

POTENTIAL FOR NOVEL FEED INGREDIENTS IN DAIRY CATTLE DIETS:
CALCAREOUS MARINE ALGAE AND MALE STERILE SORGHUM

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ABSTRACT

Dairy cattle often face negative energy balance around parturition. Without proper nutrition, periparturient diseases develop and negatively impact farm profitability due to treatment costs and reduced milk production. This study evaluates calcium-supplementing ration concentrates and cost-effective sorghum silage sources to maintain healthy, high-producing cows during the transition period and early lactation.

In an eight-month study, dietary impacts on dry matter intake (**DMI**), blood and urine metabolites, and milk yield and components were recorded for 31 multiparous and 9 primiparous Holstein cows. Data were collected from 21 treatment (**CMA**) and 19 control (**CON**) cows, balanced for parity and projected calving date. Total mixed rations were adjusted to partially replace calcium carbonate, magnesium oxide, and sodium bicarbonate with CMA.

The GLIMMIX procedure of SAS 9.4 ($P \leq 0.05$) was used to analyze treatment effects, maximum ambient temperature humidity index, projected 305-day mature equivalent milk, and their two-way interactions. No significant impacts of treatment on blood or urine metabolite status, DMI, colostrum quality or quantity, body weight, milk, fat, or protein yield were found between treatment groups ($P > 0.05$). Under non-heat stress, CMA cows ate more than CON cows from 31 to 120 DIM (29 vs. 25.3 ± 0.7 kg/d; $P < 0.01$). Treatment cows maintained ECM efficiency in heat stress from 31 to 120 DIM ($P = 0.01$), unlike CON cows that decreased from non-heat stress to heat stress. However, treatment milk efficiency was decreased compared to CON under heat stress (1.6 vs. 1.8 ± 0.1 ; $P < 0.01$) and non-heat stress (1.6 vs. 2.0 ± 0.1 ; $P < 0.01$).

The nutritional content of male sterile brown midrib sorghum (**BMR**) and corn was evaluated using wet chemistry and near-infrared spectroscopy. Corn had higher crude protein concentration than BMR harvested at 8 wk post heading ($P = 0.01$) but was comparable to BMR harvested at 7 wk post heading. No significant differences in dry matter, acid detergent fiber, neutral detergent fiber, or starch concentration were observed among forage type ($P > 0.05$). Suggesting that BMR was a viable alternative forage, though protein supplementation adjustments may be needed.

KEYWORDS: ration concentrates, sorghum, transition period, and early lactation

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CHAPTER ONE

Review of literature

INTRODUCTION

Profitable milk production depends upon efficient, high-producing cows consuming less DM (Eastridge, 2006). Metabolic disorders, often caused by dietary deficiencies from poor ration formulation, hinder DMI and maximum milk production potential (Fikadu et al., 2016). Disease-related milk yield reductions and incurred treatment expenses detrimentally impact farm profitability (Liang et al., 2017). Periparturient diseases that develop from inadequate Ca and mineral levels, such as hypocalcemia (Liang et al., 2017), hyperketonemia (McArt et al., 2015), mastitis (Miller et al., 1993), metritis (Pérez-Báez et al., 2021), and DA (Geishauser et al., 2000) are costly for farmers. Thus, disease prevention through adequate nutrition is crucial to maximizing milk yield (Fikadu et al., 2016). Limiting Ca intake in the last week of gestation and providing bioavailable Ca after parturition is linked to lower hypocalcemia and secondary periparturient disease incidence (Goings et al., 1974). Additionally, protein concentration and milk fat levels are significantly impacted by feeding management techniques (Varga and Ishler, 2005). Nutritional approaches that enhance rumen function also increase the amount of milk and components produced (Varga and Ishler, 2005).

However, purchasing and growing feedstuffs continuously constitutes the highest operating cost for dairy farms (USDA-ERS, 2023). Maximized efficiency from purchased feedstuffs and high-quality forages reduce feed costs (Gislon et al., 2020). Milk-to-feed price ratio (**MF**) is an indicator of dairy farm profitability reported monthly by the USDA National Agricultural Statistics Service (Wolf, 2010). The primary drivers of MF fluctuation are the volatility of corn and soybean prices (Wolf, 2010). When corn prices are high, hay and soybean prices rise because farmers remove hay and soybean acreage to plant corn (Wolf, 2010). Even

farms that grow their own crops face increased opportunity costs when corn grain and alfalfa hay prices rise (Wolf, 2010). Dairy farmers tend to see narrower profit margins when MF indicates higher feed prices relative to the milk price received (Wolf, 2010). Brown midrib sorghum (**BMR**) species are an alternative silage source to corn silage (**CS**) (Oliver et al., 2004, Colombini et al., 2010). Compared to CS, BMR forage yielded greater biomass and demonstrated greater water efficiency and drought tolerance during warm growing season temperatures (Sanderson et al., 1992). Identifying cost-effective ration concentrates and silage sources that provide available, essential nutrients is vital for maintaining healthy, high-yielding animals.

CALCAREOUS MARINE ALGAE

Produced from *Lithothamnion calcareum*, calcareous marine algae (**CMA**; Calmin, Feedworks USA, Ltd., Cincinnati, OH) contains 30% Ca, 0.5% P, 6.0% Mg, 0.1% K, and trace minerals (DM basis). Calcareous marine algae collectively represent several species of calcified red seaweed harvested off Irish and Icelandic coasts. The unique honeycomb structure and large surface area of CMA enables the plant to capture and retain Ca and Mg from seawater (Neville et al., 2022). The high mineral content in the cell wall slowly releases these minerals during digestion (Neville et al., 2022). Cruywagen et al. (2015) depicted CMA as a sparingly water-soluble Ca source and ruminal buffer due to its distinct marine-based physical structure and chemical characteristics. The incorporation of CMA as a supplemental Ca source had demonstrated promising results in maintaining serum metabolite levels pre- and postpartum (Bernard et al., 2014, Neville et al., 2022) and preventing rumen pH depression (Bernard et al., 2014, Cruywagen et al., 2015, Loučka et al., 2024), thereby supporting the overall productivity and health of dairy cows. Additionally, CMA maintained and exceeded milk yield and other

production parameters compared to sodium bicarbonate and limestone. (Cruywagen et al., 2015, Wu et al., 2015, Neville et al., 2022).

Periparturient cow diseases

Dairy cows transitioning from 3 wk before to 3 wk after calving are highly susceptible to periparturient diseases due to increased metabolic and nutrient needs. This crucial physiological phase triggers sudden shifts in energy, protein, and mineral metabolism, impacting health, immune function, and productive performance after parturition (Drackley et al., 2005, Zhang et al., 2022). At the start of lactation, the metabolites suddenly released into the udder reduce blood mineral levels, challenging mineral homeostasis (Goff, 2008, Cardoso et al., 2020). These metabolite deficiencies negatively affect several physiological processes in the cow, such as nervous system and muscle function, and cause severe issues that lead to death if untreated (Neville et al., 2022). The occurrence of transition cow diseases consequently causes a reduction in milk yield. Periparturient disease includes reproductive-related conditions, such as dystocia, uterine prolapse, retained placenta (**RP**), and metritis (Drackley, 1999, Fikadu et al., 2016, Cardoso et al., 2020) and metabolic disorders, such as SARA, hyperketonemia, or displaced abomasum (**DA**) (Goff, 2014). These health events negatively affect fertility and milk production, decreasing the cow's future value (Seifi and Kia, 2018, Cardoso et al., 2020). Incorporating effective buffering agents and extra Ca sources in the TMR improve recovery and resistance to adverse health events (Cardoso et al., 2020).

Hypocalcemia. Hypocalcemia, commonly known as milk fever in the clinical state, is a metabolic disease resulting from low serum Ca concentrations. Hypocalcemia is one of the most common health conditions during the periparturient period (Goff, 2008). Calcium is a vital macromineral involved in smooth muscle contraction, milk synthesis, and immune cell activation

(Goff, 2008). Hypocalcemia typically occurs at the start of lactation when nutrients rapidly move into the udder to meet the mammary glands' high Ca demand for milk production. In the beginning stages of lactogenesis, the Ca requirement often exceeds the animal's body stores and ability to promptly replenish Ca levels for maintenance (Seifi and Kia, 2018). Generally, the adult cow should maintain serum Ca levels between 8.5 and 10 mg/dL (Goff, 2008).

Hypocalcemia results when the concentration fell below that range for an extended period (Goff, 2008). Subclinical hypocalcemia (**SCH**) has no recognizable symptoms, making it more challenging to detect than clinical hypocalcemia. However, SCH can be detected through blood tests for serum Ca levels between 5.5 and 8.0 mg/dL (Fikadu et al., 2016). Clinical symptoms appear when serum Ca drops below 5 mg/dL (Fikadu et al., 2016). The most apparent clinical symptom of milk fever is the loss of ability to stand, as Ca is necessary for nerve and muscle function (Goff, 2008). Other clinical signs include lethargy, cold extremities, and rumen atony (Fikadu et al., 2016). Primiparous heifers rarely experience clinical hypocalcemia. First-calf heifers produce less colostrum and milk, allowing them to mobilize Ca more rapidly from their growing skeleton (Reinhardt et al., 2011). Reinhardt et al. (2011) observed that the prevalence of clinical hypocalcemia from 1,462 Holstein cows was 1% for first-lactation, 4% for second-lactation, 7% for third-lactation, and 10% for fourth-lactation cows. Subclinical hypocalcemia affected about 47% of all fresh cows in their second lactation or beyond (Reinhardt et al., 2011). Clinical symptoms of milk fever appeared in 5 to 7% of fresh cows (Reinhardt et al., 2011).

The inherent physiological response to low serum Ca levels at lactation onset involves the parathyroid hormone (**PTH**) regulating Ca mobilization from bones to restore normocalcemia (Goff, 2008). This process is termed lactational osteoporosis, accompanied by enhanced efficiency in dietary Ca absorption (Goff, 2008). The PTH increases Ca reabsorption in

the nephron, minimizing urinary Ca excretion, even in small quantities (Goff, 2008). To enhance intestinal Ca absorption, the kidney produces 1,25-dihydroxyvitamin D from vitamin D in response to elevated blood PTH levels (Goff, 2008, 2014).

Cows are predisposed to milk fever and SCH if metabolic alkalosis raises serum bicarbonate, inhibiting the physiological response to PTH (Craigie and Stoll, 1947). Metabolic alkalosis facilitates structural changes in the PTH receptor that reduce tissue response to PTH (Goff, 2008). If PTH cannot signal effectively to bone tissue, osteoclastic bone resorption is repressed (Goff, 2008). The kidneys also fail to respond to PTH, decreasing the glomerular filtration rate of Ca and reducing renal absorption (Goff, 2008). Additionally, the kidneys do not activate 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, impairing dietary Ca absorption in the intestine and preventing normalization of blood Ca levels (Goff, 2008). Another consequence of SCH is adipose tissue mobilization, raising blood nonesterified fatty acid concentrations (**NEFA**) and increasing the risk of hepatic steatosis, hyperketonemia, and DA (Goff, 2008, 2014). Hepatic steatosis, or fatty liver disease, affects high-producing dairy cows due to an excessive negative energy balance (**NEB**) at the onset of lactation. Insufficient dietary energy supply mobilizes large amounts of body fat reserves and transferred fatty acids to the liver (Goff, 2014). Data from Chamberlain et al. (2013) suggests that SCH may be linked to changes in lipid metabolism and increased risk for fatty liver development. This was supported by elevated plasma NEFA levels at calving and increased lipid accumulation in the liver at 7 and 35 DIM in the hypocalcemic group (Chamberlain et al., 2013).

Metritis and mastitis are inflammatory diseases prevalent around the periparturient period. Hypocalcemia is a predisposing factor to developing these inflammatory diseases due to the significant impact on early lactation cow health (Martinez et al., 2016). Metritis is

categorized as an inflammatory infection of the uterus. It mainly occurs in high-risk cows who present at least one of the following conditions at or after the time of calving: dystocia, twins, stillbirth, vaginal-vulvar laceration, or RP (Martinez et al., 2016). Venjakob et al. (2021) highlighted RP as a significant risk factor for metritis, as 102 out of 105 cows that incurred RP in the data set consequently developed metritis. The incidence of RP increased in cows experiencing SCH, attributed to weak muscle contractions resulting from depleted Ca levels (Martinez et al., 2016). Thus, the manifestation of SCH increased uterine disease susceptibility for metritis, among other periparturient diseases (Martinez et al., 2012). Martinez et al. (2012) concluded that the development of metritis increased by 22% for every 1 mg/dL decrease in serum Ca below 8.59 mg/dL. In SCH cows, where serum Ca levels were decreased, the risk of uterine disease was 66.6% for metritis and 91.3% for puerperal metritis (Martinez et al., 2012). Mastitis is defined as an inflammatory infection of the udder. Mastitis economically devastated farmers in the dairy industry by decreasing milk yield, reducing milk quality, and raising costs spent on veterinary services and antibiotics (Rahman et al., 2009). Older cows advancing in lactation are more prone to developing udder infections (Rahman et al., 2009). Cows diagnosed with a history of periparturient diseases are shown to be more susceptible to mastitis due to impaired immunity status (Martinez et al., 2012). In cows diagnosed with SCH, the risk of mastitis is greater since Ca ions played a necessary role in muscle contraction, specifically the constriction of the teat orifice after milking to prevent pathogens from entering the teat. When the teat end remains open, environmental pathogens are more likely to enter the mammary gland and cause infection (Goff, 2014). Cows experiencing SCH tend to have more frequent lying time than those with normal Ca levels (Barraclough et al., 2020, Hendriks et al., 2020). This increased lying behavior elevates the risk of teat-end exposure to opportunistic environmental organisms.

Inflammatory diseases, such as metritis or mastitis, significantly inhibit reproductive success and maintenance of pregnancy (Ribeiro et al., 2016). Farmers effectively using nutritional techniques to compensate for the loss of Ca and other metabolites during the transition period may help reduce the prevalence of hypocalcemia and secondary periparturient diseases.

Calcium supplementation by CMA was shown to successfully maintain healthy Ca concentrations around the time of calving, indicating its potential to reduce the incidence of hypocalcemia (Bernard et al., 2014, Wu et al., 2015, Neville et al., 2022). Bernard et al. (2014) observed no differences in serum metabolites among dietary treatments of CMA, sodium bicarbonate, and limestone. Wu et al. (2015) supplemented prepartum diets of multiparous and primiparous Holstein cows with calcium carbonate (CON) or 50 g/d of CMA, and postpartum diets provided either 317 g/d of sodium bicarbonate and calcium carbonate (NBC) or 100 g/d of CMA. Although prepartum serum Na was higher ($P = 0.05$) for CON (143.32 mEq/L) compared with CMA (142.55 mEq/L), no differences were observed pre- or postpartum in the other metabolites and minerals between experimental diets (Wu et al., 2015). Neville et al. (2022) compared a control (CON) limestone diet and a treatment CMA diet on serum metabolite concentration differences. Similar to (Bernard et al., 2014), CON and CMA groups yielded almost identical serum concentrations (mM) for Ca, Mg, Na, K, and Cl in the prepartum and postpartum periods. Although, CMA treatment increased the postpartum serum concentration of P to 2.13 mM compared to 1.93 mM control ($P < 0.01$) (Neville et al., 2022). A study focusing on dairy heifers by Boccardo et al. (2022) examined the effects of feeding CMA on blood total Ca (tCa) compared to calcium carbonate (CC) and calcium propionate (CP) diets. It was observed that CMA achieved a faster tCa peak on d 1 ($P = 0.001$) at 2.41 mmol/l compared to CC (2.38 mmol/l) and tended to be higher ($P = 0.07$) compared to CP (2.39 mmol/l). No

differences between CC and CP ($P = 0.38$) (Boccardo et al., 2022). Calcareous marine algae demonstrated the ability to maintain serum metabolite levels compared to traditional dietary buffers and Ca sources. Thus, authors suggest that CMA may be used as dietary Ca supplementation to replace Ca provided by limestone (Bernard et al., 2014, Neville et al., 2022) and Ca salts (Boccardo et al., 2022).

Hypomagnesemia. Hypomagnesemia is a metabolic condition defined by low blood Mg concentration. Magnesium is an intracellular cation cofactor for essential enzymatic reactions of metabolic pathways. A healthy dairy cow should maintain serum Mg concentrations between 1.8 and 2.4 mg/dL (Goff, 2008). When Mg levels fall below the normal range, PTH cannot effectively communicate with target tissues, disrupting Ca metabolism (Goff, 2008).

Periparturient cows are more susceptible to hypocalcemia when serum Mg concentrations are below 1.5mg/dL (van de Braak et al., 1987). Unlike Ca, Mg cannot be sourced from bone during deficit, as bone reabsorption solely responds to maintain Ca homeostasis (Goff, 2008).

Hypomagnesemia reduces the productivity of lactating cows because Mg is necessary for the efficient function of ATP, or energy metabolism, which is essential in milk production (Goff, 2014). There are few apparent clinical symptoms when blood Mg fell between 1.15 and 1.8 mg/dL, although cows eat slower and produced less milk (Goff, 2008). The clinical signs of hypomagnesemia, such as recumbency, convulsions, and nystagmus, are observed when blood Mg range falls between 0.9 to 1.15 mg/dL (Goff, 2008).

Hypomagnesemia is prevented by increasing available forms of dietary Mg. Adequate plasma Mg levels depend on dietary absorption. Magnesium, typically included as magnesium oxide in the TMR, positively affects rumen pH and milk fat concentration when supplemented (Erdman et al., 1982, Teh et al., 1985). Calcareous marine algae is a bioavailable source of Mg,

so a novel study by Neville et al. (2019) focused on rumen fermentation and milk production effects comparing CMA and marine magnesium oxide (**MM**; precipitated magnesia from seawater). The treatment diets included CMA, sodium bicarbonate, and a combination of CMA and MM (Neville et al., 2019). All treatment groups successfully increased total milk fat, and both the combination of CMA-MM and CMA alone increased total milk protein (Neville et al., 2019). Adding MM to diets with CMA may offer limited benefits to milk production parameters, as CMA alone effectively improves milk fat and protein (Neville et al., 2019).

Hyperketonemia. Hyperketonemia, or ketosis, is defined as elevated blood ketones, including acetone, acetoacetate, and beta-hydroxybutyrate (BHBA). Hyperketonemia develops due to poor adaptation to NEB when the liver is overwhelmed with NEFA from adipose tissue mobilization (McArt et al., 2012). During periods of NEB, ruminants convert fat into carbohydrates to obtain additional energy. Ketones are produced as a byproduct of this process. While an increase in ketones after parturition is a normal adaptive response, excessive accumulation of ketones is abnormal (Duffield et al., 2009). The clinical symptoms of hyperketonemia manifest as decreased appetite and milk production, weight loss, depression, and an odor of acetone on the breath, milk, or urine (Fox, 1971, McArt et al., 2012). However, cows frequently experience subclinical ketosis, characterized by an excess of circulating ketone bodies without displaying clinical signs (McArt et al., 2012).

Elevated ketone levels are closely tied to other metabolic conditions. Cows with ketosis are at an increased risk of developing other periparturient diseases, such as DA and metritis (Duffield et al., 2009). Hepatic steatosis and hyperketonemia are interrelated periparturient lipid-related disorders associated with elevated NEFA and BHBA levels (Grummer, 1995). Additionally, primiparous and multiparous cows see an association between reduced serum Ca

dynamics and the occurrence of ketosis independent of other periparturient diseases (Venjakob et al., 2021). Field studies have shown that serum Ca concentrations were significantly decreased between d 1 and 7 post-calving in cows diagnosed with ketosis (Venjakob et al., 2021). Rodríguez et al. (2017) found an association between SCH and ketosis when serum Ca concentrations were < 2.14 mmol/L between 24 and 48 hr after calving. There was limited research available showing the influence of CMA on hyperketonemia in dairy cattle.

Subacute ruminal acidosis. Nutrient requirements shift as the cow enters lactation, so the diet must be adjusted to meet increased requirements. To satisfy the increased nutrient demand, most modern dairy operations manage lactating cows by feeding a high-concentrate diet to provide extra calories and energy (Plaizier et al., 2008, Elmhadi et al., 2022). However, highly digestible diets skew the starch-to-fibrous carbohydrates ratio, heavily impacting normal rumen microbiota function by altering VFA profiles (Elmhadi et al., 2022). As VFAs accumulate in the gut, rumen acidity increases (Plaizier et al., 2008, Cruywagen et al., 2015). Feeding diets rich in highly fermentable carbohydrates after transitioning from forage diets causes the rumen fermentation rate to surpass both absorption and buffering rates, also contributing to decreased ruminal pH (Elmhadi et al., 2022). Without adequate buffering agents in the diet to mitigate low ruminal pH, SARA occurs when pH dropped below 5.5 for an extended period. (Cruywagen et al., 2015, Cardoso et al., 2020). The consequences of SARA are reduced DMI, poor digestibility, decreased milk production, and increased susceptibility to developing metabolic and infectious diseases (Goff, 2008, Martinez et al., 2012). The major consequence of reduced feed intake is significant adipose mobilization and risk of metabolic acidosis in early lactation, severely reducing body condition and negatively affecting health status (Elmhadi et al., 2022).

