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The Evaluation of Encapsulated *Megasphaera elsdenii* in an Accelerated Beef Step-Up Program and an Acidosis Challenge Model and the Evaluation of RAMP Versus a Traditional Forage Grain Adaptation Strategy on Methane and Respired Carbon Dioxide

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A metabolism experiment (Exp 1) was conducted to evaluate daily feeding of encapsulated Megasphaera elsdenii *(M. elsdenii)* NCIMB 41125 along with a one-time drench of 1×10^{11} CFU of *M. elsdenii* on dry matter intake (DMI), *in-vitro* lactate utilization, volatile fatty acid (VFA), and lactate concentration. Treatments consisted of steers which were fed no *M. elsdenii* (CONTROL), steers drenched with the commercial dose 1×10^{11} CFU of *M. elsdenii* (LactiproNXT) on d 1 of the experiment and received no other *M. elsdenii* (DRENCH), and steers drenched with a commercial dose of LactiproNXT on d 1 of the experiment and received 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top dress (LOW), 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top dress (MEDIUM), and 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top dress (HIGH). CONTROL was stepped-up to a finisher diet in 18 d and DRENCH, LOW, MEDIUM, and HIGH were stepped-up in 9 d. During the step-up, there were no differences in DMI; however, cattle fed *M. elsdenii* had increased butyrate by 3%

compared to CONTROL. After an acidosis event, DMI increased by 4.6% for LOW, MEDIUM, and HIGH steers compared to DRENCH. Steers fed *M. elsdenii* daily tended to have a 30% greater utilization of lactate compared to CONTROL. After an acidosis event, cattle fed *M. elsdenii* daily had a 10% increase in VFA concentration compared to DRENCH. An accelerated step-up was possible with DRENCH and daily feeding of *M. elsdenii*. A drench and daily feeding of *M. elsdenii* may have a positive effect during and after an acidosis event. A finishing experiment (Exp 2) was conducted to evaluate RAMP compared with a traditional forage adaptation program on methane (CH_4) emissions and respired carbon dioxide (CO_2), performance, and carcass characteristics of beef cattle. Steers were utilized in 2 adaptation treatments, using 100% RAMP or 43% forage during step 1. All cattle were adapted to the same finishing diet over 22 d. Feeding RAMP during step 1 resulted in 12% decrease in CH_4, in g/d and a 18% lower CH_4:CO_2. Steers fed RAMP during step 1 had an 8% increase in CO_2 g/d due to greater digestibility compared to traditional forage diet. Steers fed RAMP spent 45% less time ruminating and eating compared to CONTROL during step 1. For emissions while on the common finishing diet, steers that had been adapted using RAMP had a 9% lower CH_4, in g/d, 8% lower in CH_4 g/kg DMI, and a lower CH_4:CO_2 suggesting a carryover effect from adaptation. Steers adapted with RAMP tended to have a greater HCW. Feeding RAMP to cattle during the grain adaptation phase resulted in a 12% decrease in CH_4, which carried over to 9% less CH_4 during the finishing phase.

Table of Contents

List of Tables

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CHAPTER 1 - REVIEW OF THE LITERATURE

Acidosis Etiology

In 2015, digestive disorders caused 427,910 deaths in cows and calves in the dairy and beef industry (United States Department of Agriculture Animal and Plant Health Inspection Service, 2015). The digestive disorders consisted of bloat, parasites, enterotoxaemia, and acidosis. Britton and Stock (1989) defined acidosis as "an array of biochemical and physiological stresses caused by rapid production and absorption of ruminal organic acids and endotoxins when an animal overconsumes readily fermentable carbohydrates". Nagaraja and Titgemeyer (2007) suggested that acidosis is an accumulation of organic acids that causes imbalances between microbial production, utilization rate, and absorption rate of organic acids. Since acidosis is a "continuum of degree of ruminal acidity" (Britton and Stock, 1989), it can be classified in two different forms and has different effects on ruminants. Sub-acute ruminal acidosis is when ruminal pH drops below 5.6 and is considered to have a primary effect on intake (Britton and Stock, 1989; Stock et al., 1995) and increased number of liver abscesses (Nagaraja and Chengappa, 1998; Nagaraja and Titgemeyer, 2007). When ruminal pH is below 5.6, organic acids increase while there is a decrease in absorption. (Nagaraja and Titgemeyer, 2007). Lactic acid can be produced during sub-acute ruminal acidosis, but accumulation of lactic acid does not typically occur because bacteria can ferment lactate (Goad et al, 1998) and can be quickly converted to volatile fatty acids (VFA; Nagaraja and Titgemeyer, 2007). Whereas in acute ruminal acidosis, the ruminal pH drops below 5.0 and approaches 4.5 or lower (Owens et al., 1998; Krause and Oetzel, 2006; Nagaraja and Titgemeyer, 2007). Nagaraja and Titgemeyer (2007) suggested the reason for pH to reach

4.5 is due to an accumulation of lactic acid. The normal ruminal pH of fattening ruminants is between 5.8 and 6.5 (Nagaraja and Titgemeyer, 2007), but rumen pH is constantly changing throughout the day. In some animals, a single incident of acidosis will have negative impacts throughout the whole finishing period, and they are noted as "realizers" or animals who have poor performance as the animals consume a limited amount of the diet to avoid acidosis again (Owens et al., 1998). There are many factors that affect acidosis in the rumen; however, the main drivers affecting acidosis are pH, the rate of intake of rapidly fermentable carbohydrates (CHO), an individual's available rumen capacity, the rate of absorption of VFAs, and microbial populations (Nagaraja and Titgemeyer, 2007).

Rumen pH

When determining if acidosis is present, rumen pH is commonly associated with detecting acidosis. One of the factors affecting rumen pH is the buffering capacity of the blood through the bicarbonate buffering system. When there is increase in carbohydrates in the diet, coupled with an increase in dry matter intake, the ruminant must depend on the buffering capacity of the blood to buffer the rumen and to help prevent acidosis. The bicarbonate buffering system plays an important role in acidosis mitigation but is impact is dependent on the amount of saliva secreted. Of the acids that are produced in the rumen, 30 to 50% are neutralized by the buffering ability of salivary secretions which must be absorbed through the rumen wall (Hernández et al., 2014).

