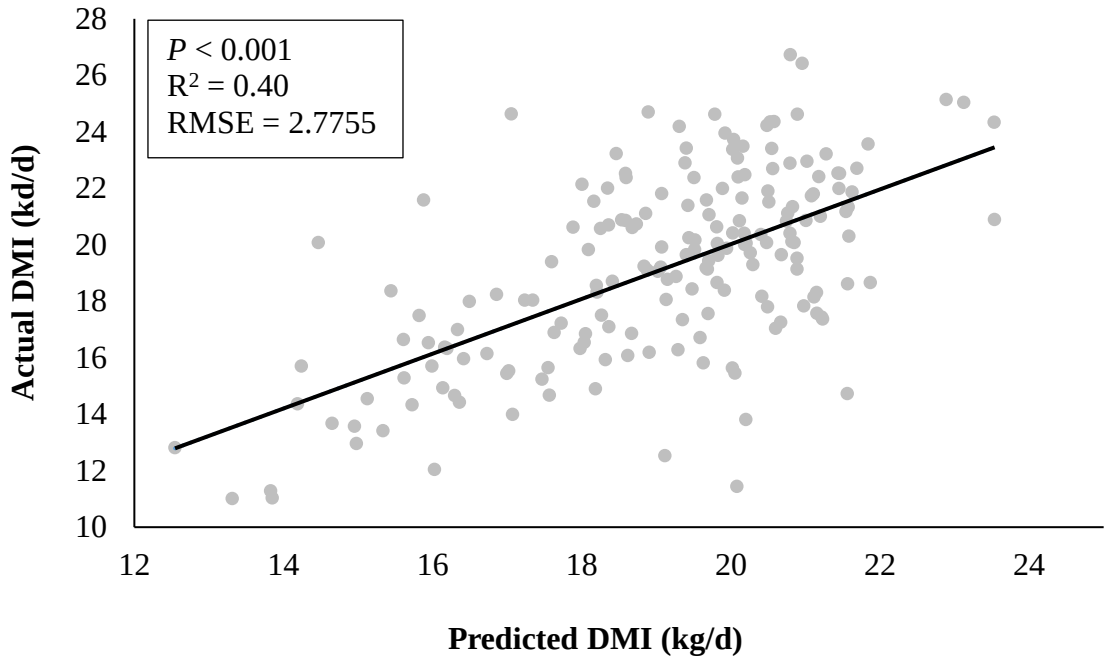


Figure 3.4: Actual versus predicted dry matter intake (DMI) from multivariate linear regression equation for significant weekly feeding behavior pattern variables.

Prediction equation: DMI = 12.19 + 0.0129*week 2 feeding time + (week 2 feeding time – 244.5)*((week 2 feeding time – 244.5)*-0.00010)) – 1.62*parity (where parity 1 = 1; 2 = -1; 3 = -1; 4 = -1) + 0.0011*PTAM + 0.226*intercept of meal frequency for week 2.