Research showed that limestone and sodium bicarbonate were the most effective buffering agents for bovine ruminants (Chalupa and Sniffen, 1996). However, more recent research by Cruywagen et al. (2015) showed that including CMA in the replacement of limestone and sodium bicarbonate significantly reduced the duration of rumen pH below 5.5, from 13 hr/d to just 4 hr/d. This demonstrated that CMA released Ca slowly, thereby maintaining a healthy pH for longer, suggesting CMA as a substitute for limestone effectively prevented ruminal acidosis (Cruywagen et al., 2015). Furthermore, buffer supplementation with CMA improved total VFA and acetate concentrations while reducing lactate concentrations, further contributing to improved rumen health (Cruywagen et al., 2015). Additionally, both CMA and sodium bicarbonate increased milk yield and milk solids to have positive effects on milk production (Cruywagen et al., 2015). Reducing the risk of SARA and increasing production value improved farm efficiency and created savings for farmers by reducing veterinary treatment costs.

Abomasal displacement. A displaced abomasum in dairy cows occurs when the abomasum, the cow's fourth stomach compartment, shifts from its normal position (Kang et al., 2019). This displacement can hinder digestion and nutrient absorption, leading to decreased milk production and overall health issues (Kang et al., 2019). Hypocalcemia increases the risk of DA in dairy cattle by reducing abomasal tone, which leads to gas accumulation, a logical risk factor for DA (Daniel, 1983, Massey et al., 1993, Seifi et al., 2011). Evidence from Holstein cows during the transition period indicated that SCH at calving increased the risk of left displaced abomasum (**LDA**) by 4.8 times compared to normocalcemic cows (Massey et al., 1993). Seifi et al. (2011) demonstrated that cows with serum Ca concentrations of 2.3 mmol/L or less in the first week postpartum had 5.1 times higher odds of developing DA.

Lower Ca levels correlate with reduced rumen and abomasal motility due to reduced smooth muscle contraction, potentially increasing the risk of DA and reduced feed intake (Daniel, 1983, Goff, 2008, Seifi et al., 2011). However, LeBlanc et al. (2005), Kang et al. (2019) did not establish a direct correlation between Ca concentrations and the incidence of DA. It was proposed that SCH might result from reduced feed intake, potentially leading to other conditions such as LDA and subclinical ketosis (LeBlanc et al., 2005). Kang et al. (2019) outlined risk factors for DA, such as higher parity cows, incidence of twin calves, and ketosis.

Transition cow nutrition

DCAD diets for prepartum cows. Parturition imposes substantial biological demands on the dairy cow. The animal's failure to recover and adapt to the substantial drop in Ca impacts her health status and future production value (Drackley et al., 2005). Maintaining blood Ca homeostasis during transition is crucial to mitigating hypocalcemia and other metabolic diseases (Zhang et al., 2022). Introducing a DCAD in the prepartum diet can control the intake of principal cations (Na^+ , K^+ , Ca^{2+} , and Mg^{2+}) and anions (Cl^- , sulfate (SO_4^{2-}), and phosphate (PO_4^{3-})) (Goff, 2008, 2014). These ions influence blood electrical charge, determining the overall acid-base status and blood pH (Goff, 2008, 2014). Blood pH depends on the diet's disparity in cation and anion particle absorption. (Goff, 2014).

When an animal consumes a diet with more cations than anions, the DCAD is positive; compared to a diet with more anions than cations, the DCAD is negative (Goff, 2014). When DCAD is positive, the renal system requires more anions, typically bicarbonate ions, for urine electroneutrality (Erdman and Iwaniuk, 2017). Conversely, with negative DCAD, additional cations, like ammonium, balanced anionic Cl^- charge (Erdman and Iwaniuk, 2017). Urine pH evaluation offers a cost-effective way to assess blood pH and determine anion supplement needs

(Goff, 2014). Cattle fed positive DCAD with high cation content have alkaline urine pH > 7, while those on negative DCAD with low cation content have acidic urine pH < 7 (Goff and Horst, 1997, Erdman and Iwaniuk, 2017).

Importance of DCAD to prevent hypocalcemia. Ender et al. (1971) and Dishington (1975) found that low-ash (low K⁺) diets reduce hypocalcemia incidence. Therefore, decreasing the intake of dietary cations and increasing the intake of dietary anions (lower K⁺ and Na⁺ than Cl⁻) reduces the incidence of SCH (Block, 1984). However, low K⁺ levels increase H⁺ secretion, decrease pH, and direct more Ca²⁺ to the urine to balance dietary Cl⁻ excretion and maintain urine neutrality (Goff and Horst, 1997, Erdman and Iwaniuk, 2017). The increased Ca²⁺ excretion upregulate metabolic processes to enhance Ca²⁺ bone reabsorption and facilitate intestinal Ca²⁺ absorption from the diet (Erdman and Iwaniuk, 2017). As demand for Ca increases during calving, the cow efficiently regulates blood Ca levels (Goff, 2008, Erdman and Iwaniuk, 2017). A prepartum diet that provides less absorbable Ca than needed results in a negative Ca balance, lowers blood Ca concentration, and prompts PTH secretion (Goff, 2008, Erdman and Iwaniuk, 2017). Elevated PTH levels stimulate bone resorption and renal 1,25-dihydroxyvitamin D production within 1 to 2 weeks (Goff, 2008, Erdman and Iwaniuk, 2017). This prepares the intestine for efficient Ca absorption once introduced into the lactating ration (Goings et al., 1974, Green et al., 1981, Kichura et al., 1982). Feeding a low-Ca, negative DCAD diet before calving then transitioning to a high-Ca diet after calving can prevent hypocalcemia and milk fever (Goff and Horst, 1997).

Reproductive health and success after transition period

Milk production depends on successful conception and parturition of calves, so optimal reproductive performance is crucial. Diet significantly impacts conception and milk yield

(Ribeiro et al., 2013). Depleted metabolite and energy stores caused by fetal growth and milk synthesis impair reproductive performance (Chapinal et al., 2012). Around the time of calving, dairy cattle commonly experience a NEB when the animal cannot consume enough energy to meet the demands of lactogenesis (Chapinal et al., 2012). The standard voluntary waiting period (**VWP**) after parturition before the first artificial insemination (**AI**) for dairy cattle is traditionally 60 d after freshening (Miller et al., 2007). Periparturient diseases keep cattle in a state of NEB at first AI, resulting in delayed estrous cyclicity recurrence and reduced pregnancy rates per AI (**P/AI**) after the VWP (Ribeiro et al., 2013). Extended NEB delays uterine involution, ovarian cyclic activity, and weakens immune responses (Kimura et al., 2006, Cardoso et al., 2020). Insufficient nutrient intake for increased energy demand depressed reproductive functions, including hormone synthesis and secretion, follicle ovulation, and embryo development. (Cardoso et al., 2020)., The animal struggles to support fetal growth and development without proper nutrient management, markedly reducing her production value within the herd (Ribeiro et al., 2013).

Transition cow diseases associated with reproductive failure. Health conditions and diseases occurring during calving or breeding hinder reproductive performance (Ribeiro et al., 2013). These issues stem from depleted energy sources or a genetic predisposition to calving difficulties. Cows diagnosed with SCH, characterized by Ca levels below 2.2 to 2.4 mmol/L, are linked to decreased pregnancy rates at first AI (Chapinal et al., 2012) and an elevated risk of pregnancy loss (Ribeiro et al., 2013). Subclinical hypocalcemia also delays the first estrus post-calving and decreases the likelihood of cows displaying estrus indicators during the VWP (Rodríguez et al., 2017). Martinez et al. (2016) counteracted the detrimental reproductive impacts of depleted mineral and nutrient status with Ca supplementation during the transition

period. Administering oral Ca to postpartum multiparous cows alleviated SCH occurrence, thereby enhancing P/AI and reducing the number of open days (Martinez et al., 2016). There is a paucity of research explicitly investigating the influence of CMA on dairy cattle reproductive performance.

Impacts of disease and CMA on production

Cost of periparturient disease. Cows in NEB experience adipose tissue and muscle depletion, consequently decreasing milk production and solids quality. Peak lactation typically occurs soon after lactation began, coinciding with peak disease incidence (Cardoso et al., 2020). Nutrient depletion and NEB negatively affect milk production, causing economic stress for farmers (Seifi and Kia, 2018, Bhat et al., 2020). The largest impact of metabolic disease felt by dairy farmers are treatment costs, culling sick animals, and reduced milk production (Liang et al., 2017). Liang et al. (2017) used a stochastic simulation model to estimate the cost of clinical disease incidence and treatment in US dairy herds for multiparous cows. Total disease costs were highly influenced by herd performance and market conditions (Liang et al., 2017). The findings concluded that the total cost for hypocalcemia was $\$246.23 \pm 52.25$ per case, with the largest portion of total costs from veterinary treatment costs and the cost of extended days open (Liang et al., 2017). Liang et al. (2017) concluded that hyperketonemia costed $\$180.91 \pm 63.74$ per case, with the largest portion from veterinary treatment and producer labor costs. McArt et al. (2015) estimated a higher cost for hyperketonemia at \$289 per case across all parity groups. This cost included expenses related to hyperketonemia, as well as the costs associated with secondary metritis and DA development (McArt et al., 2015). Primiparous cows had nearly 50% higher financial losses compared to multiparous cows, due to higher expenses related to death loss, future production, culling, and reproductive losses (McArt et al., 2015). Liang et al. (2017)

determined the total cost of mastitis was $\$426.50 \pm 80.27$ per case, metritis was $\$262.65 \pm 56.15$ per case, and LDA was $\$639.51 \pm 114.10$ per case with the two largest costs being the cost of discarded milk and veterinary treatment. Miller et al. (1993) calculated a slightly lower average cost of clinical mastitis at \$107.11 per case. The stochastic analysis by Pérez-Báez et al. (2021) reported the mean cost per case of metritis was \$513, with 95% of the scenarios ranging from \$240 to \$884. The distribution of the total cash flow variation was as follows: 59% was explained by milk price, 19% by treatment cost, 12% by replacement cost, and 7% by feed cost (Pérez-Báez et al., 2021). Geishauser et al. (2000) found that the cost of LDA varied from \$250 to \$400 per case, depending on whether surgery was required.

Impacts of CMA on milk production. Literature indicates that adding CMA effectively supports milk production and component yield at levels equivalent to or exceeding traditional mineral sources. Cruywagen et al. (2015) replaced limestone in the basal diet with CMA (90 g/d) or with limestone + sodium bicarbonate (180 g/d). Cows fed CMA saw improvements ($P < 0.01$) in milk and component yield, 4% FCM, and ECM content compared to the limestone + sodium bicarbonate control. However, no differences in milk protein or lactose percentage were associated with treatment diet (Cruywagen et al., 2015). Wu et al. (2015) observed no differences among treatments in DMI, milk yield, percentage of milk fat, protein, and lactose. Milk protein yield was higher ($P = 0.05$) for the cows fed CMA prepartum compared with those fed CON calcium carbonate, at 1.09 and 1.02 kg/d respectively. Cows fed CMA postpartum supported similar DMI, milk yield, and percentage of fat, lactose, and SNF as the CON calcium carbonate diet (Wu et al., 2015). Research by Neville et al. (2022) reported an increased ($P < 0.01$) 4% FCM yield, and milk fat percentage and yield in transition cows supplemented with CMA compared to the CON limestone diet. However, Loučka et al. (2024) observed no difference in

the performance and health measures for peak lactation cows fed a CMA-based buffer. Similarly, Bernard et al. (2014) observed no differences in milk yield and milk fat, lactose, or SNF percentages. Yet, over the last 3 wk of the trial, cows fed CMA showed improved efficiency ($P = 0.008$) compared to those on the CON sodium bicarbonate diet. This suggests that CMA could be used to replace the calcium provided by limestone and enhance production efficiency after 7 weeks (Bernard et al., 2014). Cruywagen et al. (2015) agreed that CMA treatment animals produced more milk per kilogram of DMI ($P < 0.05$) for increased efficiency of feed utilization compared to cows on the CON treatment.

NOVEL MALE STERILE SORGHUM

The choice of silage in dairy production systems is crucial in ensuring proper nutrition and maximizing milk yield. Forages are vital in dairy cattle nutrition for maintaining rumen health and minimizing feeding costs (Eastridge, 2006). Whole-plant corn silage (CS) is the most widely used major forage source for dairy farms. Corn silage is the primary forage in dairy cow TMR due to its high DM yield, energy content, palatability, and optimal DM concentration for ensiling. (Colombini et al., 2010). The CS typically accounts for 40 to 60% of the forage DM in the diet (NASEM, 2021). Due to global climate change effects, arid environments demand alternative forage crops that thrive in high growing season temperatures and drought conditions compared to traditional forage options, like corn. Brown midrib sorghum is a primary focus of research due to its lower lignin concentration and improved fiber digestibility, which benefits high-yielding cows (Cherney et al., 1991). Sorghum produces a higher biomass yield and demonstrates greater water efficiency and drought tolerance compared to corn (Sanderson et al., 1992). Experiments on lactating cows have shown that replacing CS with sorghum silage (SS), when properly supplemented with starch sources, did not impact milk yield or composition

(Grant et al., 1995, Oliver et al., 2004, Colombini et al., 2010). Sorghum can lower feed costs by providing a highly digestible fiber that grows better and yields more under dry, hot conditions.

Role of quality forage in dairy cattle diets

Forages are economically and nutritionally important for dairy cattle health, offering cost-effective sources of protein, energy, and fiber compared to most concentrates. According to the USDA-ARS (2012), legume forages meet up to 75% of the protein needs of lactating dairy cows, while corn silage fulfill up to 25%. As the primary fiber source in a cow's diet, forages are essential for slowing feed passage, thereby enhancing nutrient absorption (USDA-ARS, 2012). Adequate dietary fiber significantly lowers the risk of acidosis and laminitis (USDA-ARS, 2012). Additionally, forages promote cud chewing and rumination, which boosts appetite, maximizes feed intake, and buffers rumen acids, preventing acidosis (USDA-ARS, 2012). The rumen, functioning as a fermentation vat, converts indigestible plant material and byproducts into usable food (USDA-ARS, 2012). Forages are also vital energy sources, with corn silage providing up to 50% of energy and alfalfa silage up to 40% (USDA-ARS, 2012). By supplying essential nutrients and supporting rumen function, forages contribute to overall herd well-being.

The inclusion of high-quality forages is critical, as dairy cattle production heavily rely on forage quality (Allen, 1996). Digestibility, an estimate of forage energy concentration, is often predicted from fiber concentration as a measure of forage quality (Allen, 1996). Equations have been developed to predict digestibility based on various fiber measures, with fiber being less digestible than non-fibrous components, thereby negatively affecting digestibility (Allen, 1996). Although high forage digestibility reduces feed costs, forages fed to lactating cows are supplemented to achieve similar dietary energy levels, thus mitigating the impact of forage digestibility on dairy cattle production (Allen, 1996).

Nutritive comparison of sorghum and corn silages

Silage-type sorghums are high-biomass yielding, drought-tolerant, and adaptable to late planting after winter crops, making them viable alternative forage crops (Sanderson et al., 1992). However, the nutritional variations between CS and SS exhibit notable differences in literature, specifically differences in DM digestibility. Lignin is the primary indigestible component of plant cell walls, and large amounts hindered ruminal digestion of cell wall carbohydrates (Aydin et al., 1999). Sorghum species contain higher lignin and lower grain concentrations compared to CS, resulting in decreased DM digestibility (Aydin et al., 1999). The high lignin concentration reduces fiber digestion potential, resulting in increased gut fill, reduced DMI, and reduced milk production (Aydin et al., 1999). Chemical and genetic manipulation reduced lignification to improve fiber digestibility in sorghum forages. The development of BMR genotypes contained less lignin (6.1% of DM) with altered lignin chemical composition compared to standard sorghum silage (7.1% of DM) (Aydin et al., 1999). Brown midrib forages demonstrated greater aNDF digestion than standard sorghum genotypes through in situ and in vitro studies (Fritz et al., 1988, Cherney et al., 1991, Grant et al., 1995). Colombini et al. (2010) observed that BMR had a faster aNDF degradability rate and equivalent effective rumen degradability compared to CS. Lusk et al. (1984) observed no differences in DM digestibility between cows fed CS or BMR. These findings underscore the diverse nutritional profiles and potential benefits of BMR in livestock diets.

Brown midrib sorghum silage is not a direct replacement for CS in the TMR due to differences in starch and sugar contents. Studies suggest that BMR silage can be a suitable replacement for CS without compromising milk production (Grant et al., 1995, Aydin et al., 1999, Colombini et al., 2010). However, adjustments in starch supplementation may be

necessary to maintain optimal milk yield. Colombini et al. (2010) found that CS had higher starch concentration than BMR silage (22.8 vs. 2.8% of DM, respectively). Therefore, additional corn meal was added to the BMR silage diet (5.7 vs. 8.0 kg/d for CS and BMR diets, respectively) to achieve equivalent levels of dietary starch and attain milk production values comparable to CS. (Colombini et al., 2010). Additionally, consideration for the stage of lactation and the specific nutritional requirements of dairy cows is warranted. When balancing TMR for the replacement of CS with BMR silage, it was essential to monitor nutrient intake, milk production, and composition to ensure overall nutritional needs were effectively met.

Production effects of feeding brown midrib sorghum

In most lactation and feeding trials, BMR maintained and even improved milk production and DMI. Grant et al. (1995) compared BMR, alfalfa, normal sorghum, and corn fed to mid-lactation (90 ± 5 DIM) Holstein cows. Data showed that DMI was greater ($P < 0.1$) for the corn and BMR (4% of BW/d) than for the alfalfa and normal sorghum diets (3.4% of BW/d). The BMR supported milk yield, fat, protein, and 4% FCM production that was similar to ($P < 0.1$) that of cows on corn and alfalfa diets, but cows fed normal sorghum produced less milk and fewer milk components (Grant et al., 1995). Aydin et al. (1999) completed two experiments on short-term and long-term lactational performance. The short-term study demonstrated that a corn silage diet (65% of dietary DM) led to an increase in milk yield and protein concentration compared to a BMR diet (65% of dietary DM). In the long-term study, a lower inclusion of forages (35% of dietary dry matter) had no significant impact ($P < 0.05$) on milk and 4% FCM yield. However, BMR led to greater milk and 4% FCM yield ($P < 0.05$) compared to normal sorghum (Aydin et al., 1999). Research from Lusk et al. (1984), Oliver et al. (2004), and Colombini et al. (2010) agreed that milk yield did not differ between BMR diet and CS diets.

Lusk et al. (1984) observed average milk production for corn and BMR to be 24 kg/d. Oliver et al. (2004) observed slightly higher milk yields at 34.1 kg/d for BMR-6 sorghum, 32.2 kg/d for BMR-18 sorghum, and 33.8 kg/d for corn. Similarly, Colombini et al. (2010) observed milk yields for corn and BMR at 32.6 and 33.1 kg/d, respectively.

CONCLUSIONS

Effective nutrient management plays a pivotal role in dairy cattle health, particularly during the transition period. Optimizing dietary inclusions and forage silage selections could minimize inputs and maximize outputs to lower operating costs. The incorporation of CMA as a supplemental Ca source demonstrates promising results in maintaining blood Ca levels and improving rumen health, thereby supporting overall productivity and health in dairy cows (Cruywagen et al., 2015, Wu et al., 2015). The strategic use of DCAD diets, coupled with the benefits of CMA, offers a comprehensive approach to enhancing cow health and farm profitability. The economic implications of periparturient diseases cannot be overstated. The cost of treating and managing these diseases, including the potential loss of milk production and increased veterinary expenses, places a significant burden on dairy farmers (Liang et al., 2017). Implementing effective nutritional strategies, such as the inclusion of CMA and careful DCAD management, reduces these costs by preventing disease onset and improving overall herd health. As research continues to explore the benefits of novel feed additives and nutritional approaches, it becomes increasingly clear that proactive nutrient management is essential for maintaining healthy, high-yielding dairy cows and ensuring the economic viability of dairy farms in a volatile market environment. By reducing the incidence of periparturient diseases and improving metabolic efficiency, these nutritional interventions contribute to higher milk production and

better reproductive outcomes, ultimately leading to a more sustainable and profitable dairy farming practice.

Forage silage is crucial in dairy production for nutrition and cost-efficiency, with CS being the most common due to its high yield and energy content, making up 40-60% of forage DM in diets (NASEM, 2021). Forages, including CS and alfalfa, are vital for protein, energy, and fiber, promoting rumen health, nutrient absorption, and preventing acidosis (USDA-ARS, 2012). However, climate change necessitated alternatives like sorghum, which offered higher biomass yield, better water efficiency, and improved fiber digestibility due to lower lignin concentrations (Sanderson et al., 1992). While sorghum has higher lignin and lower digestibility than CS, BMR variants improved this, making them viable alternatives without compromising milk production, although adjustments in diet formulation are needed (Grant et al., 1995, Aydin et al., 1999). Studies showed that substituting CS with BMR silage, supplemented with starch, maintained milk yield and composition, reducing feed costs in arid conditions (Lusk et al., 1984, Oliver et al., 2004, Colombini et al., 2010).

