Nutritional Mitigation of Heat Stress-Induced Leaky Gut: The Role of DCAD and Dietary Buffer

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Introduction

Heat stress (**HS**) in lactating dairy cows is a multifactorial disorder that can lead to reductions in milk yield of up to 40% (Tao et al., 2018) and negatively impacts reproductive success thereby imposing a large financial burden at the farm level. Even dairy cattle in temperate climates, including northern regions of the United States and Canada experience mild to moderate HS, and it can be challenging to maintain milk production and performance during summer months (Ominski et al., 2002). While barn design and implementation of heat abatement systems largely dictate severity of heat stress exposure, nutritional strategies to mitigate heat stress may also be applied (Baumgard and Rhoads, 2009).

In response to elevated heat load, cows adapt by reducing DMI, increasing water intake, increasing respiration rate, and increasing sweating (Bernabucci et al., 2010). Increased respiration rate has been a particular focus and reduces pCO_2 in blood inducing respiratory alkalosis. Secondary to respiratory alkalosis is a compensatory metabolic acidosis as urinary HCO_3 excretion increases (Silanikove, 2000; Kadzere et al., 2002). Sweating and panting contribute to the loss of electrolytes including Na^+, K^+, Ca^{2+}, Mg^{2+}, P, Cl^-, HCO^-_3, and HPO^{4-} (Bailey and Balch, 1961) and altered concentrations along with reduced ruminal motility have been suggested to increase risk for ruminal acidosis during heat stress exposure (Burhans et al., 2022).

To offset losses of electrolytes the DCAD concentration of diets may be increased during months with risk for heat stress. Common dietary ingredients to increased DCAD include $NaHCO_3$, $KHCO_3$, and K_2CO_3 (West et al., 1987). Increasing DCAD has been reported to help cattle cope with heat stress as indicated by maintenance of blood acid-base balance (Wildman et al., 2007), greater DMI (Hu and Murphy, 2004), and greater water intake (Tucker et al., 1998). However, use of Na+ or K+ coupled with carbonates add an inherent confounding effect as they also provide a dietary buffer. Typically, the added buffer is viewed as being a supportive strategy to mitigate risk for ruminal acidosis co-occurring with heat stress, but this precludes confirmation if responses are driven through cations or dietary buffer.

The loss of electrolytes reduces the buffer supply to the rumen which has been postulated to increase the risk for subacute ruminal acidosis (**SARA**) leading to gastrointestinal tract (**GIT**) barrier dysfunction (Burhans et al., 2022; Plaizier et al., 2022). Ruminal acidosis may trigger local inflammation and increased GIT permeability thereby allowing for the translocation of endotoxins and live bacteria into circulation

eliciting systemic inflammation (Liu et al., 2013; Burhans et al., 2022; Plaizier et al., 2022). In addition to ruminal hyperpermeability, compromised intestinal barrier function resulting from HS has been investigated in non-ruminant species including humans (Lim, 2018) and pigs (Pearce et al., 2012; Mayorga et al., 2020); however, there is a paucity of data for ruminants. Gastrointestinal tract permeability and resulting systemic inflammation is suspected to be the etiology of HS associated milk production losses, mortality, and morbidity (Baumgard and Rhoads, 2013; Burhans et al., 2022).

Is Na^+ Limiting in the Rumen?

Regulation of ruminal pH is complex an involves acid removal mechanisms arising from short-chain fatty acid absorption, neutralization of acid via salivary buffer, passage of acid out of the rumen, and in buffering driven by other ions such as ammonia/ammonium and phosphates (Allen, 1997; Aschenbach et al., 2011). It has been proposed that cattle experiencing heat stress may suffer from a ruminal Na deficiency that compromises short chain fatty acid (**SCFA**) absorption (Mooney, 2006) increasing susceptibility to SARA (Burhans et al., 2022). While not evaluated under heat stress conditions, a recent ex vivo study reported no evidence for Na+ to affect the uptake of acetate or butyrate (Bertens et al., 2023) supporting previous results of Sehested et al. (1999) indicating Na+ concentration did not affect flux of acetate or butyrate across the isolated ruminal epithelium.

To further test this concept, we exposed cows to moderate heat stress (temperature humidity index between 68 and 72) and evaluated short-chain fatty acid absorption using the temporarily isolated and washed reticulo-rumen technique under low (45.9 mmol) and high Na (85.3 mmol) buffer conditions (Bertens et al., 2022). In that study, altering the concentration of Na+ in the incubation buffers had no effect on the rates of SCFA absorption in vivo. Collectively, these data suggest that it is unlikely that ruminal Na+ concentration limits SCFA absorption.