Rumen Volatile Fatty Acids

Another factor that affects the rumen pH are volatile fatty acids (VFA). There are three primary VFAs that are found in the rumen in large concentrations, which are

acetate, propionate, and butyrate. Other VFAs such as isobutyrate, valerate, isovalerate, and 2-methylbutanoic all exist in smaller amounts in the rumen. The primary VFAs, acetate, propionate, and butyrate generate energy for the animal when they are transported to the liver. Propionate can be used as a precursor for producing glucose, which is dependent on the make-up of the diet but also intake (Dijkstra, 1994). Volatile fatty acids provide energy for the animal; however, how much energy is dependent on the absorption and buffering ability of the ruminant. Volatile fatty acids buffer at a pH of 4.9, where the acid dissociates (pKa), so the VFA becomes more undissociated which will cause an increase in their absorption rate during sub-acute ruminal acidosis (Bergman, 1990). With a drop in pH, there is an exchange of unionized acid for ionized VFA which causes a decrease in absorption and makes it even harder for bicarbonate from the blood to buffer the rumen (Hernández et al., 2014).

Acute and subacute acidosis can cause laminitis, polioencephalomalacia, rumenitis, and liver abscesses (Brent, 1976). Polioencephalomalacia is a neurologic disease usually caused by high sulfur intake from feed or water. In acidosis polioencephalomalacia is due to a decrease in thiamine. Laminitis causes lameness and feet issues as acidosis can lead to endotoxins and lactate being released into the bloodstream which will affect blood flow to the feet. Polioencephalomalacia and laminitis both can lead to death of the animal. Rumenitis and liver abscesses are also caused from acidosis. Rumenitis may damage the rumen wall and cause lesions, which will result in a decrease in VFA absorption. Not only can a decrease in VFA absorption occur when there is a decrease in pH, but there can be a long-term decrease in VFA absorption if acidosis is severe. In a study by Krehbiel et al. (1995a) on acidosis in sheep,

they reported a tendency for a reduction of propionate ($P = 0.06$) and a numerical difference in acetate and butyrate three months after an acidosis event. Six months after an acidosis event there were still numerical decreases in the absorption of VFAs for lambs that went through an acute acidosis event. Bergman (1990) suggested that 65 to 75% of total metabolizable energy (ME) supplied to ruminants comes from VFA absorption. Since VFA absorption is responsible for about ¾ of the total ME supplied to the ruminant, Krehbiel et al. (1995a), found a 23 to 32 percent reduction in total energy. This data (Krehbiel et al., 1995a) suggests acute acidosis can cause long term effects to the animal because of poor VFA absorption rates.

Rumen Lactic Acid

Lactic acid starts accumulating at the ruminal pH of 4.5 (Nagaraja and Titgemeyer, 2007). Lactic acid has two forms that are similar in structure, L-lactate (L(+)) and D-lactate (D(−)), which will buffer at the PKA of 3.8, but have largely different impacts on the rumen. L-lactate can be converted to pyruvate rapidly and eventually goes into gluconeogenesis to make glucose. Since the metabolism of L-lactate occurs quickly because of L-lactate dehydrogenase in the liver, it can be produced at a faster rate (Ewaschuk et al., 2005). On the other hand, D-lactate will accumulate in the rumen because it is metabolized by D-α-hydroxy acid dehydrogenase at one-fifth of the rate of L-lactate metabolism (Ewaschuk et al., 2005). In a study conducted by Cori and Cori (1929), it was noted that ruminants are poor at metabolizing D-lactate. The lack of metabolism of D-lactate could be the reason why ruminants are not able to recover from acute ruminal acidosis and have a buildup of D-lactate.

Rumen Microbes

During sub-acute and acute ruminal acidosis, there is a large population of bacteria that is growing and proliferating in the rumen. A rapid consumption of readily fermentable CHOs, when acidosis is starting to occur will cause an increase in the number of lactate producing bacteria and amylolytic bacteria. Nagaraja and Titgemeyer (2007) suggest that lactate producing bacteria utilize soluble sugar and starches to be able to produce lactic acid and VFAs. When pH is below 5.0, *Streptococcus bovis*, (*S. bovis*) and Lactobacilli, which are lactate producing bacteria, will start to accumulate rapidly. Driving sub-acute ruminal acidosis to become acute ruminal acidosis. The gram-positive, lactate producing bacteria increase in population during acute ruminal acidosis but are at lower levels during sub-acute ruminal acidosis. As *S. bovis* and other lactate producing bacteria increase, so do lactate levels in the rumen, which will consistently hinder the ability of *Megasphaera elsdenii* (*M. elsdenii*) to survive at a low pH. The lactate producing bacteria cause a continual decrease in pH making it challenging for the animal to recover and can lead to death. Lactobacilli will be produced once pH decreases below 5.6 and is more acid tolerant compared to *S. bovis.* Not as many cattle experience acute ruminal acidosis and have a pH that falls below 5.6 compared to the number of cattle that experience sub-acute ruminal acidosis. Once an animal enters acute ruminal acidosis there is potential for lactic acid accumulation (Beauchemin and Penner, 2009), it is often hard for the animal to make a full recovery. With a majority of fattening ruminants going through sub-acute ruminal acidosis and in addition to the few that go through acute ruminal acidosis, acidosis has a significant impact on the cattle. Thus, acidosis becomes even more important for the beef industry to try to prevent from occurring.

Megasphaera elsdenii (*M. elsdenii*), *S. ruminantium spp., Lactilyatuc, and Anaerovirbro lipolytica* are just some of the lactate utilizers (Huber et al., 1976). Lactic acid utilizers will produce VFAs and will increase in population size when cattle are adapted to high-concentrate diets (Huber et al., 1976: Counotte and Prins, 1981). *M. elsdenii*, a gram-negative lactate utilizing bacteria, is a slow growing bacterium that is negatively affected by low pH, 5.5. *Megasphaera elsdenii* is decreased in acute ruminal acidosis and has higher levels during sub-acute ruminal acidosis (Hernández et al., 2014). *Megasphaera elsdenii* decreases the number of carboxyl groups in lactate, which allows for lactate to be converted to VFAs, mainly butyrate (Weimer et al., 2015), which may cause an increase in ruminal pH. If there is a gradual increase of a high concentrate diet, there is a minimal amount of lactate being produced, so the ruminal pH has not decreased below the ability of *M. elsdenii* to convert lactate to butyrate. When there is a large increase in rapidly fermentable carbohydrates, there will be an increased number of amylolytic bacteria to produce pyruvate, through glycolysis, and eventually into organic acids (Wang et al., 2015; Mickdam et al., 2016). *Streptococcus bovis* will produce lactate (lactic acid) rapidly and has the capability to double its numbers within nine minutes under favorable conditions (Russel and Robinson, 1984). Consequently, *Streptococcus bovis* will cause a decrease in ruminal pH and decrease the ability of *M. elsdenii* to utilize lactate to produce VFAs because of the low pH (Mills et al., 2014) making it more difficult for the animal to recover from acute ruminal acidosis.