CHAPTER TWO

Impact of feeding calcareous marine algae to Holstein cows during the transition period

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ABSTRACT

Calcified red seaweed remains (*Lithothamnion calcareum*; calcareous marine algae, **CMA**) provide bioavailable essential macrominerals. This study evaluated feeding CMA compared to traditional mineral sources on cows experiencing heat stress from late gestation to early lactation (-21 to 30 DIM). Thirty-one multiparous and nine primiparous Holstein cows were studied to evaluate feeding CMA on dry matter intake (**DMI**), blood and urine metabolites, and milk yield and components. Data were collected from 21 treatment (**CMA**) and 19 control (**CON**) cows balanced for parity and projected calving date. Pre- and postpartum total mixed rations (**TMR**; 57.1% and 38.2% forage dry matter (**DM**), respectively) partially replaced calcium carbonate (**CaCO₃**), magnesium oxide (**MgO**), and sodium bicarbonate (**NaHCO₃**) with CMA. The CON TMR was formulated to provide 1.8% DM CaCO₃ and 0.07% DM MgO in the prepartum diet and 1.5% DM CaCO₃, 0.3% DM MgO, and 1.5% DM NaHCO₃ in the lactating diet. The CMA TMR was formulated with 0.8% DM CMA in the prepartum diet (0.7% CaCO₃ and 0.07% MgO DM replaced, respectively) and 0.4% DM CMA in the lactating diet (0.3% CaCO₃, 0.1% MgO, and 0.7% NaHCO₃ DM replaced, respectively). The GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used to determine the impact of treatment, maximum ambient temperature humidity index (**MaxTHI**), and projected 305-day mature equivalent milk (**PMEM**). The two-way covariate interactions of TRT by PMEM and MaxTHI by PMEM were removed by stepwise backward elimination if non-significant. Results were significant if $P \leq 0.05$. No DMI differences ($P > 0.05$) were observed between the experimental diets prepartum (-30 to 0 DIM) or in early lactation (4 to 30 DIM). No differences ($P > 0.05$) between the experimental diets in early lactation were observed in colostrum quality or quantity, body weight, total milk, fat, or protein yield. Cows fed CMA maintained similar ($P > 0.05$)

production efficiency values in early lactation compared with CON cows. There were no significant impacts ($P > 0.05$) on blood or urine metabolite status between treatment groups pre- or postpartum. Transition cows fed CMA performed as well as their counterparts fed traditional dietary sources of CaCO_3 , MgO , and NaHCO_3 .

INTRODUCTION

Produced from *Lithothamnion calcareum*, calcareous marine algae (**CMA**; Calmin, Feedworks USA, Ltd., Cincinnati, OH) contains 30% Ca, 0.5% P, 6.0% Mg, 0.1% K, and trace minerals (DM basis). Calcareous marine algae collectively represent several species of calcified red seaweed harvested off Irish and Icelandic coasts. The unique honeycomb structure and large surface area of CMA enable the plant to capture and retain Ca and Mg from seawater (Neville et al., 2022). The high mineral content in the cell wall slowly releases these minerals during digestion (Neville et al., 2022). Cruywagen et al. (2015) depicted CMA as a sparingly water-soluble Ca source and ruminal buffer due to its distinct marine-based physical structure and chemical characteristics. The incorporation of CMA as a supplemental Ca source demonstrated promising results in maintaining serum metabolite levels pre- and postpartum (Bernard et al., 2014, Neville et al., 2022) and preventing rumen depression (Bernard et al., 2014, Cruywagen et al., 2015, Loučka et al., 2024), thereby supporting the overall productivity and health of dairy cows. Additionally, CMA has been shown to maintain and exceed milk yield and other production parameters (Cruywagen et al., 2015, Wu et al., 2015, Neville et al., 2022). Compared to the cows on the control treatment, the CMA treatment produced more milk per kg of DMI (Cruywagen et al., 2015). Bernard et al. (2014) found improved efficiency for CMA-fed cows compared to sodium bicarbonate control during the final 3 wk of the trial. These literature

indicated that adding CMA effectively supported milk production and component yield at levels equivalent to or exceeding traditional mineral sources.

Limited research exists on the impact of CMA on blood and urine metabolite status, health, and production performance of transition cows under natural heat stress conditions, particularly in the southeast. Therefore, this study hypothesized that the effects of feeding CMA will meet or outperform traditional sources of CaCO_3 , MgO , and NaHCO_3 . The objective was to evaluate the effects of feeding CMA on transition cow health, blood mineral status, and postpartum production from 0 to 30 DIM compared with traditional sources of CaCO_3 , MgO , and NaHCO_3 under heat stress and non-heat stress conditions.

MATERIALS AND METHODS

Animals and housing

From May to December 2022, primiparous and multiparous Holstein cows were used in a feeding trial beginning 21 d pre-partum through 120 d postpartum. Cows were housed at the East Tennessee Research and Education Center Little River Dairy (**LRD**) in a naturally ventilated sand-bedded freestall barn with supplemental evaporative cooling provided by fans and misters. At calving, cows remained in the freestall area, and calves were moved to individual pens following parturition. Following parturition, all cows were managed according to LRD protocols including administration of a Bovicalc bolus following calving, routine blood monitoring on d 3 and 7 for β -hydroxybutyrate, and temperature and displaced abomasum checks during the first week after calving.

Cows were moved into the project pen 35 ± 3 d before their projected calving date. Animals were trained during the first 7 d to eat behind Calan Doors (American Calan Inc., Northwood, NH). The prepartum control diet was fed during the training period. During d 1 and

2, the Calan Doors were fully open, and feed was placed inside the doors to encourage exploration. On d 3 and 4, the doors were closed but left unlocked, with feed behind them, prompting cattle to nudge them open. From d 5 to 7, doors were fully closed and locked. Cows had Calan door keys attached to their neck chains and were assisted by project volunteers to locate and unlock their designated doors. By the end of the training period, cows that proficiently used the Calan Doors were enrolled in the study. However, the failure of a cow to independently eat out of her Calan door provided the basis for her removal from the project. Seventy-two cows were initially trained; however, only 49 were successfully trained and enrolled in the study through rolling enrollment. Rolling enrollment ensured cows were balanced evenly across both treatment groups based on current parity and projected calving date. Cows were sorted into parity groups (**ParG**). Parity 1 included primiparous heifers, parity 2 included second-lactation cows, and parity 3+ included third- and fourth-lactation cows. There were not enough fourth-lactation cows enrolled to form a distinct parity group (n = 2). All protocols were approved by the University of Tennessee, Knoxville Institutional Animal Care and Use Committee under protocol #2842-0621.

Experimental diets

The supplemental rates of CMA (calcified remains of *Lithothamnion calcareum*; Feedworks USA, Ltd.) were 100 g/d, according to manufacturer recommendations (Table 2.1). The CMA partially replaced calcium carbonate (**CaCO₃**), magnesium oxide (**MgO**), and sodium bicarbonate (**NaHCO₃**). Prepartum and postpartum TMR (Table 2.1; 57.1% and 38.2% forage DM, respectively) were formulated to partially replace CaCO₃, MgO, and NaHCO₃ with CMA. The CON TMR was formulated to provide 1.8% DM CaCO₃ and 0.07% DM MgO in the prepartum diet and 1.5% DM CaCO₃, 0.3% DM MgO, and 1.5% DM NaHCO₃ in the lactating

diet. The CMA TMR was formulated with 0.8% DM CMA in the prepartum diet (0.7% CaCO_3 and 0.07% MgO DM replaced, respectively) and 0.4% DM CMA in the lactating diet (0.3% CaCO_3 , 0.1% MgO, and 0.7% NaHCO_3 DM replaced, respectively). An anionic mineral was added to the prepartum TMR.

Feeding management

Basal prepartum and postpartum forage diets were mixed daily (VSL 150 Single-Auger Vertical Mixer, Kuhn North America, Brodhead, WI). Treatment grain premixes (Table 2.1) were included with the other forage ingredients during final mixing using a Calan Data Ranger (American Calan Inc., Northwood, NH) to individually deliver TMR diets to Calan Doors. The Data Ranger barrel spun for 10 minutes to evenly mix ingredients before feeding. The ration for prepartum cows was balanced at 12.34 kg DM per cow per d (10 kg DM forage, 2.3 kg DM grain mix (CON or CMA), and 0.04 kg DM anionic mineral). The ration for postpartum cows was balanced at 28.33 kg DM per cow per d (20.01 kg DM forage and 8.32 kg DM grain (CON or CMA)). Cows were fed once daily in amounts to provide a minimum of 5% refusal and had ad libitum access to water. The required amount of forage and grain was calculated daily based on the number of cows enrolled. From April 19 to May 27, 2022, prepartum cows enrolled and training to be enrolled in the study were fed at 16:00. From May 28 to September 26, 2022, prepartum CON and CMA cows were fed at 08:00, and postpartum CON and CMA cows were fed at 16:00. After the last cow in the study calved on September 26, 2022, CMA lactating cows were fed at 08:00 and CON lactating cows were fed at 16:00. The amount of feed offered and refusals were recorded daily.

Data collection

The data collected throughout this study included DMI, BW, BCS, colostrum quality and quantity, milk yield and components, blood metabolites, urine metabolites, health events, ambient temperature and humidity, and TMR diet sampling.

Orts. Cows had ad libitum access to TMR behind the Calan Doors. Feed replacement occurred daily, with a 24 hr interval between each replacement. The amount of feed offered was recorded at each feeding and adjusted daily to provide a minimum 5% refusal based on orts. Orts were any residual TMR from the preceding feeding session that was extracted and weighed before replenishing the feed. Any feed remaining within the bin was designated as refused by the cow. The rejected feed from each cow was transferred into an empty plastic container, which was positioned on a calibrated small-scale device (Smart Weigh Digital Heavy Duty Shipping, and Postal Scale) zeroed to account for the weight of the plastic container, facilitating accurate weight recording. Orts were recorded before the 08:00 and 16:00 feedings.

Body weight and body condition score. Body weight and BCS were recorded on three consecutive days at the beginning of the trial, immediately after parturition, and on 30, 60, 90, and 120 DIM. Body weight was recorded after the 05:00 milking with a walk-over livestock scale. Body condition scoring was conducted to evaluate the relative amount of subcutaneous body fat using the 5-point scale in 0.25 increments according to Elanco Animal Health (1997), where 1 = emaciated, 2 = thin, 3 = moderate, 4 = borderline obese, and 5 = extremely obese. A single, trained individual scored all BCS values to prevent discrepancies.

Colostrum quality and quantity. Colostrum from the first postpartum milking was collected between 4 and 12 hr after calving. The colostrum was analyzed onsite with a Brix refractometer and the total volume of colostrum from the first milking was recorded. In a 100

mL sample tube, 20 mL of fresh colostrum was diluted with 20 mL phosphate-buffered saline solution. Samples were taken to the University of Tennessee Precision Dairy Quality Lab (Knoxville, TN) for analysis of fat, protein, lactose, SNF, and SCC concentrations by mid-infrared spectrophotometric analysis using a Bentley DairySpec Combi instrument (Bentley Instruments Inc., Chaska, MN, USA) as described by AOAC International (2000).

Fresh colostrum was aliquoted into a snap vial and labeled with the cow ID and sample date, then placed in the -80°F freezer until the end of the study for immunoglobulin G (**IgG**) content analysis via radial immunodiffusion (Triple J Farms, Bellingham, WA, USA). The radial immunodiffusion plates were removed from the refrigerator to room temperature approximately 30 minutes before filling the wells. Three wells were filled with reference sera standards (5 mg/mL, 25 mg/mL, 50 mg/mL) each time this process was conducted to calibrate the end-point readings. Each specimen was delivered into duplicate wells with a 5-microliter pipette by placing the pipette tip at the bottom of the well and allowing the specimen to fill to the agar surface. After all the wells were filled with samples, the cover was replaced, and the plate was incubated at room temperature for 24 hr to achieve the most equilibrated end-point readings. Following incubation, the diameter of the rings diffused around the wells was measured with a ruler to the nearest mm by a single, trained individual.

Milk yield and components. After parturition, cows were milked two times daily, at 05:00 and 17:00. Daily milk yields were recorded electronically by BouMatic (BouMatic LLC, Madison, WI) and SmartDairy parlor system (Valley Agricultural Software, Tulare, CA) with the PCDart Interface (Dairy Records Management Systems, Raleigh, NC). Milk samples were collected once per week at 05:00 and 17:00 milkings. Samples were taken to the University of Tennessee Precision Dairy Quality Lab (Knoxville, TN) for analysis of fat, protein, lactose, SNF,

and SCC concentrations by mid-infrared spectrophotometric analysis using a Bentley DairySpec Combi instrument as described by AOAC International (2000).

Blood metabolites. Blood samples were collected from the coccygeal vein after the 08:00 feeding on d 7 ± 3 pre-calving and d 2, 7, and 14 post-calving. One blood sample per cow was collected in a 10mL red-top vacutainer tube per cow. Samples were settled at room temperature for one hour and then centrifuged onsite at 1200 rpm for 10 minutes at 32°C or until sera separated. Sera was separated, aliquoted into two snap vials, and frozen at -80°C. Samples were shipped to the University of Georgia Tifton Veterinary Diagnostic and Investigational Laboratory (Athens, GA) for metabolite analysis. Samples were assayed for determination of serum calcium (**Ca**), magnesium (**Mg**), sodium (**Na**), potassium (**K**), and chloride (**Cl**). Additional metabolites were glucose, anion gap, creatinine kinase, urea nitrogen, creatinine, urea nitrogen/creatinine ratio, total protein, globulin, total bilirubin, aspartate aminotransferase, gamma-glutamyltransferase, albumin.

Urine metabolites and pH. Urine samples were collected after the 08:00 feeding on d 21, 14, and 7 ± 3 pre-calving and d 2 post-calving. The vulva was sterilized with chlorhexidine solution before manual stimulation to induce urination into a 10mL sample tube. Sample pH measurements were conducted using a Mettler Toledo S20 SevenEasy pH Meter (Mettler Toledo, Columbus, OH). The pH meter was calibrated before each measurement session using a standard buffer solution (pH 7.0). Samples were shipped to the University of Georgia Tifton Veterinary Diagnostic and Investigational Laboratory (Athens, GA) for metabolite analysis. Samples were assayed to determine Ca, Mg, Na, K, Cl, and phosphorus (**PO₄**).

Health events. Health events were recorded and reported by LRD staff. Recorded health events were categorized as transition cow diseases. Transition cow diseases (**TCD**) included the

incidence of RP, metritis, DA, vaginal tear, and low ketone levels. Somatic cell count and clinical signs of mastitis were monitored throughout the study to indicate infection. Other events included fever, pinkeye, digestive issues, and injury. The study design did not intentionally induce incidence of transition cows diseases, such as SARA or hypocalcemia. Post-partum routine supportive therapies were still applied after calving according to LRD farm protocols.

Ambient temperature and relative humidity. Ambient conditions of the freestall barn were recorded using Hobo Pro V2 loggers (Onset Computer Corporation, Bourne, MA). Four loggers were evenly placed throughout the project pen. All loggers recorded the ambient temperature, relative humidity, and dew point every 15 minutes. Data from loggers was collected every 2 wk.

Forage sampling. Samples of CON and CMA TMR diets were collected three times each week. Weekly samples were combined into a weekly composite sample by using a subsample from each level of a Penn State Shaker Box. Composite samples were shipped to Cumberland Valley Analytical Services (Waynesboro, PA) for DM and wet chemistry analysis.

Statistical analysis

Data cleaning. Cows unable to utilize the Calan Doors were not enrolled in the study ($n = 23$). Four cows with less than 30 d of data were removed (CON = 1; CMA = 3). Two cows with continuous variability in DMI traditionally below the threshold level required for milk production were removed (CON = 2; CMA = 0). These occurrences were due to inadequate Calan door use. Daily milk yield data was inaccessible for three cows, as they were sold after the study ended, so data from animals were removed (CON = 2; CMA = 1). Daily milk yields < 20.4 or > 66.7 kg were deemed unrealistic and removed. The value was considered unrealistic and removed if the difference between daily milk yields was < -18.5 or > 15.5 kg/d. Daily milk

yields for the first 3 DIM after parturition were inconsistently recorded, so milk yields for DIM < 4 were removed. Daily DMI values were removed if < 4.53 kg due to unrealistic intake amounts from Calan door misuse. After data cleaning, 19 CON animals and 21 CMA animals remained in the dataset. Parity 1 included nine primiparous heifers (CON = 4; CMA = 5), parity 2 included 19 second-lactation cows (CON = 10; CMA = 9), and parity 3+ included 12 third- and fourth-lactation cows (CON = 5; CMA = 7).

Dry matter intake and milk production. The independent variables of DMI, milk yield (**MilkYield**), milk fat (**MilkFat**), milk protein (**MilkProt**), milk efficiency (**MilkEff**), ECM, and FCM were analyzed for the fixed effects of treatment group (**TRT**), max daily temperature humidity index (**MaxTHI**), and projected 305 d mature equivalent milk (**PMEM**). Ambient temperature and relative humidity data captured from the Hobo Pro V2 loggers created the MaxTHI. If $\text{MaxTHI} \geq 68$, heat stress occurred, and if $\text{MaxTHI} < 68$, then heat stress did not occur. The values for PMEM were captured by PCDart. To calculate individual DMI for each feeding, the ort amount was subtracted from the initial amount offered. The DMI for each cow was calculated by multiplying individual intake by DM content of the TMR, which was determined by standard wet chemistry analysis. Intake was calculated using the Eq. 2.1:

$$\text{Intake (kg/cow/d)} = \text{Feed offered} - \text{Ort} \quad \text{Equation 2.1}$$

Dry matter intake was calculated using the Eq. 2.2:

$$\text{DMI (kg/cow/d)} = \text{Intake} * \% \text{DM} \quad \text{Equation 2.2}$$

Milk fat and protein percentages were transformed using the arcsine transformation to approximate the normal distribution using Eq. 2.3 and 2.4. Means were backtransformed to the original scale for presentation:

$$\text{Fatarc} = \arcsin\left(\frac{\sqrt{\text{MilkFat}(\%)}}{100}\right) \quad \text{Equation 2.3}$$

$$\text{Protarc} = \arcsin\left(\frac{\sqrt{\text{MilkProtein}(\%)}}{100}\right) \quad \text{Equation 2.4}$$

Values for ECM and 4% FCM were calculated using the Eq 2.5 (Tyrrell and Reid, 1965) and Eq 2.6 (Gaines and Davidson, 1923):

$$\text{ECM (kg/cow/d)} = (0.327 * \text{MilkYield(kg)}) + (12.95 * \text{MilkFat(kg)}) + (7.65 * \text{MilkProtein(kg)}) \quad \text{Equation 2.5}$$

$$4\% \text{ FCM (kg/cow/d)} = (0.4 * \text{MilkYield(kg)}) + (16.216 * \text{MilkFat(kg)}) \quad \text{Equation 2.6}$$

The MilkEff ratio was calculated using Eq 2.7:

$$\text{MilkEff} = \text{MilkYield(kg)} \div \text{DMI(kg)} \quad \text{Equation 2.7}$$

The random effects of ParG, the interaction of TRT by parity group, and cow nested within the interaction of TRT by parity group were included in model notation. Covariates included in the model were the interaction of TRT by PMEM and the interaction of MaxTHI by PMEM. Model notation (Eq. 2.8):

$$Y_{ijk} = \mu + T_i + P_j + M_k + u_l + e_{ijkl} \quad \text{Equation 2.8}$$

where Y = observation of DMI, MilkYield, MilkFat, MilkProt, MilkEff, ECM, FCM, and SCC; μ = the overall mean; T = TRT; P = PMEM; M = MaxTHI; u = random effects of ParG, the interaction of TRT by ParG, and cow nested within the interaction of TRT by ParG; and e = the residual effect for DIM week nested in cow, TRT, ParG, and MaxTHI.

To evaluate the effect of heat stress days vs non-heat stress days, the interaction of TRT by MaxTHI was included as an additional fixed effect in model notation using Eq. 2.9:

$$Y_{ijk} = \mu + T_i + P_j + M_k + (TM)_{ik} + u_l + e_{ijkl} \quad \text{Equation 2.9}$$

where Y = observation of DMI, MilkYield, MilkFat, MilkProt, MilkEff, ECM, FCM, and SCC; μ = the overall mean; T = TRT; P = PMEM; M = MaxTHI; TM = the interaction of TRT by MaxTHI; u = random effects of ParG, the interaction of TRT by ParG, and cow nested within the interaction of TRT by ParG; and e = the residual effect for DIM week nested in cow, TRT, ParG, and MaxTHI.

Body weight and body condition score. To analyze BW and BCS, the fixed effects of TRT, DIM of sample collection, and the interaction of TRT by DIM of sample collection were included in Model 3 with the random effects of ParG, the interaction of TRT by ParG, and cow nested within the interaction of TRT by ParG. Covariates included in the model were initial BW or initial BCS, MaxTHI, PMEM, the interaction of TRT by PMEM and the interaction of MaxTHI by PMEM. Model notation (Eq. 2.10):

$$Y_{ij} = \mu + T_i + D_j + (TD)_{ij} + u_k + e_{ijk} \quad \text{Equation 2.10}$$

where Y = observation of BW and BCS; μ = the overall mean; T = TRT; D = DIM of sample collection; TD = interaction of TRT by DIM of sample collection; u = random effects of ParG, the interaction of TRT by ParG, and cow nested within the interaction of TRT by ParG; and e = the residual effect.