Comparing DCAD and Dietary Buffer

We conducted an experiment to separate out effects arising from DCAD and dietary buffer using a 2×2 factorial treatment arrangement within a replicated (16 cows; 8 primiparous and 8 multiparous) 4×4 Latin square. Part of these data were reported in Bertens et al. (2022) with the remainder being unpublished. In this study, we altered the DCAD primarily with the inclusion or exclusion of Na-acetate and altered dietary buffer supply by including or excluding $CaMg(CO_3)_2$ (0 vs. 1% of dietary DM, MIN-AD, Papillon Agriculture Company, Easton, MD). Achieved DCAD values were 17.6 and 39.6 mEq/100 g for the low and high DCAD treatment, respectively. All cows were exposed to a temperature-humidity index (THI) averaging 73 ± 1.4 from 0600 h to 1600 h with allowance for natural night cooling from 1601 h to 0559 h achieving a mean THI of 67 ± 2.5. As evidence for heat stress conditions, cows had elevated rectal temperatures throughout the study averaging 39.1°C with no effect of DCAD, buffer, or their interaction.

In the above-mentioned study (Bertens et al., 2022), providing added buffer or increasing the DCAD did not affect DMI with an average intake of 25.1 kg/d. This result differs from several past studies where increasing DCAD stimulates DMI (Hu and Murphy, 2004; Iwaniuk and Erdman, 2015). Given the quadratic relationship between DMI and DCAD (Hu and Murphy, 2004), it is possible that the difference in DCAD was not sufficiently different to detect differences. While we could not measure water intake in this study, we did observe that cows fed greater DCAD had urine output that was 3.8 L/d greater (P =0.02) than when fed the low DCAD treatment. Increased water intake is one strategy cows can employ to mitigate heat stress as they transfer body heat to the water and excrete it as urine (McDowell et al., 1969; Bernabucci et al., 2010). Increasing DCAD with Na-acetate did not affect serum Na+ concentrations but resulted in lower concentrations of serum K (4.5 vs. 4.6 mmol/L; P = 0.02) and Cl (96.1 vs. 98.2 mmol/L; P < 0.01). Increasing dietary buffer increased serum Ca concentration (2.8 vs. 2.4 mmol/L; P = 0.04). There was an interaction between buffer inclusion and DCAD (P = 0.03) such that cows fed added buffer had greater serum HCO_3 when fed high DCAD than low DCAD, while there were no effects of DCAD when fed without added dietary buffer. Moreover, values for the low buffer treatments (low and high DCAD) were intermediate and not different to the high buffer with high DCAD and high buffer with low DCAD. Changes in the concentration of Ca with high buffer may have affected the concentration of HCO_3 according to the strong ion theory (Goff, 2018). Given the changes in serum mineral concentrations, the calculated anion gap was above 21.1 mEq/L suggesting cows were all in a very mild metabolic acidosis (Goff, 2018).

Milk yield was not affected by treatments averaging 36.9 L/d (Bertens et al., 2022). That said, providing a greater DCAD increased milk fat yield (1.53 vs. 1.50 kg/d; P = 0.03) and reduced the proportion of preformed milk fatty acids (45.5 vs. 47.4%; P < 0.01) but there were no effects on other milk components with greater DCAD or buffer inclusion. Wildman et al. (2007) reported increased milk fat with elevated DCAD using $NaHCO_3$ during heat stress in which they attributed the improvement in milk fat to enhanced ruminal buffering. Ruminal pH was not affected in the present study challenging whether DCAD directly would affect buffering. That said, we used Na-acetate to increase DCAD and a previous study using slightly higher inclusion rates of Na-acetate reported an increase in milk fat and reduced preformed fatty acids (Urrutia et al., 2019). As such, it is not possible to attribute milk fat responses directly to DCAD in the present study.

Ruminal pH

It is commonly reported that cows exposed to heat stress experience ruminal acidosis due to loss of electrolytes and consequently reduced salivary buffer supply (Burhans et al., 2022) along with altered sorting behavior (Baumgard and Rhoads, 2009), and feeding patters (Frazzi et al., 2000). Although we could not evaluate the effect of heat stress on ruminal pH as all cows were exposed to heat stress conditions, there were no effects of DCAD or dietary buffer inclusion on ruminal pH with mean ruminal pH averaging 6.39 (Bertens et al., 2022). While DCAD alone would not be expected to alter pH, the efficacy of dietary buffers to act within the rumen relies on pH