Acidosis Management Strategies

With acidosis being common in the feedlot industry and causing major health issues and economic losses, it becomes even more important to find strategies to mitigate

acidosis. Some of the widely used strategies to mitigate acidosis are feed additives, grain processing methods, and inclusion of forages. Some less common strategies that are becoming more widespread are the addition of by-products and the feeding of direct-fed microbials.

Starch Digestion

Starch digestion is dependent on many variables such as ruminal microbial population, amount of feed consumed per unit of time, composition of the diet, and grain processing types (Huntington, 1997). Concentrates have different amounts of starch and rate of starch digestion, which play an important role in sub-acute ruminal acidosis. Dry rolled sorghum and dry whole corn have the slowest rate of digestion (Stock, 2000) and dry rolled wheat has the highest rate of digestion (Aimone and Wagner, 1977; Huntington, 1997; Stock, 2000). Feeds that have a faster rate of starch digestion are more likely to cause acidosis compared to the slower starch digestion rates. A common feed management practice is to mix feeds that have a fast starch digestion rate with feeds that have a slower starch digestion rate. In a study by Stock et al. (1987), dry-rolled sorghum (slow rate) was combined with high moisture corn (fast rate). It was noted that acidosis was reduced with the addition of 25% sorghum due to the slower rate of starch digestion. This was due to the balance of starch availability which led to a decrease in acidosis.

Corn Processing and By-Products

Another acidosis mitigation strategy is processing methods, particle size, and flake density of grains. When feeding steam flaked grain sorghum to finishing cattle at three different flake densities (22, 25, or 28 lb/bushel), Reinhardt et al. (1997) saw that steers fed 28 lb/bushel flakes had a greater DMI, greater ADG, and similar F:G compared

to cattle who were fed sorghum grain at 22 lb/bushel flakes, which were more intensively processed. The authors also noted that there was a decrease in acidosis, as cattle fed 28 lb/bushel flake density spent less hours below a pH of 5.0 and less hours at a pH of 5.5 (Reinhardt et al., 1997). A smaller flake density could have led to an increase in acidosis as the starch was more available to the microbes.

There are many corn processing methods and grain by-products that can help reduce acidosis. The wet milling process can be found in detail in Blanchard (1992); however, products like steep, bran, and wet corn gluten feed (WCGF) are just some of the by-products from the wet milling industry. In a study conducted by Scott et al (1997), 60 steers were fed a control diet comprised of 85% dry rolled corn (DRC), 10% alfalfa silage and 5% supplement. The treatments consisted of replacing DRC with either 15% or 30% corn bran and/ or steep liquor. Steers that received 15% bran had increased DMI, ADG, and efficiency, which Scott et al. (1997) suggested was from a decrease in acidosis. Dry rolled corn has a greater starch content compared to bran and steep, bran is lower in energy and steep is higher in energy, which is part of the reason for increased acidosis in cattle. In a metabolism study conducted by Krehbiel et al. (1995b), steers were withheld from feed for 1 day and then they were intraruminally dosed with either 100% DRC, 50:50 DRC/WCGF (wet bran, steep liquor, distillers solubles), or 100% WCGF at 7.9 kg (DM). It was reported that all cattle experienced some level of acidosis; however, cattle dosed with WCGF recovered from an acidosis event faster than steers dosed with DRC. The starch from the corn was replaced with wet corn gluten feed which is a highly digestible fiber and kept acidosis from being as severe.

Direct Fed Microbes

Adding direct- fed microbes (DFM) is another acidosis mitigations strategy that is less common but has potential to become more popular among feedlot producers. Direct-fed microbes have been defined in different ways over the years. The definition has been narrowed to “a source of live, naturally occurring microorganisms” by the FDA (Yoon and Stern, 1995). The original use for DFM was to accelerate the growth of intestinal microflora in feed digestion and increase gut health in younger ruminants (McAllister et al., 2011). Today, DFM are targeted for improving fiber digestion and decreasing acidosis (McAllister et al., 2011). Direct-fed microbials have been developed for many different problems and diseases in the ruminant production system. One of the most well-known DFM is bacterium; this review will focus on specific bacterium that utilize lactic acid.

Studies have been conducted that involved the addition of DFM, that have either increased the utilization of lactic acid or increased the utilization of starch to prevent the buildup of lactic acid. Some of the bacteria that utilize starch are *Ruminococcus albus* (Krause et al. 2001; McAllister et al., 2011) and *Prevotella bryantii 25A* (Chiquette et al. 2008; McAllister et al., 2011). The more common use of DFM are the lactate utilizing bacteria such as *Propionibacterium freudenreichii,* (Raeth-Knight et al., 2007) *Selenomonas ruminantium,* and one of the most common bacteria being *M. elsdenii.*

Megasphaera Elsdenii

A producer may feed *M. elsdenii* during times of stress such as on reimplant day or a weather event that would cause the cattle to go off-feed for longer periods of time or cause irregular intake. Another reason to feed *M. elsdenii* would be during diet adaption to reduce the chance of acidosis. These events cause stress on the animal which allows

for an increase in the production of lactic acid. Counotte et al. (1981) suggested that *M. elsdenii* can utilize around 60-80% of lactic acid that was produced. In the rumen, *M. elsdenii* is able to utilize the lactate and convert it to end products such as VFAs.