Colostrum quality and quantity. The duplicate diameters from the radial immunodiffusion plates were averaged for each sample. Colostrum IgG concentrations were calculated from the standard sera line of “best fit,” where concentration (mg/mL) was plotted on the x-axis and diameter² (mm²) was plotted on the y-axis. The diameter was input as “x” in the slope-intercept equation using Eq. 2.11:

$$y = m(x) + b \quad \text{Equation 2.11}$$

To analyze colostrum IgG concentration, the volume of colostrum produced, and colostrum %Brix, the fixed effect of TRT was included in model notation. Covariates included in the model were MaxTHI, PMEM, the interaction of TRT by PMEM and the interaction of MaxTHI by PMEM. Model notation (Eq. 2.12):

$$Y_i = \mu + T_i + e_i \quad \text{Equation 2.12}$$

where Y = observation of IgG, colostrum volume, and %Brix; μ = the overall mean; T = TRT; and e = the residual effect.

Blood and urine metabolites. To analyze the blood metabolites of Ca, Mg, Na, K, and Cl, and urine metabolites of pH, Ca, Mg, Na, K, Cl, and PO_4 , the fixed effects of TRT, sample day (**DIMcode**), and the interaction of TRT by DIMcode were included in Model 5 with the random effect of ParG in model notation. Covariates included in the model were MaxTHI, PMEM, the interaction of TRT by PMEM and the interaction of MaxTHI by PMEM. Model notation (Eq. 2.13):

$$Y_{ij} = \mu + T_i + S_j + (TS)_{ij} + u_k + e_{ijk} \quad \text{Equation 2.13}$$

where Y = observation of blood metabolites, urine metabolites, and urine pH; μ = the overall mean; T = TRT; S = sample day; (TS) = the interaction of TRT by sample day; u = the random effect of ParG; and e = the residual effect.

The GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with the ILINK option was used to obtain solutions for all independent variables previously described. An LSMeans statement was included in the model to describe significance between treatment groups. Results were significant if $P \leq 0.05$ and had tendency if $P \leq 0.10$. Non-significant covariates were removed with stepwise backward elimination. The FREQ procedure of SAS 9.4

with chi-square analysis and Fisher's exact test was used to analyze the interaction of TRT by health event. Results were significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

Dry matter intake

The chemical composition of the experimental diets is presented in Table 2.2. Nutrient compositions were similar for prepartum and postpartum diets with or without partial replacement of CMA. Dry matter intake is presented in Table 2.3. Transition period DMI was not different among treatments for CON and CMA, averaging 19.4 ± 1.24 and 20.6 ± 1.20 kg/d, respectively. Additionally, no differences between treatments and heat stress versus non-heat stress conditions were observed for DMI.

These results agree with Wu et al. 2015 who saw no DMI differences pre- or postpartum feeding CMA versus sodium bicarbonate control, and Cruywagen et al. (2015) who saw no statistically significant differences in lactating DMI between CMA, sodium bicarbonate, and limestone diets. Meanwhile, Neville et al. (2022) observed increased prepartum and postpartum DMI. Basal diet differences must be considered to accurately compare those experiment results to the current study. Neville et al. (2022) compared a control diet containing limestone to one containing CMA. No sodium bicarbonate was included in either experimental diet. In the current study, CON contained 1.5% and CMA contained 0.8% sodium bicarbonate in the postpartum diets. The inclusion of sodium bicarbonate has been shown to increase DMI ($P < 0.10$) compared to control diets, with values of 22.6 kg/d versus 20.7 kg/d, respectively (Neville et al., 2019). Furthermore, diets containing sodium bicarbonate exhibited DMI levels similar to those containing CMA during early lactation, with values of 23 kg/d versus 22.3 kg/d, respectively (Bernard et al., 2014). Including sodium bicarbonate in both experimental diets may contribute to

comparable DMI between groups. No differences were found among major serum mineral concentrations, so it is challenging to relate mineral status changes to DMI differences in this experiment.

Available Ca in the early postpartum period was essential for rumen contractions that dictated DM consumption (Goff, 2008). Enhanced ruminal pH and fermentation, as well as quicker rumen passage rate due to improved nutrient digestibility, may be associated with increased DMI. Although we found no significant differences in mineral concentrations throughout the study, increased availability of Ca and Mg from the CMA treatment may have contributed to the slight numerical increase in DMI. However, further research is needed to investigate this.

Milk production

Postpartum milk yield and composition are presented in Table 2.3. No differences ($P = 0.49$) among treatments were observed in milk yield, percentage of milk fat, protein, and lactose. No differences ($P = 0.29$) between treatment groups were observed in milk fat, protein, and lactose yield. No differences ($P = 0.93$) between ECM and 4% FCM were seen among the treatment groups. No differences between treatment, MaxTHI, or the interaction of treatment and MaxTHI for heat stress versus non-heat stress conditions were observed for milk production measures or efficiency metrics.

The impact of CMA on milk production has been studied in early lactation (14 to 100 DIM) (Bernard et al., 2014, Wu et al., 2015, Neville et al., 2022), peak lactation (45 to 90 DIM) (Loučka et al., 2024), and mid-lactation (100 to 200 DIM) cows (Cruywagen et al., 2015). These studies differed in their findings related to milk production and milk composition. Bernard et al. (2014) compared three treatment groups including either 87 g/d of CMA, 204 g/d of sodium

bicarbonate, or no supplement. No differences were observed for milk yield, component yield, or ECM. However, cows fed the non-supplemental diet tended to have higher milk protein composition ($P = 0.08$) than those fed the CMA-containing and sodium bicarbonate diets (Bernard et al., 2014). Wu et al. (2015) compared prepartum diets supplemented with calcium carbonate or 50 g/d of CMA and postpartum diets supplemented with 317 g/d of sodium bicarbonate and calcium carbonate or 100 g/d of CMA. No differences were observed among treatments in milk yield, percentage of milk fat, protein, and lactose. Milk protein yield was higher ($P = 0.05$) for the cows fed CMA prepartum than those fed calcium carbonate prepartum, at 1.09 and 1.02 kg/d, respectively (Wu et al., 2015). Cows fed CMA postpartum supported similar milk yield and fat, lactose, and SNF percentage as the sodium bicarbonate and calcium carbonate postpartum diet (Wu et al., 2015). Cruywagen et al. (2015) observed that cows fed the CMA-supplemented diet had improved ($P < 0.01$) milk yield, milk fat, protein, lactose yield, ECM, and 4% FCM compared to sodium bicarbonate and limestone diets. Although CMA treatment saw increased milk fat percentage ($P = 0.01$), no milk protein or lactose composition differences were associated with treatment diet (Cruywagen et al., 2015). This difference in milk production parameters was most likely due to CON cows experiencing extended periods of rumen pH below 5.5 (13.8 hr/d) and exhibiting signs of SARA, such as panting and drooling, during the study. It is widely recognized that SARA has a detrimental impact on milk production (Kleen et al., 2003, Krause and Oetzel, 2006). In a recent CMA trial, Loučka et al. (2024) observed no difference in milk production parameters at peak lactation similar to our study.

Milk, energy, and fat efficiencies are presented in Table 2.3. No difference ($P = 0.60$) in efficiency values for milk, ECM, or 4% FCM per kg of DMI was observed between treatment groups. This agrees with the results of Wu et al. (2015). Conversely, Bernard et al. (2014)

observed improved efficiency ($P = 0.008$) in cows fed CMA compared to those on the sodium bicarbonate diet during the last three weeks of the trial. The authors attributed this to supplemental sodium bicarbonate having increased DMI during the latter weeks of the trial, thus reducing the efficiency of milk production (ECM/DMI) compared with CMA (Bernard et al., 2014). Longer trials were necessary to validate these observations and identify underlying reasons for the improved efficiency (Bernard et al., 2014). According to Cruywagen et al. (2015), CMA supplementation of 90 g/d in TMR improved feed efficiency for milk production ($P = 0.006$) and composition ($P < 0.001$) compared to sodium bicarbonate and limestone diets. As previously stated, CON cows on this trial experienced symptoms of SARA, thus reducing milk production compared to cows supplemented with CMA. This may contribute to CMA appearing more efficient than CON cows. In the current trial, the partial replacement of CMA throughout the transition period was as effective as CON for maintaining milk yield and composition.

Colostrum, body weight, and body condition score

Colostrum quality and quantity, BW, and BCS are presented in Table 2.3. No differences among treatment groups were observed in colostrum IgG concentration, Brix score, or volume. Cows fed CON tended to produce increased amounts at 30.6 ± 2.15 mg/mL diets compared to those supplemented with CMA produced an average serum IgG concentration of 23.8 ± 2.39 mg/mL ($P = 0.06$). Insufficient consumption of IgG and failure to transfer passive immunity resulted if serum IgG concentration was < 10 mg/mL (Weaver et al., 2000, Calloway et al., 2002). Both treatments produced IgG concentrations sufficient to transfer passive immunity to calves. A Brix score $\geq 22\%$ ensured good quality colostrum (Bielmann et al., 2010). Control and CMA cows yielded Brix scores above 22%, providing good quality colostrum at $22.4 \pm 2.28\%$

and $23.2 \pm 2.17\%$, respectively ($P = 0.79$). It is interesting that the CON cows tended to produce higher IgG concentrations but had numerically lower Brix score than CMA cows. This could be explained by the fact that Brix score considers all immunoglobulins (A, D, E, G, and M), not just IgG. The approximate colostrum quantity produced by dairy cows was between 8.5 and 10 kg (Pritchett et al., 1991, Godden et al., 2019). Although the colostrum production was slightly lower than this standard value, CON and CMA cows produced 7.67 ± 1.11 kg and 8.4 ± 1.05 kg, respectively, with no significant difference ($P = 0.64$) between the two groups. There was a paucity of research explicitly investigating the influence of CMA on colostrum metrics to compare to the results of this study.

No differences were observed among treatments in BW or BCS throughout the trial. These results agree with Bernard et al. (2014) and Wu et al. (2015). Body weight at calving was not significantly different ($P = 0.90$) between CON and CMA cows, at 639.2 ± 14.4 kg and 635.8 ± 13.8 kg, respectively. Body weight at 30 DIM, the end of the transition period, was also not significantly different ($P = 0.90$) for CON and CMA cows, at 612.8 ± 14.4 kg and 612.2 ± 13.8 kg, respectively. Bernard et al. (2014) also found no differences in BW among the treatment groups. However, BW values in Bernard's study were slightly higher than those in our trial (660 kg average across treatments), likely because Bernard et al. (2014) recorded BW at 98 DIM, giving cows more time to regain weight compared to our measurements taken at calving and at 30 DIM. Additionally, herd management differences could account for the increased mean BW of cows in Bernard's study compared to our current study, which could explain why both studies reported no differences between treatment groups. Weights at calving were above the healthy weight for mature cows (Hoffman, 1997). Although BW at 30 DIM was slightly below the recommended level, body condition is typically at its lowest from 30 to 120 DIM (Elanco

Animal Health, 1997). At the time of calving, BCS for cows on the CMA diet was 3.17, and those on the CON diet were 3.09 ($P = 0.49$). At 30 DIM, CON cows had a BCS of 3.0 and CMA had a BCS of 3.04 ($P = 0.49$). The optimum calving BCS was between 3.0 and 3.25 (Roche et al., 2009). A lower calving BCS was linked to decreased production and reproduction, while a BCS of 3.5 or higher was associated with reduced early lactation DMI, lower milk production, and a higher risk of metabolic disorders (Roche et al., 2009). Both treatments maintained BCS within the optimum range from the time of calving until the end of the transition period. Both treatments maintained BW and BCS within an optimum range from the time of calving until the end of the transition period.

Blood and urine metabolites

Serum mineral concentrations did not differ among CON and CMA treatments, consistent with findings by Bernard et al. (2014) and Neville et al. (2022). Mineral-related challenges in dairy cows often occurred in the days following parturition, as mineral metabolism was more stressed after giving birth than in the weeks before calving (Goff, 2008). Consequently, differences in Ca and Mg concentrations between CMA and CON treatments due to variations in bioavailability may not have been noticeable before freshening. Differences were observed in serum Ca and Na levels relative to the day around calving for both treatment groups. Serum Ca on d 2 after calving was lower ($P < 0.01$) compared to d -7 before calving and d 7 and 14 after calving (Figure 2.1a). Blood Ca concentrations were known to reach their lowest levels between 24 and 48 hr postpartum due to the demand for colostrum and milk production, typically stabilizing to 8.5 to 10 mg/dL within 3 to 4 d postpartum (McArt, 2019). In the current study, the dip in serum Ca on d 2 still remained in the healthy range and returned to prepartum levels by d 7, reflecting healthy physiological function for both treatment groups. Serum Na was lower ($P <$

0.05) on d 7 and 14 after calving compared to d -7 and 2 around the time of calving (Figure 2.1c). The reason for this decrease in serum Na as lactation advances was unclear, as dietary Na concentrations were similar for pre- and postpartum diets. Wu et al. (2015) reported differences in serum Na between treatment groups. Serum Na was slightly increased for cows fed sodium bicarbonate prepartum ($P = 0.05$) and cows fed no sodium bicarbonate postpartum ($P < 0.05$) compared to those fed CMA. The authors noted that the reason for this was uncertain, as dietary Na concentrations were similar for both CON and CMA (Wu et al., 2015). Regardless, serum Na levels in the current study remained in the normal range of 136 to 144 mEq/L throughout the transition period for both treatments. All other serum metabolites remained within the recommended range for healthy animals (Merck Animal Health, 2022).

Urine mineral concentrations did not differ among treatments. On d 2 after calving, urine Ca (Figure 2.2a) and Cl (Figure 2.2e) levels were lower ($P < 0.01$) compared to d -21, -14, and -7 before calving. This possibly indicates reduced availability of Ca for excretion due to calving and milk synthesis. It could also suggest that cows utilized extra Ca in the postpartum diet to retain Ca after calving rather than excreting it. Decreased Cl excretion postpartum is likely attributed to the DCAD lactating diet formulated to contain less Cl than the prepartum diet. As demonstrated by Silankikove et al. (1997), a lower intake of Cl ions resulted in lower Cl excretion in the urine.

The urine pH did not differ between treatment groups (Figure 2.3). On d 2, urine pH for both CMA and CON was significantly higher ($P < 0.01$) than on d -21, -14, and -7 before calving. To effectively control hypocalcemia, cows were fed DCAD-formulated diets, with high anionic levels prepartum and high cationic levels postpartum. The average urine pH of close-up cows should be between 5.8 and 6.3 (Goff, 2008). A urine pH of 5.0 to 5.5 indicates excessive

anions and could cause uncompensated metabolic acidosis, leading to a decline in DMI (Goff, 2008). Typically, urine pH on high cation diets is around 8.2 (Goff, 2008). In this study, prepartum urine pH ranged from 6.0 to 6.9, while postpartum urine pH levels were slightly lower than Goff (2008) recommendation, at 7.6 for CON and 7.8 for CMA. This discrepancy is likely due to the sample being taken early in lactation (d 2), when cows may not have fully adjusted to the high cation diet. Wu et al. (2015) reported prepartum urine pH values higher than the current study, with 7.35 for CON and 7.0 for CMA. Postpartum pH was closer to the recommended value and slightly higher than the current study, averaging 8.01 across treatment groups (Wu et al., 2015).

Health events

Health events recorded throughout the study were not significantly different ($P = 0.35$; Table 2.5) between treatment groups. There were 6 TCD recorded for CON and 6 for CMA. Low cases of TCD were further evidence that the DCAD diet worked as expected and standard postpartum practices encouraged healthy transition cows. One case of mastitis was recorded for CON, and no cases were recorded for CMA. There were 3 other diseases recorded for CON and 5 for CMA.

LIMITATIONS

The study utilized a single Calan Data Ranger for both experimental diets (CON and CMA). The barrel was fully emptied before switching diets to prevent cross-contamination, which likely did not significantly affect the study results. Some cows stole feed, primarily because older, taller cows bullied heifers and newly enrolled cows away from their designated doors. Additionally, the low-set Calan Doors, designed for larger-headed cows, made it easier for others to reach in and steal feed. Despite attempts to separate these groups, challenges persisted

due to the rolling enrollment and difficulty retraining cows to new door locations. Some Calan Doors did not fully close and lock, allowing manipulation by cows. To mitigate this, door springs were tightened for quicker closure. The authors noted that feeding behavior might have been affected by changes in feeding times during the study.

CONCLUSIONS

In summary, partially replacing conventional mineral ingredients with CMA in dairy cow diets during the transition period resulted in similar DMI and milk production metrics compared to control diets. Both treatments maintained serum mineral concentrations, BW, and BCS within optimal ranges, with no significant differences observed. The inclusion of sodium bicarbonate in both diets likely contributed to the comparable DMI among groups. Additionally, the quality of colostrum produced by cows in both treatments was adequate for transferring passive immunity to calves, with both groups achieving Brix scores above 22%. Although serum Ca and Na levels showed expected fluctuations around calving, all measured serum and urine metabolites remained within healthy ranges throughout the study. These findings suggest that CMA can be effectively used as a partial dietary replacement without negatively impacting key health and production parameters in dairy cows during the critical transition period. Future research is needed to better understand the effects of CMA on transition cow health. A study that more diligently monitors health events and observes serum and urine mineral levels until the end of the transition period may help elucidate the underlying mechanisms of CMA supplementation on Ca homeostasis.

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APPENDIX A

Table 2.1. Balanced ration ingredients (%DM) for pre- and postpartum diets for CON¹ or CMA¹.

Ingredient	Prepartum		Postpartum	
	CON ¹	CMA ¹	CON ¹	CMA ¹
Corn silage	36.45	36.45	28.50	28.50
Wheat straw	20.63	20.63		
Oat silage			9.69	9.69
Ground corn	1.59	1.59	22.48	22.48
Soybean hull pellets	5.06	5.06	8.33	8.33
Cottonseed hulls	7.58	7.51		0.65
Hominy feed			4.41	4.41
Soybean meal, 48% CP	5.32	5.32	7.49	7.49
SoyPlus ²	13.16	13.16	5.65	5.65
Roasted soybeans			6.26	6.26
CMA ³		0.82		0.37
Calcium carbonate* ⁴	1.78	1.11	1.50	1.20
Magnesium oxide*	0.07		0.33	0.28
Sodium bicarbonate*			1.50	0.83
Calcium sulfate dihydrate* ⁴	0.55			
Di calcium phosphate			0.13	0.13
Potassium chloride			0.50	0.50
Animate ⁵	3.15	3.15		
AjiPro-L Gen 3 ⁶	0.04	0.04		
K-Carb plus ⁷			0.33	0.33
White salt (NaCl)			0.23	0.23
Spectrum fusion ⁸			1.25	1.25
Molasses	0.96	0.96	0.48	0.48
Reashure choline ⁹	0.44	0.44		
Urea	0.19	0.19	0.50	0.50
ETX-5 ¹⁰	0.15	0.15		
Vistacell yeast ¹¹	0.11	0.11		
RA-251 PMX .60 08-24-20 ¹²	2.22	2.22		
RA-CCF AC Trace PMX 12-21 ¹³			0.22	0.22
Aliment (MHA) ¹⁴	0.04	0.04	0.10	0.10
Dynamate ¹⁵			0.07	0.07
Mepron ¹⁶	0.07	0.07		
ClariFly ¹⁷	0.07	0.07	0.03	0.03
Total Concentrate	40.97	40.42	61.81	61.81
Total Forage	57.07	57.07	38.19	38.19

*Ingredient differences between CON and CMA diets.

¹CON = control diet; CMA = calcareous marine algae partial replacement.

²Soybean-based bypass protein, Landus, Des Moines, IA.

³Calcareous marine algae, Calmin, Feedworks USA, Ltd., Cincinnati, OH.

⁴Inorganic calcium salts.

⁵Anionic mineral supplement, Phibro Animal Health Corp., Teaneck, NJ.

⁶Rumen-protected lysine, Ajinomoto Health & Nutrition North America, Inc., Raleigh, NC.

⁷Feed-grade protected granular potassium carbonate, K-Carb Plus, Woodbury, MN.

⁸Fatty acid supplement, Perdue AgriBusiness, LLC., Salisbury, MD.

⁹Encapsulated choline, Balchem Corp., Green Pond, SC.

¹⁰Manages mycotoxin-related disease, Feedworks USA, Ltd., Cincinnati, OH.

¹¹*Saccharomyces cerevisiae* granular live yeast, AB Vista, Plantation FL.

¹²Prepartum mineral-vitamin premix contained (DM basis) 24% Ca; 1.88% P; 6.02% Mg; 0.09% K; 0.13% S; 4% Na; 6.14% Cl; 904.7 mg/kg of Fe; 1423.7 mg/kg of Zn; 125 mg/kg of Cu; 610.4 mg/kg of Mn; 14.6 mg/kg of Se; 23.1 mg/kg of Co; 35.9 mg/kg of I; 201.2 KIU of vitamin A; 66.5 IU/kg of vitamin D; 7405.7 IU/kg of vitamin E.