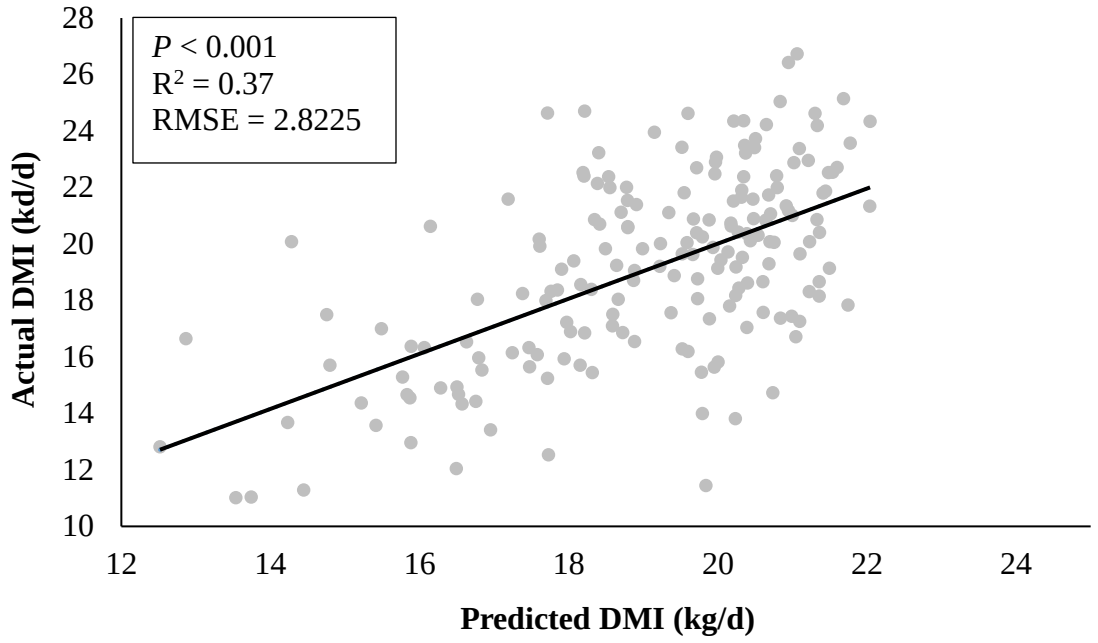


Figure 3.5: The quadratic function of feeding time for prediction of dry matter intake (DMI).

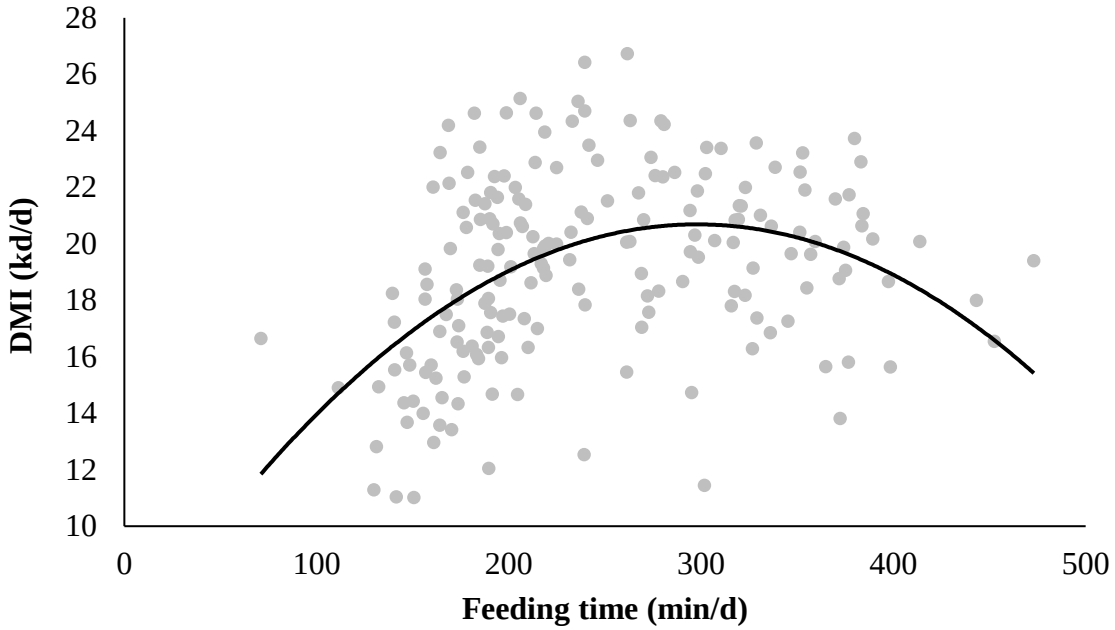


Figure 3.6: The quadratic function of the intercept of meal frequency for prediction of dry matter intake (DMI).