Research suggests that the addition of *M. elsdenii* could help with the reduction of rumen acidosis (Leedle et al., 1990; Horn et al., 2009; Long et al., 2014; Weimer et al., 2015; Chen et al., 2019). In a study conducted by Chen et al. (2019), there were positive effects for animals in sub-acute ruminal acidosis when there was an increased amount of *M. elsdenii* and *Butyrivibrio fibrisolvens*. The amount of lactate being produced decreased and VFAs increased, mainly butyrate, causing the pH to increase. Surprisingly, when butyrate increased, there was also an increase in the *M. elsdenii* populations. Other studies such as Minuti et al. (2014) noted higher levels of acetate and propionate. Kung and Hession (1995) conducted an in vitro study that used rumen fluid from steers fed alfalfa grass hay for 60 d. The rumen fluid was collected then buffer and rapidly degradable substrates (starch, glucose, cellulose, cellobiose, and trypticase) were added to the fluid to simulate a diet that was similar to a high concentrate diet. The combined culture was inoculated with M. *elsdenii* at different dose amounts. They concluded that the *M. elsdenii* prevented a rapid drop in pH which would decrease the chance of acute ruminal acidosis from occurring. Samples at low levels of inoculated *M. elsdenii* had greater concentrations of lactate five hours post feeding, while feeding higher levels *M. elsdenii* did not observe this effect. After twenty-four hours of fermentation, samples were transferred to a fresh buffer and six hours later there was a reduced amount of lactate built-up in both levels of the inoculated *M. elsdenii* treatments compared to the control group. Hibbard et al. (1993) reported cattle that were stepped up from a 50% to

90% concentrate diet and were orally drenched with *M. elsdenii* led to an increase in DMI which suggested that acidosis occurred less during the transition period.

M. Elsdenii NCIMB 41125

Most of the studies presented earlier involved many strains of *M. elsdenii*. One of the more common strains of *M. elsdenii* is NCIMB 41125. M.S. Biotech (Lactipro, M.S. Biotech, Wamego, KS) is a company that is using the strain NCIMB 41125 of *M. elsdenii* to help control acidosis in cattle. Meissner et al., (2010), suggested that the NCIMB 41125 strain of *M. elsdenii* was not affected by ionophores and its fast-growing rates allow for the use of lactate to make VFAs and other end products when the pH is lower than 5.5 in the rumen.

In a study by Henning et al. (2010a), 24 ruminally cannulated wether lambs were utilized in a 2 x 2 factorial design. The wethers were either fed forage at *ad-libitum* or a controlled amount, then one treatment of *ad-libitum* and one treatment of controlled received a dose with 1×10^{11} CFU of *M. elsdenii.* The main feed ingredients in the concentrate diet were whole kernel corn, slaked lime, and molasses products. Lambs received the dose of *M. elsdenii* on day 1 and 2 of concentrate feeding. On d 1 of starting the concentrate diet, all lambs overate, which was expected. On day 2 through 11, lambs that were drench with *M. elsdenii,* had increased DMI of concentrates by 46% ($P < 0.001$). The lambs still had an 11% increase in concentrate intake compared to the control lambs until slaughter (50 d). In other studies, feeding *M. elsdenii* led to an increase in DMI (Henning et al., 2010b; Miller, 2013; Mazon et al., 2020). Other studies have shown a decrease or no difference in DMI in cattle that received *M. elsdenii* while on different rations and after different step-up regimes (Leeuw et al., 2009; McDaniel et al., 2009;

Henning et al., 2010a; Drouillard et al., 2012; Miller, 2013; Ellerman et al., 2017; DeClereck et al., 2020; Wagner et al., 2022). For dry matter intake, there are varying results, for the specific strain of *M. elsdenii* NCIMB 41125.

When feeding *M. elsdenii* NCIMB 41125, there can be mixed results in terms of volatile fatty acid concentrations. In a second study conducted by Henning et al. (2010a), 12 steers were utilized with 4 treatment groups that consisted of control (no drench), low (drenched at 1.72×10^9 CFU), medium (drenched at 1.72×10^{10} CFU), and high (drenched at 1.72×10^{11} CFU). Steers went through a 24- hr feed withdrawal and then treatments were given on day 2. Cattle were stepped-up to the finisher diet in 16 days with four step-up diets. Control steers had lower concentration of acetate compared to that of the *M. elsdenii* cattle ($P < 0.05$), but propionate was higher for the control cattle ($P < 0.05$). Butyrate increased for the *M. elsdenii* treatment ($P < 0.04$), which was consistent with other studies when cattle were fed *M. elsdenii* NCIMB 41125 (Weimer et al., 2015; Wagner et al., 2022). A study was conducted on mid-lactating Holstein cows, that were ruminally cannulated and fed a different strain of *Megasphaera elsdenii* called *M. elsdenii* ATCC 25940. Eight cows were utilized in a paired 2 x 2 crossover design with two different treatments of either 35 ml saline or 35 ml suspension per day of *M. elsdenii* ATCC 25940 (at 1×10^8 CFU) which was dissolved in saline (Zebeli et al., 2012). Acetate decreased for cattle that were dosed with *M. elsdenii* ($P < 0.01$) and a shift in the VFA production with an increase in butyrate ($P < 0.01$). An increase in butyrate is not surprising because many lactate utilizing bacteria metabolize lactate to butyrate and propionate (Satter and Esdale 1968). Counotte et al. (1981) suggested that butyrate is a major end product from *M. elsdenii* being metabolized in the rumen.

In terms of total VFA concentrations, there have been mixed results for cattle fed *M. elsdenii*. Ellerman et al. (2017) reported an increase in total VFA concentration for cattle that were drenched or received a daily supplement of *M. elsdenii* NCIMB 41125 ($P < 0.01$). Wagner et al. (2022) reported a tendency ($P = 0.10$) for an increase in the total VFA concentration for the treatment that received the commercial dose of *M. elsdenii* NCIMB 41125 as a drench, 1 day before a challenge. The treatment that received the commercial drench at 10x the dosed amount had similar concentration of VFAs as the control treatment (Wagner et al., 2022). However, several studies have shown no differences in total VFA concentrations (Henning et al., 2010a; Weimer et al., 2015). Outside of observing increased concentrations of butyrate, there are inconsistencies in VFAs from cattle fed *M. elsdenii*, which suggests that the concentration of VFAs are being affected by many factors in the rumen.