¹³Postpartum mineral-vitamin premix contained (DM basis) 24.5% Ca; 0.02% P; 10.86% Mg; 0.04% K; 0.03% S; 0.56% Na; 0.44% Cl; 227.4 mg/kg of Fe; 17,280.6 mg/kg of Zn; 1444.9 mg/kg of Cu; 12,602.2 mg/kg of Mn; 195.4 mg/kg of Se; 232.4 mg/kg of Co; 399.1 mg/kg of I; 688.9 KIU of vitamin A; 281 IU/kg of vitamin D; 17,997 IU/kg of vitamin E.

¹⁴Rumen-protected methionine, Novus International, Chesterfield, MO.

¹⁵Feed-grade potassium sulfate and magnesium sulfate mineral, Mosaic Global Sales, LLC., Lithia, FL.

¹⁶Rumen-protected methionine, Evonik Industries, Etowah, TN.

¹⁷Larvacide fly control, Central Life Sciences, Schaumburg, IL.

Table 2.2. Chemical composition of experimental diets fed pre- and postpartum and supplemented with or without CMA¹.

Ingredient, % of DM	Prepartum ²		Postpartum ³	
	CON ¹	CMA ¹	CON ¹	CMA ¹
DM, %	43.3 ± 1.9	42.1 ± 4.4	46.1 ± 2.6	45.9 ± 3.3
CP	16.1 ± 1.7	16.8 ± 1.6	16.1 ± 1.7	16.5 ± 1.0
ADF	22.5 ± 2.4	27.1 ± 3.9	24.1 ± 5.4	20.5 ± 2.2
NDF	36.9 ± 3.1	42.0 ± 4.8	37.4 ± 7.2	34.1 ± 3.6
Ash	7.9 ± 1.2	8.8 ± 0.9	8.0 ± 0.8	8.0 ± 0.6
Ca	1.0 ± 0.3	1.3 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
P	0.3 ± 0.04	0.4 ± 0.03	0.3 ± 0.03	0.3 ± 0.03
Mg	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
K	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.2	1.6 ± 0.2
S	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Na	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Cl	0.6 ± 0.2	0.9 ± 0.2	0.6 ± 0.3	0.6 ± 0.1
TDN	68.0 ± 4.0	68.0 ± 4.0	69.5 ± 4.2	69.6 ± 4.1
NE _L , Mcal/lb	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.05	0.7 ± 0.05
NE _M , Mcal/lb	0.8 ± 0.07	0.8 ± 0.07	0.8 ± 0.07	0.8 ± 0.07

¹CON = control diet; CMA = calcareous marine algae partial replacement.

² Values are averaged from analyzed prepartum (n = 36; CON = 18, CMA = 18) TMR composite samples throughout the entire study.

³ Values are averaged from analyzed postpartum (n = 62; CON = 31, CMA = 31) TMR composite samples throughout the entire study.

Table 2.3. Performance of cows fed diets CON¹ or CMA¹ pre- and postpartum from 0 to 30

DIM.

Item	CON ¹	CMA ¹	<i>P</i> -value
DMI, kg/d	19.41 ± 1.24	20.56 ± 1.20	0.19
Yield, kg/d			
Milk	34.60 ± 5.26	34.56 ± 5.23	0.98
Fat	1.62 ± 0.27	1.63 ± 0.27	0.98
Protein	1.00 ± 0.14	1.00 ± 0.14	0.95
Lactose	1.60 ± 0.22	1.53 ± 0.22	0.49
ECM	40.20 ± 6.26	40.43 ± 6.22	0.93
4% FCM	41.81 ± 6.62	41.55 ± 6.58	0.93
Milk Composition, %			
Fat	4.61 ± 0.16	4.60 ± 0.15	0.96
Protein	2.92 ± 0.06	2.85 ± 0.05	0.34
Lactose	4.35 ± 0.08	4.49 ± 0.08	0.29
Milk efficiency, kg/kg			
Milk/DMI	1.81 ± 0.19	1.75 ± 0.19	0.60
ECM/DMI	2.09 ± 0.22	2.03 ± 0.22	0.72
FCM/DMI	2.15 ± 0.24	2.11 ± 0.24	0.78
Colostrum IgG concentration, mg/mL	30.56 ± 2.51	23.78 ± 2.39	0.06
Colostrum quality, %Brix	22.42 ± 2.28	23.24 ± 2.17	0.79
Colostrum volume, kg	7.67 ± 1.11	8.40 ± 1.05	0.64
BW, kg			
1 DIM	639.24 ± 14.4	635.80 ± 13.8	0.90
30 DIM	612.84 ± 14.4	612.23 ± 13.8	0.90
BCS			
1 DIM	3.09 ± 0.16	3.17 ± 0.16	0.49
30 DIM	3.00 ± 0.16	3.04 ± 0.16	0.49
SCC, cells/mL	527,377 ± 286,514	388,097 ± 283,878	0.38

¹CON = control diet, n = 19; CMA = calcareous marine algae partial replacement, n = 21.

Table 2.4. Performance of cows fed diets CON¹ or CMA¹ pre- and postpartum from -21 to 30 DIM under heat stress² and non-heat stress conditions³.

Item	Heat stress ²		Non-heat stress ³		P-value		
	CON ¹	CMA ¹	CON ¹	CMA ¹	TRT	MaxTHI	TRT*MaxTHI
DMI, kg/d	18.64 ± 1.15	19.74 ± 1.11	19.68 ± 2.02	21.40 ± 1.37	0.44	0.16	0.75
Yield, kg/d							
Milk	33.66 ± 5.23	33.58 ± 5.21	34.25 ± 5.87	35.84 ± 5.37	0.76	0.35	0.58
Fat	1.57 ± 0.27	1.57 ± 0.27	1.58 ± 0.31	1.71 ± 0.28	0.69	0.35	0.47
Protein	0.99 ± 0.14	1.00 ± 0.14	1.00 ± 0.15	1.02 ± 0.14	0.87	0.58	0.80
Lactose	1.49 ± 0.22	1.56 ± 0.22	1.51 ± 0.25	1.65 ± 0.23	0.44	0.45	0.65
ECM	39.16 ± 6.23	39.32 ± 6.20	39.54 ± 6.81	41.99 ± 6.37	0.68	0.34	0.47
4% FCM	40.33 ± 6.58	40.50 ± 6.55	40.73 ± 7.31	43.66 ± 6.76	0.65	0.33	0.45
Milk Composition, %							
Fat	4.55 ± 0.14	4.54 ± 0.13	4.59 ± 0.35	4.69 ± 0.21	0.88	0.62	0.76
Protein	2.94 ± 0.05	2.87 ± 0.05	2.94 ± 0.13	2.82 ± 0.08	0.39	0.74	0.73
Lactose	4.34 ± 0.07	4.48 ± 0.12	4.39 ± 0.20	4.48 ± 0.06	0.49	0.81	0.84
Milk efficiency, kg/kg							
Milk/DMI	1.80 ± 0.19	1.74 ± 0.18	1.73 ± 0.27	1.78 ± 0.21	0.96	0.85	0.60
ECM/DMI	2.08 ± 0.22	2.03 ± 0.21	2.00 ± 0.31	2.07 ± 0.24	0.98	0.90	0.63
FCM/DMI	2.14 ± 0.23	2.09 ± 0.23	2.05 ± 0.34	2.15 ± 0.26	0.92	0.94	0.59

¹CON = control diet, n = 19; CMA = calcareous marine algae partial replacement, n = 21.

²Heat stress conditions were max daily temperature humidity index > 68.

³Non-heat stress conditions were max daily temperature humidity index < 68.

Table 2.5. Health events of pre- and postpartum cows fed diets CON¹ or CMA¹ from -21 to 30

DIM.

Item	CON ¹	CMA ¹	<i>P</i> -value
TCD ²	6	6	0.35
Mastitis	1	0	0.35
Other ³	2	5	0.35

¹CON = control diet, n = 19; CMA = calcareous marine algae partial replacement, n = 21.

²TCD = transition cow disorders; retained placenta, metritis, displaced abomasum, vaginal tear and low ketones.

³Other = injury, pinkeye, fever, and digestive issues.

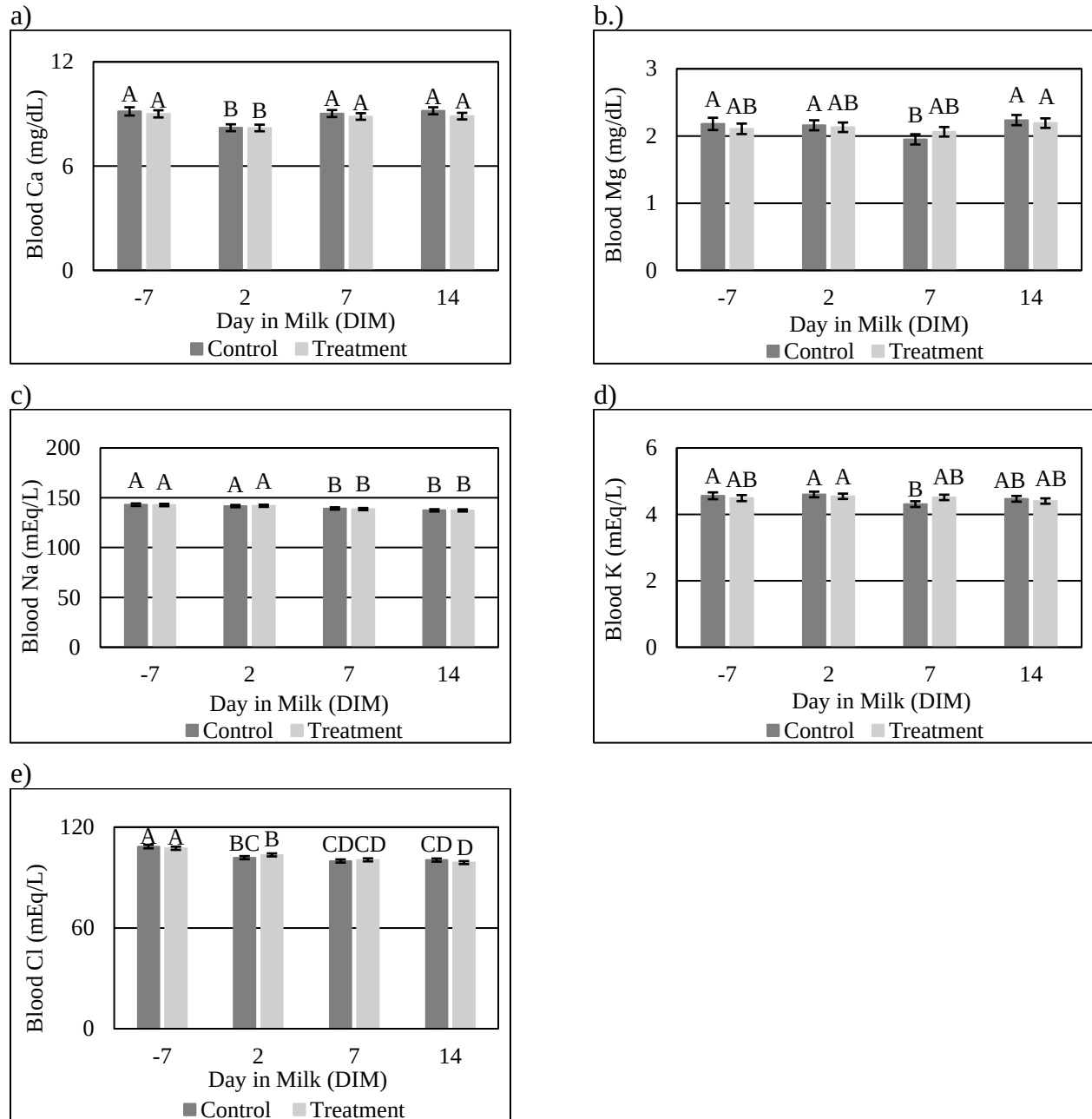


Figure 2.1. Blood metabolite results (a = calcium, mg/dL; b = magnesium, mg/dL, c = sodium, mmol/L, d = potassium, mmol/L, and e = chloride, mmol/L) from 19 control and 21 treatment cows during the prepartum (-7 DIM) and postpartum (2, 7, and 14 DIM) period from May to December 2022. Means within a figure marked with different letters differ significantly ($P \leq 0.05$).

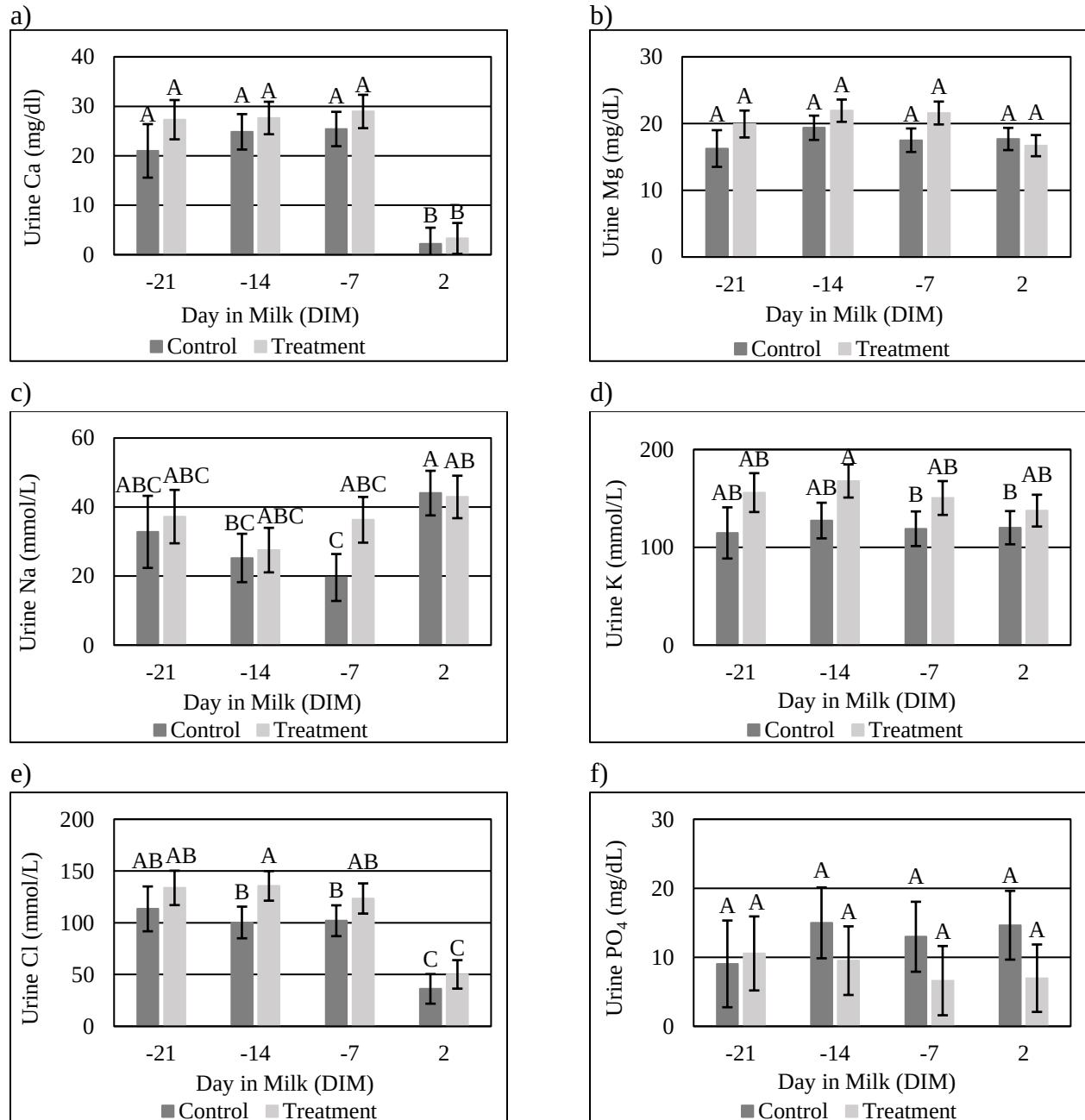


Figure 2.2. Urine metabolite results (a = calcium, mg/dL; b = magnesium, mg/dL, c = sodium, mmol/L, d = potassium, mmol/L, e = chloride, mmol/L, and f = phosphate, mg/dL) from 19 control and 21 treatment cows during the prepartum (-21, -14, and -7 DIM) and postpartum (2 DIM) period from May to December 2022. Means within a figure marked with different letters differ significantly ($P \leq 0.05$).

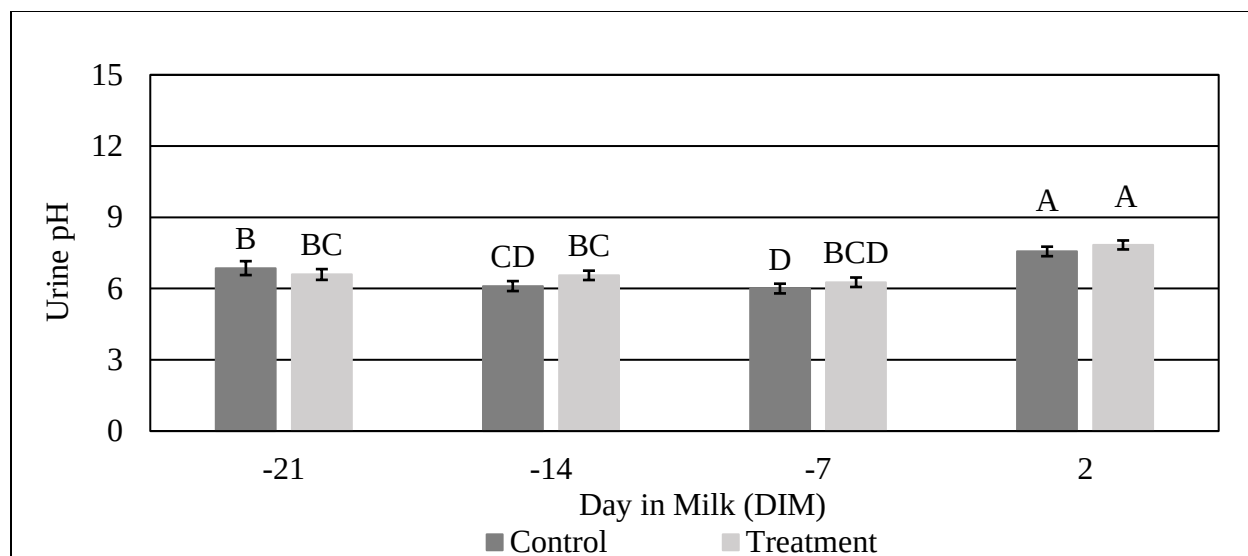


Figure 2.3. Urine pH results from 19 control and 21 treatment cows during the prepartum (-21, -14, and -7 DIM) and postpartum (2 DIM) period from May to December 2022. Means within a figure marked with different letters differ significantly ($P \leq 0.05$).

CHAPTER THREE

Impact of feeding calcareous marine algae to Holstein cows on lactational performance

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ABSTRACT

This study evaluated the production effects of feeding calcareous marine algae (**CMA**) compared to traditional ingredients on lactating Holstein cows. Twenty-five multiparous cows and 8 first-lactation heifers were housed and fed CMA (calcified remains of *Lithothamnion calcareum*) using Calan gates at the East Tennessee Research and Education Center Little River Dairy Unit from 31 to 120 days in milk (**DIM**). Data on dry matter intake (**DMI**), milk production and composition, and production efficiency (**MilkEff**) were collected from 16 treatment cows and 17 control cows, matched with a diet using paired rolling enrollment based on parity and projected calving date. Total mixed rations (**TMR**) (38.2% forage dry matter (**DM**)) were formulated to equalize mineral availability for the partial replacement of calcium carbonate (**CaCO₃**), magnesium oxide (**MgO**), and sodium bicarbonate (**NaHCO₃**) with CMA. The control TMR (**CON**) concentrate portion was formulated to provide 1.5% dietary DM as CaCO₃, 0.3% dietary DM as MgO, and 1.5% DM as NaHCO₃. The TMR concentrate treated with CMA (**CMA**) was formulated to provide 1.2% dietary DM as CaCO₃, 0.4% dietary DM as CMA, 0.2% dietary DM as MgO, and 0.8% dietary DM as NaHCO₃. Data was analyzed using proc GLIMMIX to determine treatment impacts in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The GLIMMIX procedure was used to determine the impact of treatment, maximum ambient temperature humidity index (**MaxTHI**), and projected 305-day mature equivalent milk (**PMEM**). The two-way covariate interactions of TRT by PMEM and MaxTHI by PMEM were removed by stepwise backward elimination if non-significant. Results were significant if $P \leq 0.05$. No differences ($P > 0.05$) in DMI were observed between the experimental diets in mid-lactation (31 to 60 DIM). Under non-heat stress conditions, CMA cows ate more compared to CON (29 vs. 25.3 ± 0.7 kg/d; $P < 0.01$). Treatment MilkEff was decreased compared to CON

under heat stress (1.6 vs. 1.8 ± 0.1 ; $P < 0.01$) and non-heat stress (1.6 vs. 2.0 ± 0.1 ; $P < 0.01$). However, CMA cows maintained ECM efficiency in heat stress compared to non-heat stress conditions (1.8 vs. 1.8 ± 0.2 ; $P = 0.7$) while CON cows reduced MEff during heat stress (1.9 vs. 2.0 ± 0.2 ; $P < 0.01$). Experimental diets did not significantly impact body weight, milk, fat, or protein yield. These results indicated CMA can partially replace CaCO_3 , MgO , and NaHCO_3 in the ration with minimal impacts on performance.