to allow for complete or partial solubilization (Le Ruyet and Tucker, 1992). For $CaMg(CO_3)_2$, reductions in pH increase solubilization (Altland and Jeong, 2016). In the present study, mean ruminal pH was 6.39 and minimum pH was 5.85 which likely resulted in only partial ruminal solubilization of Ca and Mg $(CO_3)_2$. Hence, the chemical properties of $CaMg(CO_3)_2$ most likely explains the undetected effect of buffer to modulate ruminal pH; however, the increase in serum Ca with greater buffer inclusion suggests that, at least in the total tract, Ca supply was improved with buffer supply. These findings are supported by Crawford et al. (2008) in which there was limited effect of $Ca(CO_3)_2$ on ruminal pH in growing yearling steers (mean ruminal pH of 6.0). Razzaghi et al. (2021) evaluated the effect of $NaHCO_3$, MgO, and $CaMg(CO_3)_2$ on ruminal pH, measured continuously, in lactating dairy cattle fed diets containing 34% starch. They found no difference in mean ruminal pH (5.76 ± 0.04) but saw increases in maximum pH and minimum pH with all three buffer supplements. In addition, they found that the area below 5.8 was reduced with all buffer supplementation when compared to the control, with the greatest effects seen for $NaHCO_3$ followed by lesser but similar effects seen for MgO and $CaMg(CO_3)_2$.

Permeability of the Gastrointestinal Tract

There has been a growing body of evidence supporting that heat stress increases permeability of the gastrointestinal tract. In fact, direct effects of heat stress have been reported to compromise gastrointestinal barrier function in ruminants (Koch et al., 2019), rodents (Lambert et al., 2002), pigs (Pearce et al., 2012), and humans (Lim, 2018). Therefore, heat stress-induced leaky gut may result independently of ruminal acidosis. As an approach to separate ruminal and post-ruminal permeability, we developed a novel technique utilizing ruminally infused Cr-EDTA to indicate total tract permeability (Zhang et al., 2013) along with a simultaneous abomasal infusion of Co-EDTA to indicate post-ruminal permeability (Bertens et al., 2022). In the heat stress study evaluating buffer and DCAD describe above, we detected tendency ($P = 0.098$) for added $CaMg(CO_3)_2$ to reduce the amount Cr-EDTA recovered in urine and a reduction in the recovery of Co-EDTA ($P < 0.01$). These data suggest that that dietary buffer may help improve barrier function of the gastrointestinal tract largely by reducing post-ruminal permeability. These data are supported by a recent study showing that heat stress alters intestinal barrier function in the jejunum along with changes in the microbial community structure in the colon. Other studies in our laboratory have further confirmed that intestinal regions may be more sensitive to barrier dysfunction than ruminal regions (Penner et al., 2014; Lambert et al., 2023) supporting the results of Bertens et al. (2022).

While we cannot confirm the mechanisms involved in promoting barrier function with added dietary buffer (Bertens et al., 2022), the potential for $CaMg(CO_3)_2$ to solubilize in the abomasum may lead to potential buffering effects more distally in the gastrointestinal tract. In agreement, Rauch et al. (2012) utilized an in vitro technique with $CaMg(CO_3)_2$ and reported no change in ruminal pH. However, in the same paper, the authors tested the effects of different buffer supplements fed to lactating cows under thermoneutral conditions and demonstrated elevated fecal pH in cows supplemented

with $CaMg(CO_3)_2$ when compared to cows fed $NaHCO_3$ and control diets. It may be possible that dietary buffers that solubilize post-ruminally may help regulate intestinal fermentation and regulation of the intestinal barrier. Future research is needed to confirm these findings and to evaluate potential mechanisms for such an effect.

Conclusion

In summary, it does not appear that ruminal Na will limit SCFA absorption or potential effects on ruminal pH. Provision of $CaMg(CO_3)_2$ as a dietary buffer and altering DCAD do not interact to affect DMI, ruminal fermentation, and GIT permeability in lactating dairy cattle exposed to mild heat stress. However, elevated DCAD as affected by Na-acetate increased urine output and increased milk fat yield. Despite the lack of ruminal acidosis in the present study, $CaMg(CO_3)_2$ reduced intestinal permeability and tended to reduce total tract permeability while DCAD had no effect. These findings highlight the pitfalls of the current literature emphasizing ruminal permeability associated with ruminal acidosis without sufficient acknowledgement for post-ruminal permeability. Consequently, these findings may extend value of dietary buffer beyond the rumen and help to provide new information on the individual contributions of dietary buffer and DCAD under mild heat stress conditions.

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