In cattle, ruminal pH results have been variable due to differences in time of dosing, amount of dosing, the acidosis challenge the cattle went through, and the transition diets the animals were adjusted to. In a study by Wagner et al. (2022), 24 ruminally canulated steers were utilized in a randomized complete block design that had 4 treatments (6 head per treatment). The four treatments consisted of control treatment that received no *M. elsdenii*, a treatment that received a commercial dose 4 days before the start of the study, a treatment that was dosed at 10 times the amount of one dose on 1 day before the study, and a treatment that received a commercial dose on 1 day before the study. The commercial dose contained 1×10^{10} CFU of *M. elsdenii*. Steers were adjusted to a common finisher diet for 32 days before the start of the challenge. The challenge that cattle underwent was a 36-hour 50% feed restriction, then they were offered 175% of

their average DMI, from the 7 days before the start of the challenge. The treatment that received the normal amount of the commercial dose 1 d before the challenge, had the largest variance in rumen pH during the challenge ($P \leq 0.06$). The treatment that was dosed 4 days before the start of the trial had the highest minimum and maximum pH compared to the other treatments throughout the duration of the study ($P \leq 0.01$), suggesting that the *M. elsdenii* dose had time to proliferate in the rumen before undergoing the acidosis challenge. In another study, Mazon et al. (2020), suggested that *M. elsdenii* needs 4-5 days in the rumen before there is an adequate supply of *M. elsdenii.*

Ellerman et al. (2017) conducted a study that contained 435 steers in a randomized complete block design with 4 treatments. The treatments consisted of cattle that received no *M. elsdenii*, cattle that were drenched with a fresh dose of Lactipro advance (fresh), cattle that received rehydrated lyophilized culture once (rehyd), and cattle that received the rehydrated lyophilized culture once in combination with lyophilized culture powder and fed as a top-dress daily (rehyd + daily). Control cattle went through a 22-day step-up period and *M. elsdenii* cattle went through a 10-day accelerated step-up period. The rehydrated culture and the dose contained 1×10^{10} CFU of *M. elsdenii*, which is the same amount fed in two of the treatments in the Wagner et al. (2022) study. The treatment that received the top-dress and the rehydrated lyophilized culture received 1×10^{10} CFU of *M. elsdenii* once and received 2.19×10^{8} CFU of *M. elsdenii* daily. For this study, 3 pH thresholds were set (5.6-5.2, 5.2-5.0, 5.0-below). During the step-up period, there were no differences in the pH thresholds that were set ($P > 0.05$). From grain adaptation until the cattle were reimplanted Ellerman et al. (2017) found that the rehyd and rehyd + daily cattle spent less time with a rumen pH of 5.5-5.6

compared to the fresh and the control treatment. Cattle that received *M. elsdenii* spent less time in an acidotic pH state compared to the control during the step-up period. Ellerman et al. (2017) suggested that when *M. elsdenii* is dosed at the correct time, it can be beneficial to the rumen during an acidosis event. McDaniel et al. (2009) showed a higher rumen pH for the first 24 hours after receiving *M. elsdenii* but after that saw no differences; however, Wagner et al. (2022) found a continuum of higher pH.

When cattle receive *M. elsdenii* there are positive effects on the animal's rumen health. As mentioned earlier, *M. elsdenii* can decrease acidosis which might affect liver abscesses (LA). There are many factors that influence liver abscesses, however LA are commonly associated with acute acidosis (Jensen et al., 1954). *Fusobacterium necrophorum (F. necrophorum)*, an anaerobic gram-negative bacterium, is the main bacteria that causes LA. The rumen wall can become damaged during acidosis which allows for bacteria such as *F. necrophorum* to invade the blood and enter into the portal vein to form lesions on the liver and eventually turn into abscesses, and decrease liver functions (Nagaraja and Chengappa, 1998). Not only do liver abscesses decrease the value of the animal at slaughter, but they also negatively impacts performance traits, such as a decrease in feed efficiency and daily gain by 9.7% and 11%, respectively (Brink et al., 1990). Because *M. elsdenii* is able to reduce lactate build up in the rumen which decreases acidosis, there should be a decrease in the damage of the rumen wall. Thus *M. elsdenii* is not impeding the production of *F. necrophorum (*Chaucheyras-Durand and Durand, 2010), just decreasing the opportunity for the bacteria to be absorbed across the rumen wall and enter the blood supply, which may result in fewer liver abscesses. However, steers who were orally dosed with *M. elsdenii* had no differences in percent of

liver abscess compared to the control treatment, from 11.8% for control to 10.8% for dosed cattle (P = 0. 75; Miller, 2013).

Transition Diets

Another factor that becomes prevalent when mitigating acidosis is the grain adaptation phase. Acidosis becomes the most prevalent during diet adaptations. Traditionally, ruminants in the feedlot industry are stepped up gradually (3-4 weeks) from a high forage diet to a high concentrate diet (HCD). However, with a shorter adaptation period, there can be an increase in efficiency of gain of the ruminants on rapidly fermentable carbohydrates (Bevans et al., 2005) and possibly decreased days on feed. A high concentrate diet will cause an increase in amylolytic bacteria, which breaks down sugars and starches, and causes a decrease in fibrolytic bacteria, which breaks down fiber and cellulose (Goad et al., 1998; Tajima et al., 2001). When animals are on a low concentrate diet (LCD)**,** for example brome grass hay, intake is controlled by gut fill. When transitioning to HCD, the animal can no longer eat to gut fill without experiencing acidosis. Instead, the animal must be gradually adjusted to increase the intake of a HCD (Nagaraja and Titgemeyer, 2007). If intake of the HCD constantly continues without allowing enough time for digestion and absorption of feed, the animal can become acidotic.

When adapting cattle at a fast rate, there is typically a decrease in DMI during the transition period and sometimes through the entire feeding phase. Accordingly, there may be a decrease in body weight (BW) if acidosis continually occurs. In a study conducted by Bevans et al. (2005), heifers were adapted from 40 to 90% barley-based concentrate diet in either 3 days, rapid adaptation (RA), or over 19 days, slow adaptation (SA). The

RA diets consisted of 65% concentrates for d 1-3 and 90% concentrates on d 4-19. The SA diets contained 48.3, 56.7, 65.0, 73.3, 81.7, and 90% concentrate, each diet fed for 3 days. Dry matter intake did not differ between treatment groups but differed greatly among individuals within the treatments. Tremere et al. (1968) and Hironaka (1969) reported a decrease in DMI among individual animals and treatment groups. In the Bevans et al. (2005) study, on certain days there was a greater range of day-to-day intake on the RA group compared to SA, which might explain why feed intake and total efficiency of the heifers were variable and not significantly different. An increase in variation of intake per individual or by treatment group, is an indicator that sub-acute ruminal acidosis is occurring (Britton and Stock, 1987; Stock et al., 1995), which may result in a 10% decrease in gain and efficiency of fattening ruminants (Galyean et al., 1992). In another study, Burrin et al. (1988), noted a 60% decrease in DMI for steers fed a 75% concentrate diet for 6 days and then a 95% concentrate diet. The authors also concluded that over the 21-day adaptation period, ADG and gain to feed efficiency decreased by 10% and 9%, respectively.