INTRODUCTION

Produced from *Lithothamnion calcareum*, calcareous marine algae (**CMA**; Calmin, Feedworks USA, Ltd., Cincinnati, OH) contains 30% Ca, 0.5% P, 6.0% Mg, 0.1% K, and trace minerals (DM basis). Calcareous marine algae collectively represent several species of calcified red seaweed harvested off Irish and Icelandic coasts. The unique honeycomb structure and large surface area of CMA enables the plant to capture and retain Ca and Mg from seawater (Neville et al., 2022). The high mineral content in the cell walls slowly releases these minerals during digestion (Neville et al., 2022). Cruywagen et al. (2015) depicted CMA as a sparingly water-soluble Ca source and ruminal buffer due to its distinct marine-based physical structure and chemical characteristics. The incorporation of CMA as a supplemental Ca source has demonstrated promising results in preventing rumen depression (Bernard et al., 2014, Cruywagen et al., 2015, Loučka et al., 2024) to support the productivity and health of dairy cows. Additionally, CMA has been shown to maintain and exceed milk yield and other production parameters (Cruywagen et al., 2015, Wu et al., 2015, Neville et al., 2022). Compared to the cows on the control treatment, the CMA treatment produced more milk per kilogram of DMI (Cruywagen et al., 2015). Bernard et al. (2014) found improved efficiency for CMA-fed cows compared to sodium bicarbonate control during the final 3 wk of the trial. These literature

indicated that adding CMA effectively supported milk production and component yield at levels equivalent to or exceeding traditional mineral sources.

Limited research exists on the impact of CMA on the health and production performance of lactating cows under natural heat stress conditions, particularly in the southeast. Therefore, this study hypothesized that the effects of feeding CMA will meet or outperform traditional sources of CaCO_3 , MgO , and NaHCO_3 . The objective was to evaluate the effects of feeding CMA on post-partum milk production and efficiency from 31 to 120 DIM compared with traditional sources of CaCO_3 , MgO , and NaHCO_3 under heat stress and non-heat stress conditions.

MATERIALS AND METHODS

Animals and housing

This study was a continuation of the work outlined in Foley (2024) focused on 31 to 120 d post-partum. In brief, from May to December 2022 primiparous and multiparous Holstein cows were used in a feeding trial beginning 21 d pre-partum through 120 d postpartum. Cows were housed at the East Tennessee Research and Education Center Little River Dairy (**LRD**). Detailed Calan door training and enrollment protocols can be found in Foley (2024). Forty-nine cows were successfully trained and enrolled in the study. Cows were sorted into parity groups (**ParG**). Parity 1 included primiparous heifers, parity 2 included second-lactation cows, and parity 3+ included third- and fourth-lactation cows. All protocols were approved by the University of Tennessee, Knoxville Institutional Animal Care and Use Committee under protocol #2842-0621.

Experimental diets

The supplemental rates of CMA (calcified remains of *Lithothamnion calcareum*; Feedworks USA, Ltd.) were 100 g/d, according to manufacturer recommendations (Table 3.1).

Postpartum TMR (Table 3.1; 38.2% forage DM) was formulated to partially replace CaCO_3 , MgO , and NaHCO_3 with CMA. The CON TMR was formulated to provide 1.5% DM CaCO_3 , 0.3% DM MgO , and 1.5% DM NaHCO_3 in the lactating diet. The CMA TMR was formulated with 0.4% DM CMA in the lactating diet (0.3% CaCO_3 , 0.1% MgO , and 0.7% NaHCO_3 DM replaced, respectively).

Feeding management

Detailed feed management information can be found in Foley (2024). In brief, basal forage diets were mixed daily (VSL 150 Single-Auger Vertical Mixer, Kuhn North America, Brodhead, WI) with treatment grain premixes (Table 3.1) added to forage ingredients during final mixing using a Calan Data Ranger (American Calan Inc., Northwood, NH) to individually deliver TMR diets to Calan Doors. The ration for postpartum cows was balanced at 28.33 kg DM per cow per d (20.01 kg DM forage and 8.32 kg DM grain (CON or CMA)). Cows were fed once daily in amounts to provide a minimum of 5% refusal and had ad libitum access to water. The required amount of forage and grain was calculated daily based on the number of cows enrolled. From May 28 to September 26, 2022, postpartum CON and CMA cows were fed at 16:00. After the last cow in the study calved on September 26, 2022, CMA lactating cows were fed at 08:00, and CON lactating cows were fed at 16:00. The amount of feed offered and orts were recorded daily.

Data collection

The data collected throughout this study included DMI, BW, BCS, colostrum quality and quantity, milk yield and components, blood metabolites, urine metabolites, health events, ambient temperature and humidity, and TMR diet sampling.

Orts. The amount of feed offered was recorded at each feeding and adjusted daily to provide a minimum 5% refusal based on orts. Any feed remaining within the bin was designated as refused by the cow. The rejected feed from each cow was transferred into an empty plastic container, which was positioned on a calibrated small-scale device (Smart Weigh Digital Heavy Duty Shipping, and Postal Scale) zeroed to account for the weight of the plastic container, facilitating accurate weight recording. Orts were recorded before the 08:00 and 16:00 feedings.

Body weight and body condition score. Body weight and BCS were recorded on 60, 90, and 120 DIM. Body weight was recorded in the afternoon after the 05:00 milking with a walk-over livestock scale. Body condition scoring was conducted to evaluate the relative amount of subcutaneous body fat using the 5-point scale in 0.25 increments according to Elanco Animal Health (1997), where 1 = emaciated, 2 = thin, 3 = moderate, 4 = borderline obese, and 5 = extremely obese. A single, trained individual scored all BCS values to prevent discrepancies.

Milk yield and components. After parturition, cows were milked two times daily, at 05:00 and 17:00. Daily milk yields were recorded electronically by BouMatic (BouMatic LLC, Madison, WI) and SmartDairy parlor system (Valley Agricultural Software, Tulare, CA) with the PCDart Interface (Dairy Records Management Systems, Raleigh, NC). Milk samples were collected once per week at 05:00 and 17:00 milkings. Samples were taken to the University of Tennessee Precision Dairy Quality Lab (Knoxville, TN) for analysis of fat, protein, lactose, SNF, and SCC concentrations by mid-infrared spectrophotometric analysis using a Bentley DairySpec Combi instrument as described by AOAC International (2000).

Health events. Health events were recorded and reported by LRD staff. Recorded health events were categorized as transition cow diseases. Transition cow disorders (**TCD**) included the incidence of RP, metritis, DA, vaginal tear, and low ketone levels. Somatic cell count and

clinical signs of mastitis were monitored throughout the study to indicate infection. Other events included fever, pinkeye, digestive issues, and injury. The study design did not intentionally induce incidence of transition cows diseases, such as SARA or hypocalcemia. Post-partum routine supportive therapies were still applied after calving according to LRD farm protocols.

Ambient temperature and relative humidity Ambient conditions of the freestall barn were recorded using Hobo Pro V2 loggers (Onset Computer Corporation, Bourne, MA). Four loggers were evenly placed throughout the project pen. All loggers recorded the ambient temperature, relative humidity, and dew point every 15 minutes. Data from loggers was collected every 2 wk.

Forage sampling. Samples of CON and CMA TMR diets were collected three times each week. Weekly samples were combined into a weekly composite sample by using a subsample from each level of a Penn State Shaker Box. Composite samples were shipped to Cumberland Valley Analytical Services (Waynesboro, PA) for DM and wet chemistry analysis.

Statistical analysis

Data cleaning. Detailed data cleaning was described in Foley (2024). After data cleaning, 19 CON cows and 21 CMA cows remained in the dataset. Parity 1 included nine primiparous heifers (CON = 4; CMA = 5), parity 2 included 19 second-lactation cows (CON = 10; CMA = 9), and parity 3+ included 12 third- and fourth-lactation cows (CON = 5; CMA = 7).

Thirty-three cows (CON = 17; CMA = 16) were enrolled in the study until 120 DIM and seven cows (CON = 2; CMA = 5) were enrolled until 90 DIM. The breakdown of these seven animals across treatment and parity groups were 1 primiparous CMA heifer, 1 second lactation CMA cow, 1 second lactation CON cow, 3 third lactation CMA cows, and 1 third lactation CON cow. Data reflected in this chapter only includes cows that remained in the study until 120 DIM.

Dry matter intake and milk production. The independent variables of DMI, milk yield (**MilkYield**), milk fat (**MilkFat**), milk protein (**MilkProt**), milk efficiency (**MilkEff**), ECM, and FCM were analyzed for the fixed effects of treatment group (**TRT**), max daily temperature humidity index (**MaxTHI**), and projected 305 d mature equivalent milk (**PMEM**). Ambient temperature and relative humidity data captured from the Hobo Pro V2 loggers created the MaxTHI. If $\text{MaxTHI} \geq 68$, heat stress occurred, and if $\text{MaxTHI} < 68$, then heat stress did not occur. The values for PMEM were captured by PCDart. To calculate individual DMI for each feeding, the ort amount was subtracted from the initial amount offered. The DMI for each cow was calculated by multiplying the individual intake by the DM content of the forage, which was determined by wet chemistry analysis. Orts were calculated using the Eq. 3.1:

$$\text{Intake (kg/cow/d)} = \text{Feed offered} - \text{Ort} \quad \text{Equation 3.1}$$

Dry matter intake was calculated using the Eq. 3.2:

$$\text{DMI (kg/cow/d)} = \text{Intake} * \% \text{DM} \quad \text{Equation 3.2}$$

Milk fat and protein percentages were transformed using the arcsine transformation to approximate the normal distribution using Eq. 3.3 and 3.4. Means were backtransformed to the original scale for presentation:

$$\text{Fatarc} = \arcsin\left(\frac{\sqrt{\text{MilkFat}(\%)}}{100}\right) \quad \text{Equation 3.3}$$

$$\text{Protarc} = \arcsin\left(\frac{\sqrt{\text{MilkProtein}(\%)}}{100}\right) \quad \text{Equation 3.4}$$

Values for ECM and 4% FCM were calculated using the Eq 3.5 (Tyrrell and Reid, 1965) and Eq 3.6 (Gaines and Davidson, 1923):

$$\text{ECM (kg/cow/d)} = (0.327 * \text{MilkYield(kg)}) + (12.95 * \text{MilkFat(kg)}) + (7.65 * \text{MilkProtein(kg)}) \quad \text{Equation 3.5}$$

$$4\% \text{ FCM (kg/cow/d)} = (0.4 * \text{MilkYield(kg)}) + (16.216 * \text{MilkFat(kg)}) \quad \text{Equation 3.6}$$

The MilkEff ratio was calculated using Eq 3.7:

$$\text{MilkEff} = \text{MilkYield(kg)} \div \text{DMI(kg)} \quad \text{Equation 3.7}$$

The random effects of ParG, the interaction of TRT by parity group, and cow nested within the interaction of TRT by parity group were included in model notation. Covariates included in the model were the interaction of TRT by PMEM and the interaction of MaxTHI by PMEM. Model notation (Eq. 3.8):

$$Y_{ijk} = \mu + T_i + P_j + M_k + u_l + e_{ijkl} \quad \text{Equation 3.8}$$

where Y = observation of DMI, MilkYield, MilkFat, MilkProt, MilkEff, ECM, FCM, and SCC; μ = the overall mean; T = TRT; P = PMEM; M = MaxTHI; u = random effects of ParG, the interaction of TRT by ParG, and cow nested within the interaction of TRT by ParG; and e = the residual effect for DIM week nested in cow, TRT, ParG, and MaxTHI.

To evaluate the effect of heat stress days vs non-heat stress days, the interaction of TRT by MaxTHI was included as an additional fixed effect in model notation using Eq. 3.9:

$$Y_{ijk} = \mu + T_i + P_j + M_k + (TM)_{ik} + u_l + e_{ijkl} \quad \text{Equation 3.9}$$

where Y = observation of DMI, MilkYield, MilkFat, MilkProt, MilkEff, ECM, FCM, and SCC; μ = the overall mean; T = TRT; P = PMEM; M = MaxTHI; (TM) = the interaction of TRT by MaxTHI; u = random effects of ParG, the interaction of TRT by ParG, and cow nested within the interaction of TRT by ParG; and e = the residual effect for DIM week nested in cow, TRT, ParG, and MaxTHI.

Body weight and body condition score. To analyze BW and BCS, the fixed effects of TRT, DIM of sample collection, and the interaction of TRT by DIM of sample collection were included in Model 3 with the random effects of ParG, the interaction of TRT by ParG, and cow

nested within the interaction of TRT by ParG in model notation. Covariates included in the model were initial BW or initial BCS, MaxTHI, PMEM, the interaction of TRT by PMEM and the interaction of MaxTHI by PMEM. Model notation (Eq. 3.10):

$$Y_{ij} = \mu + T_i + D_j + (TD)_{ij} + u_k + e_{ijk} \quad \text{Equation 3.10}$$

where Y = observation of BW and BCS; μ = the overall mean; T = TRT; D = DIM of sample collection; TD = interaction of TRT by DIM of sample collection; u = random effects of ParG, the interaction of TRT by ParG, and cow nested within the interaction of TRT by ParG; and e = the residual effect.

The GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with the ILINK option was used to obtain solutions for all independent variables previously described. An LSMeans statement was included in the model to describe significance between treatment groups. Results were significant if $P \leq 0.05$ and had tendency if $P \leq 0.10$. Non-significant covariates were removed with stepwise backward elimination. The FREQ procedure of SAS 9.4 with chi-square analysis and Fisher's exact test was used to analyze the interaction of TRT by health event. Results were significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

Dry matter intake

The chemical composition of the experimental diets is presented in Table 3.2. Dry matter intake is presented in Table 3.3. The DMI for CMA cows was 27.2 ± 0.68 kg/d compared to 25.7 ± 0.70 kg/d for CON cows, though this difference was not statistically significant ($P = 0.27$). These results agree with Wu et al. (2015) who saw no DMI differences postpartum feeding CMA versus sodium bicarbonate control, and Cruywagen et al. (2015) who saw no statistically significant differences in lactating DMI between CMA, sodium bicarbonate, and limestone diets. Meanwhile, Neville et al. (2022) observed increased prepartum and postpartum DMI. Basal diet differences must be considered to accurately compare this experiment to Neville et al. (2022). Neville et al. (2022) compared a control diet containing limestone to one containing CMA. No sodium bicarbonate was included in either experimental diet. In the current study, CON contained 1.5% and CMA contained 0.8% sodium bicarbonate in the postpartum diets. The inclusion of sodium bicarbonate has been demonstrated to raise DMI compared to control diets (Neville et al., 2019) and compared to CMA-containing diets in early lactation (Bernard et al., 2014). Including sodium bicarbonate in both experimental diets may contribute to comparable DMI between groups. It should also be noted that Neville et al. (2022) trial ended at 42 DIM and CON group may have experienced SARA, thus lower DMI due to no inclusion of sodium carbonate buffer. Calcareous marine algae has been shown to have buffering effect (Cruywagen et al., 2015), so CMA cows likely experienced less incidence of SARA and more rumen motility for high DMI.

Milk production

Postpartum milk yield and composition are presented in Table 2.3. No difference in milk yield was observed between the treatment diets ($P = 0.46$). The cows fed CMA produced 42.6 ± 4.35 kg/d and those fed CON produced 43.7 ± 4.35 kg/d. Both groups had identical fat yields at 1.86 ± 0.21 kg/d ($P = 0.98$). No differences in protein ($P = 0.66$), lactose ($P = 0.75$), ECM ($P = 0.78$), and 4% FCM ($P = 0.81$) between treatment diets. No difference in milk composition percentages of fat ($P = 0.60$), protein ($P = 0.86$), and lactose ($P = 0.34$) between treatments. Somatic cell count, an indicator of milk quality, was numerically lower for CMA cows at $130,431 \pm 46,211$ cells/mL compared to $222,079 \pm 44,986$ cells/mL for CON cows, though this difference was not statistically significant ($P = 0.39$).

The impact of CMA on milk production has been studied in early lactation (14 to 100 DIM) (Bernard et al., 2014, Wu et al., 2015, Neville et al., 2022), peak lactation (45 to 90 DIM) (Loučka et al., 2024), and mid-lactation (100 to 200 DIM) cows (Cruywagen et al., 2015). These studies differed in their findings related to milk production and milk composition. Bernard et al. (2014) compared three treatment groups including either 87 g/d of CMA, 204 g/d of sodium bicarbonate, or no supplement. No differences were observed for milk yield, component yield, or ECM. However, cows fed the non-supplemental diet had higher milk protein composition ($P = 0.08$) than those fed the CMA-containing and sodium bicarbonate diets (Bernard et al., 2014). Wu et al. (2015) compared prepartum diets supplemented with calcium carbonate or 50 g/d of CMA and postpartum diets supplemented with 317 g/d of sodium bicarbonate and calcium carbonate or 100 g/d of CMA. No differences were observed among treatments in milk yield, percentage of milk fat, protein, and lactose. Milk protein yield was higher ($P = 0.05$) for the cows fed CMA prepartum compared with those fed calcium carbonate prepartum, at 1.09 and

1.02 ± 0.04 kg/d respectively. Cows fed CMA postpartum supported similar milk yield ($P = 0.99$), and percentage of fat ($P = 0.54$), lactose ($P = 0.80$), and SNF ($P = 0.72$) as the sodium bicarbonate and calcium carbonate postpartum diet (Wu et al., 2015). Cruywagen et al. (2015) observed cows fed the CMA-supplemented diet had improved milk yield ($P = 0.006$), milk fat yield ($P < 0.001$), protein yield ($P = 0.003$), lactose yield ($P = 0.003$), ECM yield ($P < 0.001$), and 4% FCM yield ($P < 0.001$) compared to sodium bicarbonate and limestone diets. Although CMA treatment experienced increased milk fat percentage ($P = 0.014$), no milk protein or lactose composition differences ($P = 0.399$ and $P = 0.838$, respectively) were associated with treatment diet (Cruywagen et al., 2015). This difference in milk production parameters was likely due to CON cows experiencing extended periods of rumen pH below 5.5 (13.8 hr/d) and exhibiting signs of SARA, such as panting and drooling, during the study. It is widely recognized that SARA has a detrimental impact on milk production. Stone (1999) observed that SARA was linked to a 3 kg/d decrease in milk production, reduced milk fat from 37 to 34 g/kg and protein from 39 to 28 g/kg. Similarly, Kitkas et al. (2019) recorded a 0.22% decrease in milk fat content associated with SARA. In the most recent CMA trial, Loučka et al. (2024) observed no difference ($P > 0.05$) in milk production parameters at peak lactation.

Milk, ECM, and 4% FCM efficiencies are presented in Table 2.3. Milk efficiency, measured as milk yield/DMI (kg/kg), was not significantly different ($P = 0.27$) between treatment groups, 1.63 ± 0.15 for CMA cows and 1.76 ± 0.15 for CON cows. The ECM/DMI and FCM/DMI ratios were also not significant ($P = 0.32$ and $P = 0.34$, respectively) between treatment groups with CMA cows at 1.81 ± 0.71 and 1.90 ± 0.19, respectively, compared to 1.93 ± 0.71 and 2.0 ± 0.19 for CON cows. No difference occurred between efficiency values for milk, ECM, or 4% FCM per kg of DMI. This agrees with the results of Wu et al. (2015). Conversely,

Bernard et al. (2014) observed improved efficiency ($P = 0.008$) in cows fed CMA compared to those on the sodium bicarbonate diet, but only during the last three weeks of the trial. The authors attributed this to supplemental sodium bicarbonate having increased DMI during the latter weeks of the trial, thus reducing the efficiency of milk production compared with CMA. Since this trial ended at 98 DIM, longer trials were necessary to validate these observations and identify underlying reasons for the improved efficiency (Bernard et al., 2014). According to Cruywagen et al. (2015), CMA supplementation of 90 g/d in TMR improved feed efficiency for milk production ($P = 0.006$) and composition ($P < 0.001$) compared to sodium bicarbonate and limestone diets. As previously stated, CON cows on this trial experienced symptoms of SARA, thus reducing milk production compared to cows supplemented with CMA. This likely contributed to CMA appearing more efficient than CON cows (Cruywagen et al., 2015). In the current trial, the partial replacement of CMA was as effective as CON for maintaining milk yield and composition from 30 to 120 DIM.

Production parameters under heat stress. The performance of lactating cows under heat stress and non-heat stress conditions is presented in Table 3.4. Under non-heat stress conditions, CMA cows had a significantly higher DMI of 29.0 ± 0.72 kg/d compared to 25.4 ± 0.71 kg/d for CON cows ($P < 0.01$). During heat stress, there was no significant difference ($P = 0.14$) in DMI between the two groups, with CMA cows consuming 26.1 ± 0.68 kg/d and CON cows consuming 25.2 ± 0.66 kg/d.

Milk yield followed a similar pattern. The differences in milk yield across treatments was not significant ($P = 0.58$) but was significantly affected by MaxTHI ($P < 0.0001$). In non-heat stress conditions, both groups showed increased milk yield, with CON cows producing 44.1 ± 4.37 kg/d and CMA cows producing 43.8 ± 4.36 kg/d. During heat stress, milk yield was slightly

lower for both groups, with CON cows yielding 42.9 ± 4.36 kg/d and CMA cows yielding 41.6 ± 4.36 kg/d.

For milk composition, fat yield was consistent across both heat stress and non-heat stress conditions, with no significant differences between CON and CMA cows ($P = 0.98$). Protein yield was higher in non-heat stress conditions for both groups, with CON cows at 1.26 ± 0.09 kg/d and CMA cows at 1.29 ± 0.09 kg/d, compared to 1.15 ± 0.09 kg/d and 1.12 ± 0.09 kg/d during heat stress, respectively ($P < 0.0001$). The difference in protein yield between treatments was not significant ($P = 0.95$) but was significantly affected by MaxTHI ($P < 0.0001$). Similarly, lactose yield increased during non-heat stress, with CON cows at 2.02 ± 0.17 kg/d and CMA cows at 2.03 ± 0.17 kg/d, versus 1.93 ± 0.17 kg/d and 1.90 ± 0.17 kg/d during heat stress ($P = 0.88$ for TRT, $P < 0.0001$ for MaxTHI). Energy-corrected milk was also higher for CMA cows in non-heat stress conditions. The ECM for CON cows was 49.07 ± 4.87 kg/d and for CMA cows was 49.17 ± 4.87 kg/d, compared to 46.03 ± 4.86 kg/d and 45.29 ± 4.87 kg/d during heat stress ($P = 0.87$ for TRT, $P < 0.0001$ for MaxTHI). No difference in 4% FCM between treatments or heat stress versus non-heat stress conditions ($P = 0.86$).

Milk composition percentages showed that protein and lactose percentages were higher in non-heat stress conditions. CON cows had protein percentages of $2.91 \pm 0.07\%$ and CMA cows had $2.97 \pm 0.07\%$ in non-heat stress, compared to $2.74 \pm 0.07\%$ and $2.71 \pm 0.07\%$ during heat stress ($P = 0.83$ for TRT, $P < 0.01$ for MaxTHI). Lactose percentages increased to $4.63 \pm 0.08\%$ for CON and $4.68 \pm 0.08\%$ for CMA in non-heat stress, from $4.54 \pm 0.08\%$ and $4.61 \pm 0.08\%$ during heat stress ($P = 0.38$ for TRT, $P < 0.01$ for MaxTHI).

Milk efficiency, measured as milk yield/DMI (kg/kg), was higher for CON cows during non-heat stress (1.81 ± 0.15) compared to CMA cows (1.59 ± 0.15 ; $P < 0.01$ for the interaction

of TRT by MaxTHI, $P = 0.20$ for TRT, $P = 0.88$ for MaxTHI). The interaction of TRT by MaxTHI was significant ($P < 0.0001$) for ECM/DMI and FCM/DMI, with higher ratios for CON cows during non-heat stress conditions. This indicated better efficiency in CON cows converting feed to milk compared to CMA cows during non-heat stress periods.

Body weight and body condition score

Body weight and BCS are presented in Table 3.5. No differences were observed among treatments in BW or BCS throughout the trial. Body weight at 60, 90, and 120 DIM was not different ($P = 0.45$) between CON and CMA. At 60 DIM, BW was 603.2 ± 11.7 kg for CMA cows and 622.9 ± 11.3 kg for CON cows. At 90 DIM, BW was 614.6 ± 11.7 kg for CMA and 621.1 ± 11.3 kg for CO. At 120 DIM, BW was 620.07 ± 11.7 kg for CMA cows and 637.6 ± 11.4 kg for CON cows. Bernard et al. (2014) also found no differences in BW among the treatment groups. However, BW values were slightly higher than those in our trial (660 kg average across treatments). Both Bernard et al. (2014) and our trial studied primiparous and multiparous, so it is unclear why there is such a large discrepancy in weight. Herd management differences could account for the increased mean BW of cows in Bernard's study compared to our current study, which could explain why both studies reported no differences between treatment groups. Body condition score was also not significantly different between CON and CMA cows ($P = 0.39$). At 60 DIM, BCS was 3.07 ± 0.16 for CMA cows and 2.97 ± 0.16 for CON cows. At 90 DIM, BCS was 3.13 ± 0.16 for CMA and 3.04 ± 0.16 for CON. At 120 DIM, BCS was 3.20 ± 0.16 for CMA cows and 3.13 ± 0.16 for CON cows. The optimum BCS for 60 to 120 DIM was between 2.25 and 3.5 (Elanco Animal Health, 1997). Both treatments maintained BCS within the optimum range throughout the trial. Treatments maintained healthy BW and BCS from 60 to 120 DIM.

Health events

There were not enough health event observations in the postpartum period to run an analysis. Only two incidences of other diseases were recorded for CON and none for CMA.

LIMITATIONS

The study utilized a single Calan Data Ranger for both experimental diets (CON and CMA). The barrel was fully emptied before switching diets to prevent cross-contamination, which likely did not significantly affect the study results. Some cows stole feed, primarily because older, taller cows bullied heifers and newly enrolled cows away from their designated doors. Additionally, the low-set Calan Doors, designed for larger-headed cows, made it easier for others to reach in and steal feed. Despite attempts to separate these groups, challenges persisted due to the rolling enrollment and difficulty retraining cows to new door locations. Some Calan Doors did not fully close and lock, allowing manipulation by cows. To mitigate this, door springs were tightened for quicker closure. The authors noted that feeding behavior might have been affected by changes in feeding times during the study.

CONCLUSIONS

In conclusion, this study demonstrated that partially replacing traditional mineral sources with CMA in the diets of lactating cows under heat stress conditions did not significantly affect DMI, milk yield, or milk composition. Both treatment groups maintained similar levels of milk production and component yields, with no notable differences in SCC or BCS. While CMA-fed cows exhibited slightly higher DMI during non-heat stress conditions, overall milk efficiency and body weight remained consistent across treatments. These findings suggest that CMA can be an effective alternative to traditional mineral sources without compromising the health and productivity of dairy cows.

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APPENDIX B

Table 3.1. Balanced ration ingredients (%DM) for pre- and postpartum diets for CON¹ or CMA¹.

Ingredient	CON ¹	CMA ¹
Corn silage	28.50	28.50
Oat silage	9.69	9.69
Ground corn	22.48	22.48
Soybean hull pellets	8.33	8.33
Cottonseed hulls		0.65
Hominy feed	4.41	4.41
Soybean meal, 48% CP	7.49	7.49
SoyPlus ²	5.65	5.65
Roasted soybeans	6.26	6.26
CMA ³		0.37
Calcium carbonate* ⁴	1.50	1.20
Magnesium oxide*	0.33	0.28
Sodium bicarbonate*	1.50	0.83
Di calcium phosphate	0.13	0.13
Potassium chloride	0.50	0.50
K-Carb plus ⁵	0.33	0.33
White salt (NaCl)	0.23	0.23
Spectrum fusion ⁶	1.25	1.25
Molasses	0.48	0.48
Urea	0.50	0.50
RA-CCF AC Trace PMX 12-21 ⁷	0.22	0.22
Aliment (MHA) ⁸	0.10	0.10
Dynamate ⁹	0.07	0.07
ClariFly ¹⁰	0.03	0.03
Total Concentrate	61.81	61.81
Total Forage	38.19	38.19

*Ingredient differences between CON and CMA diets.

¹CON = control diet; CMA = calcareous marine algae partial replacement.

²Soybean-based bypass protein, Landus, Des Moines, IA.

³Calcareous marine algae, Calmin, Feedworks USA, Ltd., Cincinnati, OH.

⁴Inorganic calcium salt.

⁵Feed-grade protected granular potassium carbonate, K-Carb Plus, Woodbury, MN.

⁶Fatty acid supplement, Perdue AgriBusiness, LLC., Salisbury, MD.

⁷Mineral-vitamin premix contained (DM basis) 24.5% Ca; 0.02% P; 10.86% Mg; 0.04% K; 0.03% S; 0.56% Na; 0.44% Cl; 227.4 mg/kg of Fe; 17,280.6 mg/kg of Zn; 1444.9 mg/kg of Cu; 12,602.2 mg/kg of Mn; 195.4 mg/kg of Se; 232.4 mg/kg of Co; 399.1 mg/kg of I; 688.9 KIU of vitamin A; 281 IU/kg of vitamin D; 17,997 IU/kg of vitamin E.

⁸Rumen-protected methionine, Novus International, Chesterfield, MO.

⁹Feed-grade potassium sulfate and magnesium sulfate mineral, Mosaic Global Sales, LLC., Lithia, FL.

¹⁰Larvacide fly control, Central Life Sciences, Schaumburg, IL.

Table 3.2. Chemical composition of experimental diets fed postpartum and supplemented with or without CMA¹. Values are averaged from analyzed postpartum TMR composite samples (n = 62, CON = 31, CMA = 31) throughout the entire study.

Ingredient, % of DM	CON ¹	CMA ¹
DM, %	46.1 ± 2.6	45.9 ± 3.3
CP	16.1 ± 1.7	16.5 ± 1.0
ADF	24.1 ± 5.4	20.5 ± 2.2
NDF	37.4 ± 7.2	34.1 ± 3.6
Ash	8.0 ± 0.8	8.0 ± 0.6
Ca	1.0 ± 0.2	1.0 ± 0.2
P	0.3 ± 0.03	0.3 ± 0.03
Mg	0.5 ± 0.1	0.4 ± 0.1
K	1.4 ± 0.2	1.6 ± 0.2
S	0.3 ± 0.1	0.2 ± 0.1
Na	0.3 ± 0.1	0.4 ± 0.1
Cl	0.6 ± 0.3	0.6 ± 0.1
TDN	69.5 ± 4.2	69.6 ± 4.1
NE _L , Mcal/lb	0.7 ± 0.05	0.7 ± 0.05
NE _M , Mcal/lb	0.8 ± 0.07	0.8 ± 0.07

¹CON = control diet; CMA = calcareous marine algae partial replacement.

Table 3.3. Performance of lactating cows fed diets CON¹ or CMA¹ from 31 to 120 DIM.

Item	CON ¹	CMA ¹	<i>P</i> -value
DMI, kg/d	25.72 ± 0.70	27.21 ± 0.68	0.27
Yield, kg/d			
Milk	43.66 ± 4.35	42.57 ± 4.35	0.46
Fat	1.86 ± 0.21	1.86 ± 0.21	0.98
Protein	1.22 ± 0.09	1.20 ± 0.09	0.66
Lactose	1.98 ± 0.17	1.96 ± 0.17	0.75
ECM	47.67 ± 4.85	47.11 ± 4.86	0.78
4% FCM	49.05 ± 5.30	48.53 ± 5.30	0.81
Milk Composition, %			
Fat	4.27 ± 0.11	4.37 ± 0.11	0.60
Protein	2.84 ± 0.07	2.83 ± 0.07	0.86
Lactose	4.58 ± 0.08	4.65 ± 0.08	0.34
Milk efficiency, kg/kg			
Milk/DMI	1.76 ± 0.15	1.63 ± 0.15	0.27
ECM/DMI	1.93 ± 0.71	1.81 ± 0.71	0.32
FCM/DMI	1.99 ± 0.19	1.86 ± 0.19	0.34

¹CON = control diet, n = 18; CMA = calcareous marine algae partial replacement, n = 16.

Table 3.4. Performance of lactating cows fed diets CON¹ or CMA¹ from 31 to 120 DIM under heat stress² and non-heat stress³ conditions. Means within a row with different superscripts differ ($P \leq 0.05$).

Item	Heat stress ²		Non-heat stress ³		P-value		
	CON ¹	CMA ¹	CON ¹	CMA ¹	TRT	MaxTHI	TRT*MaxTHI
DMI, kg/d	25.25 ± 0.66 ^b	26.11 ± 0.68 ^b	25.36 ± 0.71 ^b	29.03 ± 0.72 ^a	0.14	<0.0001	<0.0001
Yield, kg/d							
Milk	42.91 ± 4.36 ^{bc}	41.59 ± 4.36 ^c	44.10 ± 4.37 ^a	43.84 ± 4.36 ^{ab}	0.58	<0.0001	0.01
Fat	1.79 ± 0.21	1.79 ± 0.21	1.93 ± 0.21	1.93 ± 0.21	0.98	<0.0001	0.95
Protein	1.15 ± 0.09 ^b	1.12 ± 0.09 ^b	1.26 ± 0.09 ^a	1.29 ± 0.09 ^a	0.95	<0.0001	<0.0001
Lactose	1.93 ± 0.17 ^{ab}	1.90 ± 0.17 ^b	2.02 ± 0.17 ^a	2.03 ± 0.17 ^a	0.88	<0.0001	0.154
ECM	46.03 ± 4.86 ^{ab}	45.28 ± 4.87 ^b	49.07 ± 4.87 ^a	49.17 ± 4.87 ^a	0.87	<0.0001	0.13
4% FCM	47.58 ± 5.30	46.95 ± 5.31	50.39 ± 5.32	50.23 ± 5.32	0.86	<0.0001	0.45
Milk composition, %							
Fat	4.19 ± 0.11	4.30 ± 0.11	4.38 ± 0.12	4.42 ± 0.12	0.68	<0.0001	0.28
Protein	112.74 ± 0.07 ^b	2.71 ± 0.07 ^b	2.91 ± 0.07 ^a	2.97 ± 0.07 ^a	0.83	<0.0001	<0.0001
Lactose	4.54 ± 0.08 ^c	4.61 ± 0.08 ^{bc}	4.63 ± 0.08 ^{ab}	4.68 ± 0.08 ^a	0.38	0.0006	0.63
Milk efficiency, kg/kg							
Milk/DMI	1.75 ± 0.15 ^{ab}	1.64 ± 0.15 ^{ab}	1.81 ± 0.15 ^a	1.59 ± 0.15 ^b	0.20	0.88	0.006
ECM/DMI	1.89 ± 0.17 ^b	1.79 ± 0.17 ^b	2.02 ± 0.17 ^a	1.78 ± 0.17 ^b	0.22	0.01	0.002
FCM/DMI	1.95 ± 0.19 ^b	1.86 ± 0.19 ^b	2.08 ± 0.19 ^a	1.82 ± 0.19 ^b	0.23	0.10	0.001

¹CON = control diet, n = 18; CMA = calcareous marine algae partial replacement, n = 16.

²Heat stress conditions were max daily temperature humidity index > 68.

³Non-heat stress conditions were max daily temperature humidity index < 68.

Table 3.5. Body weight and BCS of lactating cows fed diets CON¹ or CMA¹ from 31 to 120

DIM.

Item	CON ¹	CMA ¹	<i>P</i> -value
BW, kg			
60 DIM	622.87 ± 11.34	603.20 ± 11.70	0.45
90 DIM	621.15 ± 11.34	614.59 ± 11.70	0.45
120 DIM	637.63 ± 11.42	620.07 ± 11.70	0.45
BCS			
60 DIM	2.97 ± 0.16	3.07 ± 0.16	0.39
90 DIM	3.04 ± 0.16	3.13 ± 0.16	0.39
120 DIM	3.13 ± 0.16	3.20 ± 0.16	0.39
SCC, cells/mL	222,079 ± 44,986	130,431 ± 46,211	0.39

¹CON = control diet, n = 18; CMA = calcareous marine algae partial replacement, n = 16.

CHAPTER FOUR

Novel brown midrib male sterile sorghum as an alternative silage crop in the Southeast

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ABSTRACT

The nutritional content of male sterile brown midrib sorghum (**BMR**) and corn harvested at different stages of maturity was evaluated using wet chemistry (**WC**) and near-infrared spectroscopy (**NIRS**) to determine DM, crude protein (**CP**), acid detergent fiber (**ADF**), neutral detergent fiber (**aNDF**), and starch concentrations. The three treatment groups included corn harvested 8 wk after pollination, BMR harvested 7 wk post heading, and BMR harvested 8 wk post heading. The BMR was partially pollinated at the time of harvest. The GLM procedure of SAS 9.4 was used to describe the differences in DM, CP, ADF, aNDF, and starch concentration with significance set at $P \leq 0.05$. Wet chemistry analysis yielded corn CP concentration at $7.27 \pm 0.45\%$ of DM, which was significantly higher ($P = 0.01$) than the 8 wk BMR ($4.58 \pm 0.37\%$ of DM) and comparable to the 7 wk BMR ($7.19 \pm 0.45\%$ of DM). Similarly, NIRS analysis reported corn CP concentration of $8.53 \pm 0.47\%$ of DM, which was significantly higher ($P < 0.01$) than the 8 wk BMR ($4.41 \pm 0.38\%$ of DM, $P = 0.0045$) and comparable to the 7 wk BMR ($7.72 \pm 0.47\%$ of DM). No significant differences were observed in NIRS analysis ADF, aNDF, or starch concentration among the treatments. These findings suggest that while BMR has potential as an alternative silage crop, adjustments in protein supplementation may be necessary to match the nutritional profile of traditional corn silage.

INTRODUCTION

Whole-plant corn silage (**CS**) is the most widely used major forage source for dairy farms due to its high DM yield, energy content, palatability, and optimal DM concentration for ensiling (Colombini et al., 2010). Typically, CS accounts for 40 to 60% of the forage DM in the diet (NASEM, 2021). Forages offered cost-effective protein, energy, and fiber sources compared to most concentrates. However, global climate change created a demand for alternative forage crops that could thrive in high temperatures and drought conditions, unlike traditional options like corn.

Male sterile brown midrib sorghum (**BMR**) emerged as a primary focus of research due to its lower lignin concentration and improved fiber digestibility, which benefited high-yielding cows (Cherney et al., 1991). Brown midrib sorghum also produces higher biomass yields and demonstrates greater water efficiency and drought tolerance compared to corn (Sanderson et al., 1992). Studies showed that replacing CS with sorghum silage, when properly supplemented with starch sources, did not negatively impact milk yield or composition (Grant et al., 1995, Oliver et al., 2004, Colombini et al., 2010). Therefore, sorghum lowered feed costs by providing highly digestible fiber that grew better and yielded more under dry, hot conditions. Brown midrib sorghum is not a direct silage replacement for CS in the TMR due to differences in starch and sugar concentrations. Studies suggest that BMR can be a suitable replacement for CS without compromising milk production (Grant et al., 1995, Aydin et al., 1999, Colombini et al., 2010). However, adjustments in starch supplementation may be necessary to maintain optimal milk yield. Colombini et al. (2010) found that the BMR diet required additional supplementation with corn meal to attain milk production values comparable to CS (Colombini et al., 2010).

The hypothesis of this study is that male sterile BMR will meet or outperform traditional corn varieties in DM and digestibility. Additionally, it is hypothesized that wet chemistry (**WC**) and near-infrared spectroscopy (**NIRS**) will have comparable analyses of pre-ensiled BMR and corn samples. The first objective was to compare the DM, CP, aNDF, ADF, and starch composition of BMR and corn before ensiling. The second objective was to compare analyses of WC and NIRS for the pre-ensiled BMR and corn samples.

MATERIALS AND METHODS

Experimental design

Corn and BMR crops were planted at the ETREC Plant Science Unit (**PSU**) and Little River Dairy Unit (**LRD**). Male sterile BMR was planted across 2 acres at PSU and 8 acres at LRD. The seeds were donated by S&W seed company. A mixture of CS varieties (Pioneer, Johnston, IA; Dekalb, Decatur, GA) was planted across 195 acres at LRD. Sorghum seeds were planted no-till in 15-inch rows by a Kinze corn planter with sorghum plates in late May 2023. Corn and BMR samples were collected by hand 6 inches above ground from PSU 7 wk post heading (n = 2) and from LRD 8 wk post heading (n = 3). Corn samples were harvested from LRD 8 wk after pollination (n = 2).

Nutritive Analysis

Samples were dried in a 55°C forced air oven (Heratherm OMH750, Thermo Scientific, Waltham, MA) for more than 72 h and weighed once no more moisture loss was determined to occur. The DM percentage for the samples was determined by subtracting the initial weight minus final weight divided by the initial weight, multiplied by 100. The dried samples were ground through a 1-mm screen (Model 4, Wiley Mill, Thomas Scientific, Philadelphia, PA) followed by a 1-mm cyclone mill grind (Cyclotec CT293, Foss, Eden Prairie, MN) and mixed for

homogeneity before scanning on NIRS. Previously ground forage samples were dried in a forced air oven at 55°C for 1h to equilibrate moisture content with a reported DM range between 94 to 97.6% (McIntosh, 2019, McIntosh et al., 2022).