M. Elsdenii **in Transition Diets**

With the experiments discussed earlier, it leads to the conclusion that the number of step-up diets and the rate concentrates can be added to the diet, remains somewhat unclear and acidosis tends to be seen on a per animal basis rather than a group of cattle. With the addition of *M. elsdenii*, there is potential for shorter step-up periods as *M. elsdenii* has the ability to decrease acidosis. In the Ellerman et al. (2017) study, control cattle were stepped-up in 22 days and cattle fed *M. elsdenii* were stepped-up in 10 days to a finisher diet that consisted of 90% concentrates (steam-flaked corn, wet corn gluten

feed, and ground corn) and 10% corn silage. Alfalfa and corn silage decreased by 5% during each step-up diet and body weights were taken every 28 days. At d 28 cattle had similar BW, ADG, and feed efficiency ($P = 0.53$, $P = 0.71$, $P = 0.69$, respectively). Cattle that received the rehydrated lyophilized culture once in combination with lyophilized culture that was a powder and fed as a top dress daily (rehyd + daily) had a decrease in DMI compared to the control cattle ($P = 0.05$) during the step-up period. There were no differences after the accelerated step-up or during the finishing period in terms of feedlot performance, which Ellerman (2017) suggested their results were similar to the results of other studies that have completed an accelerated step-up regime (Leeuw et al., 2009; Drouillard et al., 2012; Miller, 2013). Feeding *M. elsdenii* to cattle during an accelerated step-up program can be beneficial to the animal's health without having negative effects on the animal's performance or rumen pH. MacDonald and Luebbe, (2012) suggested that 35-40% of the roughages needed in a feedlot were for the transition diets. If the step-up period could be decreased from 20-28 days to around 10 days, it could be a large difference in the amount of forages that a producer would need.

By-Products in Transition Diets

Transition diets play an important role in mitigating acidosis when stepping cattle from a high forage diet to a high concentrate diet. The dry and wet milling industry have become a popular feed source and some by-products can be beneficial during grain adaptation to a high concentrate diet (Buttrey et al., 2012; MacDonald and Luebbe, 2012; Schneider 2013; Huls et al., 2016). The use of by-products in step-up diets range from feeding Sweet Bran (Cargill Corn Milling), distillers grain, WCGF, to RAMP (Cargill Corn Milling).

In the dry milling industry, dry distillers grain (DDG), wet distillers grains with solubles (WDGS), and modified dried distillers grain with solubles (MDGS) have been utilized to transition cattle during grain adaptation. Rolfe et al. (2010) conducted a 35 d metabolism study that utilized 8 ruminally fistulated steers to determine the effect of WDGS in diet transitions. Treatments consisted of control (alfalfa hay decreased from 45% to 7.5%, DRC increased 15% to 52.5%, and WDGS was constant at 35%) and treatment (WDGS decreased from 87.5% to 35%, DRC increased from 15% to 52.5%, and alfalfa hay was constant at 7.5%). Steers were stepped-up to the finisher diet in 4 step-up diets that were each fed for 7 days and then the finishing diet was fed for 7 d. The finishing diet consisted of 52.5% DRC, 35% WDGDS, 7.5% alfalfa hay, and 5% supplement. During adaptation 1, 2, and 3, TRT had a lower DMI than CON ($P = 0.01$; $P = 0.01$; $P = 0.06$, respectively). For diet adaptation 1, no differences in rumen pH were reported, however during diet adaptation 2 and 3 the TRT treatment had a lower average rumen pH ($P = 0.01$; $P = 0.01$, respectively). No significant differences were reported for DMI or rumen pH for the adaptation 4 or during the finishing period. With a decrease in DMI and rumen pH during adaptation step 1, 2, and 3, there may not be a benefit to adapting cattle with WDGS when forage was held constant at 7.5%.

In the wet corn milling industry, there are many by-products. Wet corn gluten feed is the main component of the wet milling industry. Sweet Bran is a branded wet corn gluten feed that has more corn steep liquor, which allows for a more consistent higher energy product (Stock et al., 2000; Klopfenstein et al., 2008). Huls et al. (2016) conducted a metabolism and a finishing trial feeding WCGF (Sweet Bran) in transition diets. In the metabolism study, eight ruminally fistulated steers were utilized in a

completely randomized design. There was a 26 d grain adaptation period that utilized WCGF compared to a traditional grain adaptation with decreasing forage. Grain adaptation treatments consisted of control (alfalfa hay decreased from 45 to 15%, DRC increased from 45 to 7.5%, molasses, and supplement were included at 5%, each) or Sweet Bran (Sweet Bran decreased from 87.5 to 48.13%, DRC increased from 0 to 39.38%, and alfalfa hay and supplement were held constant at 7.5% and 5%, respectively). Steers were stepped-up to the finisher in 4 diets that were 5, 7, 7, and 7 d, then the finishing diet was fed for 7 d. The finishing diet for the control steers consisted of 82.5% DRC, 7.5% alfalfa hay, 5% molasses, and 5% supplement. For the Sweet Bran treatment, the finishing diet consisted of 52.5% DRC, 35% Sweet Bran, 7.5% alfalfa hay, and 5% supplement. The steers that were adapted with Sweet Bran had a greater DMI compared to the control steers ($P < 0.01$). Huls et al. (2016) reported that the Sweet Bran diet contains a greater concentration of crude protein and fat, and a lower neutral detergent fiber (NDF), resulting in a higher energy diet. The steers on the Sweet Bran treatment had a lower average pH, minimum pH, and a maximum pH ($P \leq 0.01$). It was suggested that the lower pH was due to the increased DMI.