The Foss DS2500 (Foss, Eden Prairie, MN) was used to determine forage nutritive values using the 2023 Haylage calibrations from the *NIRS Forage and Feed Consortium* (NIRSC, Berea, KY). The Global H statistical test compared the samples against the model and other samples within the database for accurate results, where all forage samples reported fit the equation with the ($H < 3.0$) and are reported accordingly (Murray and Cowe, 2004). Calibration statistics are included for the 2023 Haylage release (NIRSC 2023, Table 4.1). An external validation set that compared the NIRS predictions from the NIRS Forage and Feed Nutritional Analysis Laboratory (Knoxville, TN) to the WC analysis that used the same methods calibrations were developed with Dairyland Labs (Arcadia, WI) is included (Figure 4.1). This evaluation was to check the reasonable constituent predictions presented in these data utilized for statistical comparisons of the experimental lines of DM, CP, ADF, aNDF, and starch for corn and BMR sorghum (McIntosh et al., 2022).

Statistical analysis

The GLM procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) was used to describe the differences in DM, CP, ADF, aNDF, and starch concentration between the three sample groups. An LSMeans statement was included in the model to describe significant differences. Results were significant if $P < 0.05$. In Microsoft Excel, the Pearson correlation coefficient, denoted as R^2 , was used to determine the strength and direction of the linear relationship between WC and NIRS.

RESULTS

Wet chemistry analysis

The results of the WC analysis are presented in Table 4.2. The DM did not show significant differences among the treatments ($P = 0.16$). Corn had a DM of $65.0 \pm 3.57\%$, while the 8 wk and 7 wk BMR had DM of $76.0 \pm 2.92\%$ and $74.4 \pm 3.57\%$, respectively. Crude protein concentration showed significant differences between the groups ($P = 0.01$). Corn had a CP concentration of $7.27 \pm 0.45\%$, which was significantly higher than the 8 wk BMR ($4.58 \pm 0.37\%$) and comparable to the 7 wk BMR ($7.19 \pm 0.45\%$). There were no significant differences in the ADF and aNDF concentrations among the treatment groups ($P = 0.96$ and $P = 0.89$, respectively). Corn had an ADF concentration of $31.3 \pm 3.73\%$, the 8 wk BMR had $31.5 \pm 3.04\%$, and the 7 wk BMR had $30.2 \pm 3.73\%$. Corn had an aNDF concentration of $54.8 \pm 6.30\%$, while the 8 wk and 7 wk BMR had $51.8 \pm 5.14\%$ and $50.6 \pm 6.30\%$, respectively. The starch concentration varied among the groups but was not statistically significant ($P = 0.16$). Corn had a starch concentration of $3.24 \pm 1.97\%$, the 8 wk BMR had $8.24 \pm 1.61\%$, and the 7 wk BMR had $2.84 \pm 1.97\%$.

Near-infrared spectroscopy analysis

The results of the NIRS analysis are presented in Table 4.3. Significant differences were observed in CP concentration among the treatment groups, whereas ADF, aNDF, and starch concentrations showed no significant differences. Crude protein concentration significantly differed between the groups ($P < 0.01$). Corn had a CP concentration of $8.53 \pm 0.47\%$, which was significantly higher than the 8 wk BMR ($4.41 \pm 0.38\%$) and comparable to the 7 wk BMR ($7.72 \pm 0.47\%$). There were no significant differences in the ADF concentration among the treatment groups ($P = 0.99$). Corn had an ADF concentration of $34.3 \pm 3.07\%$, the 8 wk BMR

had $33.9 \pm 2.50\%$, and the 7 wk BMR had $33.7 \pm 3.07\%$. The aNDF concentration did not differ significantly between the groups ($P = 0.91$). Corn had an aNDF concentration of $56.8 \pm 5.50\%$, while the 8 wk and 7 wk BMR had $57.3 \pm 4.49\%$ and $54.4 \pm 5.50\%$, respectively. The starch concentration varied among the groups but was not statistically significant ($P = 0.08$). Corn had a starch concentration of $3.30 \pm 1.56\%$, the 8 wk BMR had $9.22 \pm 1.27\%$, and the 7 wk BMR had $4.48 \pm 1.56\%$.

Validation of wet chemistry and near-infrared spectroscopy predictions

The scatter plot for CP (Figure 4.1b) demonstrated a strong positive correlation ($R^2 = 0.76$) between WC and NIRS analysis. The data points aligned closely with the trend line, suggesting that NIRS provided highly accurate protein measurements compared to the traditional WC method. The scatter plots for ADF and aNDF (Figure 4.1c and Figure 4.1d) exhibited strong positive correlations ($R^2 = 0.89$ and $R^2 = 0.90$, respectively) between WC and NIRS measurements. The trend line closely followed the data points, indicating that NIRS was reliable for measuring digestibility with minimal deviation from the WC results. The scatter plot for starch analysis (Figure 4.1e) showed a strong positive correlation between WC and NIRS results. The data points were closely clustered around the trend line, indicating that NIRS was a precise method for determining starch concentration in corn and BMR sorghum.

DISCUSSION

Dry matter

Higher DM concentrations are beneficial for ensiling as it reduces the risk of spoilage and DM loss (Borreani et al., 2018). The DM concentrations were not significantly different between corn and sorghum in this study. This finding contrasts with Colombini et al. (2010), who reported lower DM concentration for sorghum (18.5%) compared to CS (28.2%). However, their

research focused on ensiled samples, whereas the current study analyzed pre-ensiled corn and BMR, which could account for the differences observed.

Crude protein

Significant differences were noted in CP concentration between the groups, with corn exhibiting higher CP than the 8 wk BMR. This aligns with Grant et al. (1995), who also found that BMR generally has lower CP concentration compared to corn (7.9 vs. 8.1% of DM, respectively). The lower CP in BMR may necessitate protein supplementation in dairy diets to meet the nutritional requirements of high-yielding cows.

Fiber digestibility

The fiber concentrations of ADF and aNDF did not differ significantly among the treatment groups in our study. This finding aligns with Aydin et al. (1999) and Oliver et al. (2004), who reported that BMR, despite its lower lignin concentration, has comparable fiber concentration to corn. However, our results differ from those of Grant et al. (1995), who found that BMR had higher ADF concentrations (39.8% vs. 28.7% of DM) and aNDF (60.4% vs. 55.4% of DM) compared to CS. A potential reason for these differences is that Grant et al. (1995) analyzed ensiled samples, whereas our study focused on pre-ensiled corn and BMR, which may influence fiber concentration measurements. Additionally, due to poor seed quality control, partial pollination of the BMR plots at PSU and LRD resulted in fertilized seeds sporadically appearing on the sorghum heads. This likely affected the performance of the 8 wk BMR harvest, preventing it from statistically outperforming the corn crop and the less mature 7 wk BMR. Typically, the more mature 8 wk BMR should have decreased aNDF compared to the less mature 7 wk BMR and corn (Hoffman et al., 2003).

Starch

The starch concentration showed variability among the groups but was not statistically significant. This variability is likely due to partial pollination and the presence of seeds in our BMR samples, which increased starch concentration compared to traditional male sterile BMR species. Oliver et al. (2004) observed increased starch concentration for CS (19.9% of DM) compared to BMR-6 (16.8% of DM) and BMR-18 (14.5% of DM). Similarly, Colombini et al. (2010) noted that BMR typically has lower starch concentration (2.8% of DM) compared to corn (22.8% of DM) but can be effectively supplemented with corn meal or other starch sources to meet dietary energy requirements. This supplementation strategy ensures that milk production and composition are maintained, as Colombini et al. (2010) demonstrated.

Validation of wet chemistry and near-infrared spectroscopy predictions

The analysis of corn and BMR samples using both WC and NIRS methods provided insights into the accuracy and reliability of NIRS as an analytical tool. There was a strong positive correlation between NIRS and WC for CP, ADF, aNDF, and starch. This indicates that NIRS can be accurately used for digestibility, protein, and starch analysis.

LIMITATIONS

The objective of this study was to compare the nutritional concentrations of male sterile BMR and corn. However, partial pollination of the BMR resulted in partially fertilized seed heads, likely affecting the anticipated digestibility outcomes for sterile species. This issue may have stemmed from inadequate seed quality control, leading to a mixture of sterile and non-sterile seeds. Non-sterile seeds were likely cross-pollinated, contributing to the observed effects of pollination.

CONCLUSIONS

This study compared the nutritional content of BMR and corn harvested at different stages of maturity. Significant differences were found in CP concentration among the treatment groups. Corn had a higher CP concentration than the 8 wk BMR but was comparable to the 7 wk sorghum. No significant differences were observed in DM, ADF, aNDF, or starch concentration among the groups. The partial pollination of the BMR plots likely affected the expected outcomes, particularly the performance of the 8 wk BMR harvest compared to the less mature 7 wk BMR and corn. Despite these challenges, BMR showed potential as a viable alternative forage to corn, especially in environments experiencing high temperatures and drought conditions. However, differences in starch and sugar concentration between BMR and corn necessitate adjustments in dietary supplementation to maintain optimal milk production. Future research should focus on improving seed quality control and refining dietary adjustments to maximize the benefits of BMR as a silage in dairy production systems. While BMR demonstrated promise, careful dietary planning and further studies are essential to fully harness its potential as a substitute for traditional corn. To determine if BMR is more cost-effective than corn as silage forage in dairy systems, a cost analysis comparing both in a realistic production setting should be conducted.

Overall, the results demonstrate that NIRS is an accurate method for analyzing digestibility and protein concentration in both corn and BMR. These findings support using NIRS as a reliable alternative to WC, offering a faster and less labor-intensive option for routine analysis of pre-ensiled forage samples.

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APPENDIX C

Table 4.1. Mixed Haylage Calibration Statistics for the Foss DS2500 (NIRSC 2023, Berea, KY)

Constituent	Mean	SD	SEC	RSQ	SECV
DM	94.32	1.43	0.35	0.94	0.38
CP	15.14	5.23	0.89	0.97	0.98
ADF	38.18	6.83	1.92	0.92	2.08
aNDF	49.74	11.44	2.33	0.96	2.42
Ash	10.45	3.45	1.19	0.88	1.32
Fat	2.67	0.81	0.45	0.69	0.51
Lignin	7.12	2.85	1.11	0.85	1.21
dNDF30	21.28	7.72	3.93	0.74	4.67
IVTDMD30	70.87	9.80	4.91	0.75	5.42
dNDF48	22.38	6.66	2.49	0.86	2.61
IVTDMD48	78.04	5.85	2.26	0.85	2.45
Ca	1.07	0.45	0.17	0.86	0.18
P	0.31	0.08	0.05	0.58	0.05
K	2.46	0.82	0.37	0.79	0.40
Mg	0.30	0.11	0.07	0.62	0.07
ESC	5.19	3.57	1.24	0.88	1.42
WSC	7.30	3.90	1.09	0.92	1.26
Starch	2.50	4.01	0.89	0.95	1.00
ADICP	1.04	0.55	0.15	0.92	0.26
NDICP	2.25	1.04	0.46	0.81	0.54
InsolCP	5.44	2.06	0.38	0.97	0.52
aNDFom	50.56	10.87	2.74	0.94	3.21

Table 4.2. Results of wet chemistry (WC) analysis for brown midrib sorghum (BMR) planted in Tennessee.

Item, % of DM	Corn ¹	7wk sorghum ²	8wk sorghum ³	<i>P</i> -value
DM, %	65.0 ± 3.57	74.4 ± 3.57	76.0 ± 2.92	0.16
CP	7.27 ± 0.45 ^a	7.19 ± 0.45 ^a	4.58 ± 0.37 ^b	0.01
ADF	31.30 ± 3.73	30.2 ± 3.73	31.5 ± 3.04	0.96
aNDF	54.80 ± 6.30	50.6 ± 6.30	51.8 ± 5.14	0.89
Starch	3.24 ± 1.97	2.84 ± 1.97	8.24 ± 1.61	0.16

¹Corn = Pioneer and Dekalb varieties, harvested at 8 weeks after pollination

²7wk sorghum = BMR sorghum, harvested at 7 weeks after heading, partially pollinated

³8wk sorghum = BMR sorghum, harvested at 8 weeks after heading, partially pollinated

Table 4.3. Results of near-infrared spectroscopy (NIRS) analysis for brown midrib sorghum (BMR) planted in Tennessee.

Item, % of DM	Corn ¹	7wk sorghum ²	8wk sorghum ³	<i>P</i> -value
CP	8.53 ± 0.47 ^a	7.72 ± 0.47 ^a	4.41 ± 0.38 ^b	<0.01
ADF	34.3 ± 3.07	33.7 ± 3.07	33.9 ± 2.50	0.99
aNDF	56.8 ± 5.50	54.4 ± 5.50	57.3 ± 4.49	0.91
Starch	3.30 ± 1.56	4.48 ± 1.56	9.22 ± 1.27	0.08

¹Corn = Pioneer and Dekalb varieties, harvested at 8 weeks after pollination

²7wk sorghum = BMR sorghum, harvested at 7 weeks after heading, partially pollinated

³8wk sorghum = BMR sorghum, harvested at 8 weeks after heading, partially pollinated

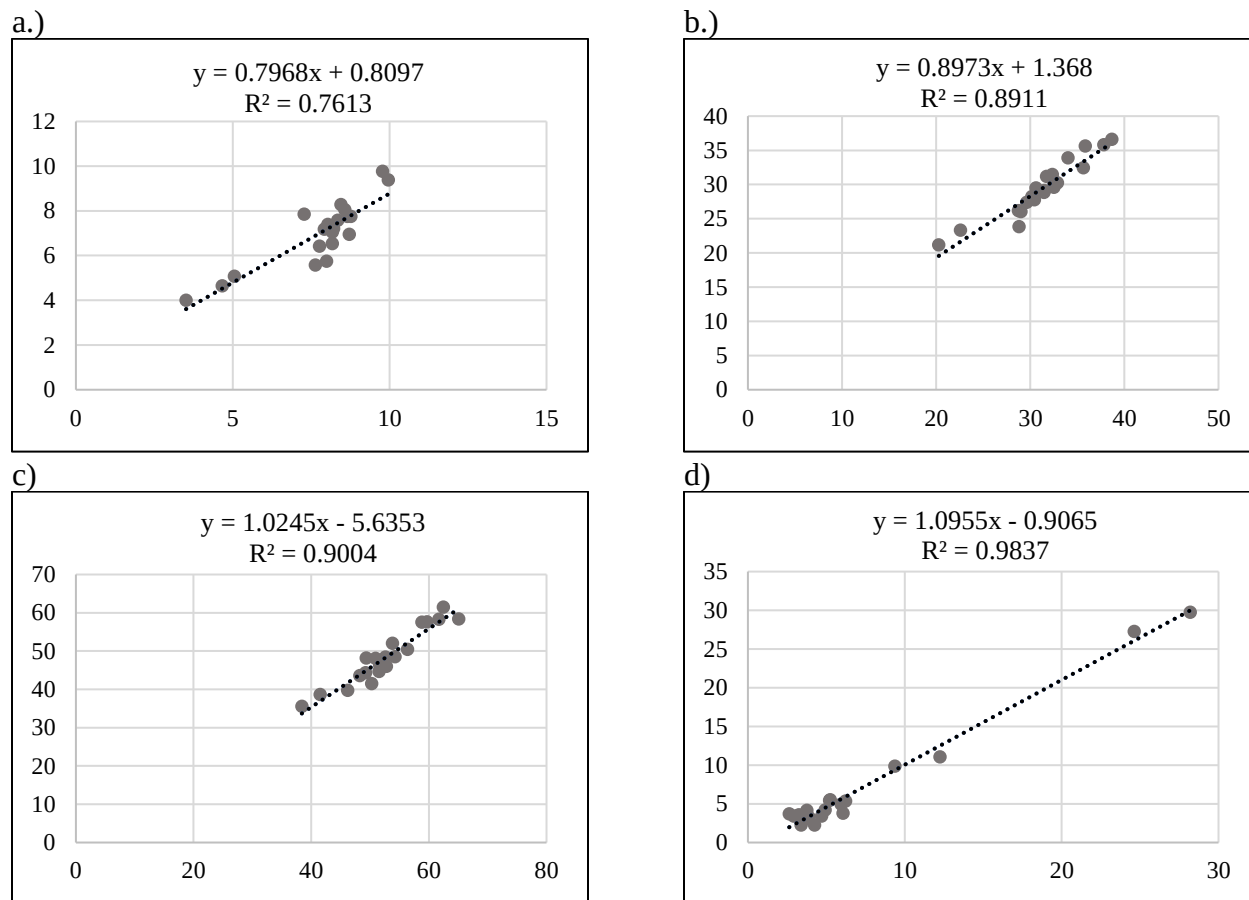


Figure 4.1. Correlations between nutrient composition metrics (a = crude protein; b = acid detergent fiber; c = neutral detergent fiber; and d = starch) from wet chemistry and near-infrared spectroscopy analyses of corn and brown midrib sorghum samples.

CHAPTER FIVE

Conclusions

Partially replacing conventional mineral ingredients with calcareous marine algae (**CMA**) in dairy cow diets during the transition period resulted in similar DMI and milk production metrics compared to control diets. Both treatments maintained serum mineral concentrations, BW, and BCS within optimal ranges, with no significant differences observed. The inclusion of sodium bicarbonate in both diets likely contributed to the comparable DMI among groups. Additionally, the quality of colostrum produced by cows in both treatments was adequate for transferring passive immunity to calves, with both groups achieving Brix scores above 22%. Although serum Ca and Na levels showed expected fluctuations around calving, all measured serum and urine metabolites remained within healthy ranges throughout the study. These findings suggest that CMA can be effectively used as a partial dietary replacement without negatively impacting key health and production parameters in dairy cows during the critical transition period.

The partial replacement of traditional mineral sources with CMA in the diets of lactating cows under heat stress conditions did not significantly affect DMI, milk yield, or milk composition. Both treatment groups maintained similar levels of milk production and component yields, with no notable differences in SCC or BCS. While cows fed CMA exhibited slightly higher DMI during non-heat stress conditions, overall milk efficiency and body condition remained consistent across treatments. These findings suggest that CMA can be an effective alternative to traditional mineral sources without compromising the health and productivity of dairy cows.

The nutritional content of male sterile brown midrib sorghum (**BMR**) and corn harvested at different stages of maturity were studied to compare nutritional concentrations as an

alternative silage forage. Significant differences were found in crude protein (**CP**) concentration among the treatment groups. Corn had a higher CP concentration compared to the 8 wk sorghum but was comparable to the 7 wk sorghum. No significant differences were observed in DM, acid detergent fiber (**ADF**), neutral detergent fiber (**aNDF**), or starch concentration among the groups. The partial pollination of the sorghum plots likely affected the expected outcomes, particularly the performance of the 8 wk sorghum harvest compared to the less mature 7 wk sorghum and corn. Despite these challenges, BMR showed potential as a viable alternative to corn, especially in environments experiencing high temperatures and drought conditions. However, differences in starch and sugar concentration between BMR sorghum and corn necessitate adjustments in dietary supplementation to maintain optimal milk production. Overall, while BMR demonstrated promise, careful dietary planning and further studies are essential to fully harness its potential as a substitute for traditional corn.

Future research

Future research is required to better understand the effects of CMA on transition cow health. A study that more diligently monitors health events and observes serum and urine mineral levels until the end of the transition period may help elucidate the underlying mechanisms of CMA supplementation on Ca homeostasis. Potential investigations should include longer-term studies that observe the entire lactation period to determine the full effects of CMA on milk production. Prospective analysis of male sterile BMR should focus on improving seed quality control and refining dietary adjustments to maximize the benefits of BMR as a silage in dairy production systems. To determine if BMR and CMA supplementation are more cost-effective than their alternatives in dairy systems, a cost analysis comparing both in a realistic production setting should be conducted.

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VITA

Loren Foley was born in Ohio to Kendra and Pat Foley, who are the best parents a girl with dreams could ever ask for. She graduated from Waynesville High School in 2019. Loren was enrolled in the five-year BS/MS degree program at the University of Tennessee, Knoxville. In 2023, she earned a Bachelor of Science degree in Animal Science. She began studying under Dr. Liz Eckelkamp in May of 2022. Her major research focused on the impact of novel calcareous marine algae on transition Holstein cow health and lactation performance. Loren was also involved in studying the nutritional performance of brown midrib sorghum compared to traditional corn varieties. She presented oral and poster presentations about her research at the 2024 American Dairy Science Association annual meeting in West Palm, FL.

Loren was the 2023 ADSA Undergraduate Student Division liaison for the University of Tennessee, Knoxville. She served as the 2023/24 Social Chair for the Animal Science Graduate Student Association at the University of Tennessee, Knoxville.

Scientific Meeting Abstracts:

Foley, L.F., K. Mason, K. McLean, and E. Eckelkamp. 2024. Impact of feeding calcareous marine algae to Holstein cows during the transition period. Abstract 1533. Oral Presentation American Dairy Science Association Annual Meeting. West Palm, FL.

Foley, L.F., K. Mason, K. McLean, and E. Eckelkamp. 2024. Impact of feeding calcareous marine algae to Holstein cows on lactational performance. Abstract 2738. Poster Presentation. American Dairy Science Association Annual Meeting. West Palm, FL.

Awards:

Foley, L. 2024. People's Choice Award. 3-Minute Thesis Competition. University of Tennessee, Knoxville, TN.