Huls et al. (2016) conducted a finishing trial that consisted of 240 steers that were utilized in two treatments; CON (in a 1:1 ratio DRC and HMC increased from 3.75 to 18.75%, alfalfa hay decreased from 37.50 % to 7.50%, corn silage, and Sweet Bran was held constant at 15% and 35%, respectively) or Sweet Bran (in a 1:1 ratio DRC and HMC increased from 0 to 16.875%, Sweet Bran decreased from 80 to 46.25%, and corn silage was held constant 15%). Steers were stepped-up to the finisher in 4 diets that were 5, 7, 7, and 7 d. Then the common finisher diet (22.5% DRC, 22.5% HMC, 35% Sweet Bran,

15% corn silage, and 5% supplement) was fed for 147 d. During the 26 d diet adaption period, DMI was only significantly higher for the CON treatment during the second diet adaptation step by 0.2 kg/d ($P < 0.01$), otherwise there were no significant differences in DMI for any adaptation phase or during the entire 173 d feeding period ($P \leq 0.19$). Cattle fed Sweet Bran treatment had greater G:F and ADG ($P < 0.01$). Huls et al (2016) suggested the greater G:F and ADG was due to the added energy provided from Sweet Bran in the adaptation diets since there were no diet difference past d 26 of the study. The Sweet Bran treatment also had increased final BW and hot carcass weight (HCW; $P \leq 0.01$) compared to CON.

Another by-product produced in the wet milling industry is RAMP, a complete starter feed that contains high levels of Sweet Bran, low levels of cottonseed hulls, alfalfa hay, minerals and vitamins. Buttrey et al. (2012) utilized 306 steers in a finishing study to evaluate the effects of diet transitions, with RAMP versus a common adaptation diet. The study included 6 treatments. The traditional starter diet consisted of 32.5% SFC, 20% Sweet Bran, and 45% alfalfa hay (CON). Steam flaked corn increased and alfalfa hay decreased over 4 steps that were 5, 5, 6, and 6 d, for a total of a 22 d step-up for the control steers. There were 5 RAMP treatments that were stepped- up in either 14, 18, 22, 26, or 30 d. With each step, RAMP decreased and SFC, Sweet Bran, and alfalfa hay increased. All treatments were transitioned to a common finishing diet consisting of 65.7 % SFC, 20% Sweet Bran, 8% alfalfa hay, the remainder, 6.3%, of the diet was made up of yellow grease, limestone, urea, and supplement. Interim weights were taken for the first 36 d on feed, RAMP treatments had a greater HCW ($P = 0.01$) resulting in a greater ADG ($P = 0.01$). The length of diet adaptation between RAMP treatments had no

significant effects ($P \geq 0.20$). Interim weights were taken on d 84 and the results for d 84 weights were similar as the weights taken on d 36 of having an increased HCW and ADG for the RAMP treatment. For the entire study, cattle fed RAMP had increased ADG ($P = 0.06$), adjusted final body weight ($P = 0.05$), and hot carcass weight (HCW; $P = 0.05$) compared to CON. From that study, there is an advantage to feeding by-products from the wet milling industry. The added energy, without increasing the starch concentration in the diets, becomes important when transitioning cattle from a high forage diet to a high concentrate diet and trying to mitigate acidosis. Not only can by-products help decrease acidosis, but also could help with mill efficiency. Decreasing the amount of expensive bulky forages needed in a feedlot will increase mill production along with decreasing the production price of cattle.

RAMP Performance

Typically, when cattle are received at a feedlot, one usually thinks about feeding forages because of acidosis; however, energy plays an important role for newly received cattle on performance. Carroll and Forsberg (2007), suggested that cattle's ability to fight off disease decreased when there was a deficiency in energy. For feedlots to change the feed ingredients, there typically must be an added performance or economic benefit. By-products from the wet milling industry can be high in energy and have an added performance benefit in either ADG, G:F, HCW, or adjusted final body weight.

MacDonald and Luebbe (2012) utilized 315 steers in a 112 d finishing study to determine the effects of feeding high amounts of Sweet Bran or 3 complete starter diets during the transition to a high concentrate common finisher diet. The finisher diet consisted of 20% Sweet Bran, 66% SFC, 8% alfalfa hay, and the remaining 6% of the

diet contained yellow grease, supplement, limestone, and urea. The control diet consisted of low levels of Sweet Bran that remained constant while SFC increased, and alfalfa hay decreased (CON). The second treatment had high levels of Sweet Bran that decreased while SFC increased, and alfalfa hay remained constant (HSB). There were three treatments that were a complete starter ration that contained Sweet Bran and 1 of 3 levels of cottonseed hulls, low level (LCS), medium level (MCS), or high level (HCS). All cattle were stepped onto the same common finisher diet in 4 steps. Significant differences were reported for 28 d performance which was expected since there were diet differences. However, after d 22, all cattle were on the same diet. The MCS performed similar to CON for ADG, BW, and G:F ($P \geq 0.20$). The LCS and the HSB treatments had a lower ADG, BW, and a higher G:F ($P \leq 0.05$). However the HCS treatment had an increased ADG, BW, and a lower G:F ($P \leq 0.05$). Cattle fed any level of cottonseed hulls had an increased marbling score compared to the control treatment. There was a performance benefit to higher levels of cottonseed hulls in combination with Sweet Bran.

Schneider (2013) completed multiple studies using RAMP during diet adaptation. The complete starter diet, RAMP, was a very similar diet composition as the complete starter diet used in MacDonald and Luebbe (2012). In one experiment by Schneider, 229 yearling steers were utilized to evaluate RAMP in a 22 day diet adaptation on performance and carcass characteristics. The three treatments consisted of steers that received a traditional step-up diet (decreased in alfalfa, increased in HMC and DRC, and Sweet Bran remained constant; CON), and two treatments that were fed 100% RAMP, then RAMP decreased and DRC, HMC, and Sweet Bran increased. The two RAMP treatments were fed as 1 diet- system, fed twice a d (RAMP-1RS) or as 2 different diets

delivered separately (1st feeding was RAMP and 2nd feeding was the finishing diet; RAMP-2RS). The common finishing diet consisted of 25% Sweet Bran, 25% DRC, 37.5% HMC, 7.5% alfalfa hay, and 3% supplement. During the 22 d diet adaption period, both RAMP treatments had decreased DMI compared to CON ($P < 0.09$). During the entire feeding period, DMI were similar between all treatments ($P = 0.39$). However, both RAMP treatments had an increased G:F ($P < 0.01$) and tended to increase HCW ($P = 0.13$) compared to CON. RAMP-1RS also had an increased ADG ($P = 0.03$) compared to the control treatment. There was an added benefit in performance and carcass characteristics for feeding RAMP to cattle during diet adaptation. The increase in HCW and ADG from adapting cattle with RAMP has also been reported in other studies. (Buttrey et al. 2012; MacDonald and Luebbe, 2012).

A follow up study conducted by Schneider (2013) used 90 yearlings to determine the effect of RAMP during an accelerated step-up program on performance and carcass characteristics. Two control treatments utilized RAMP to adapt cattle to a HMC diet that contained either 25% (CON25) or 47.5% (CON47) Sweet Bran. The diet adaptation occurred over 24 d with 4 steps. Three accelerated rations were used to transition cattle from RAMP to a finisher diet that contained 47% Sweet Bran. All accelerated treatments were fed RAMP for 10 days then either 3 blends for 3 d each, 2 blends for 2 d each, or 1 blend for 4 d. No significant differences between treatments were reported for performance traits or carcass characteristics ($P \geq 0.11$). Although the results from this study do not show an increase in performances or carcass characteristics, there were no negative effects to feeding RAMP in an accelerated step-up.

Rumination and Eating

CowManager SensOor tags were originally developed on dairy cattle that consumed high forage. Some of the first validation studies for the tags were conducted on grazing dairy cattle and lactating dairy cattle (Bikker et al., 2014; Pereira et al., 2018). In a validation study by Pereira et al (2018), 24 crossbred dairy cows were utilized in a pasture-based system. The authors concluded that the sensor can accurately monitor. rumination and eating behaviors of grazing dairy cattle, but more research was needed to measure activity and behavior for the tag and for cattle consuming a high concentrate diet. Wolfger et al. (2015) utilized 18 (326 kg) steers in a validation study on a 100% barley silage diet. The authors conclude that the CowManager SensOor tags would be able to accurately measure feeding (eating) behavior; however, more research needed to be conducted on the algorithm to differentiate rumination from eating.

It is well understood that time spent ruminating is affected by DMI, diet composition, and particle size. Poppi et al. (1980) suggested that particles that are longer than 1.18 mm, according to the critical size theory, have the most resistance to passage and can stimulate chewing and rumination. Yansari et al. (2004) noted that a reduction in particle size in alfalfa led to an increase in ruminal particle passage rate. In dairy cattle, Yansari et al. (2004), reported that a decrease in alfalfa particle size resulted in a decrease in time spent eating and ruminating, in a total mixed ration containing 20, 20, 35, 7, 7.5, 10% of alfalfa, corn silage, barley, soybean meal, beetpulp and wheat bran on a DM basis, respectively. The alfalfa was included at either a large, medium, or fine particle size. In the fine particle size alfalfa diet, the cows spent 445.5 min/d ruminating and

eating and the cows on the large particle size alfalfa spent 596.7 min/d. Yansari et al. (2004), reported an average DMI of 23 kg/d for the large and fine particle alfalfa size. Feed sources with a decreased particle size, lower concentration of forage, and diets that are more digestible could lead to less time spent ruminating and eating due to the decrease in particle size, lower concentration of forages, and the more digestible diet.

Adin et al. (2009) suggested that adult lactating dairy cattle spend around 7 to 8 h/d ruminating. The cows were on a 13.5% soybean meal, 18.4% corn grain, 10.5% barley grain, and 56.7% wheat hay diet for -21 days before calving until calving. Once the cows calved, they were fed their experimental diets which consisted of 12.2% corn silage, 14.5% soybean hulls, 2.4% vetch hay, 8.9% wheat straw, 4.5% soybean meal, 15.1% ground corn meal, 13.1% ground barley grain, 6.6% DDGS and CGF, 4.6% sunflower meal, and 3.2% rapeseed meal (DM basis) for the TMR treatment. For the control treatment, corn silage increased, wheat bran was added at 3% and soybean hulls were removed. The control treatment spent 482.6 min/d ruminating and the experimental TMR treatment spent around 428 min/d ruminating. The tags that were utilized in that study were a commercial tag (Hi-Tag, SCR Engineers, Netanya, Israel).

Gentry et al., (2016) utilized 54 steers with a collar (HR Tag; SCR Dairy, Netanya, Israel) that continuously measured rumination minutes via a sensory microphone that detected the passage of a feed bolus. Corn stalks were fed at different inclusion rates and different grind lengths. The first treatment consisted of 54% SFC, 30% WCGF, 5% short-ground corn stalks, and the remaining 11% of the diet consisted of supplement, urea, limestone, and corn oil (5SG). The second treatment consisted of 54%

SFC, 30% WCGF, 5% long-ground corn stalks (5LG) The third treatment consisted of 56% SFC, 24% WCGF, and 10% short-ground corn stalks. 5SG treatment spent 245 min/d rumination which was 15 % less time than 5LG ($P \leq 0.05$) at 289 min/d and 20% less time then 10SG at 307 min/d ($P \leq 0.05$).

Spowart at el. (2022), reported the physically effective NDF (peNDF) and time spent ruminating for finishing steers on Sweet Bran and wet distillers grains with soluble (WDGS). Rumination minutes were continuously recorded and averaged within 2 h time increments using the Data Flow II program (Allfex Livestock Intellegence) Physically effective NDF measures particle size and NDF concentration to predict rumination and the stimulation from the diet. The diets in Spowart at el. (2022), were 76.6% flaked corn grain, 8% ground corn stalks, and 6% cottonseed meal. Corn oil, molasses blend, and a supplement were also included at 9.45% of the diet (control). The second diet included less flaked corn grain, and 20% WDGS and no cottonseed meal (WDGS20). The SB20 treatment was similar to WDGS20 but contained 20% Sweet Bran and no WDGS. The fourth treatment (COMBO) was a combination of WDGS20 and SB20, COMBO contained 53.2% flaked corn grain, 20% Sweet Bran, 10% WDGS, and 8% corn stalks. Physically effective NDF was highest for the COMBO diet and WDGS20 and SB20 were similar, but all three treatments were still higher than peNDF for the control treatment. For time spent ruminating and eating Spowart at el. (2022), reported a numerical increase in WDGS20, SB20, and COMBO compared to the control treatment.

Methane and Carbon Dioxide

Methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) are the three main greenhouse gases (GHG). The GHG can either be anthropogenic, emissions linked to human activity, or natural emissions which are often referred to as the Earth's natural cycle. Worldwide agriculture is responsible for 44% of total GHG (Troy et al., 2015). Agriculture is responsible for 10-12% of anthropogenic GHG (Smith et al., 2007; Todd et al., 2011). Of the 10-12% of anthropogenic GHG from agriculture, ruminants are responsible for 96% of anthropogenic methane because of enteric fermentation (Chang et al., 2019).

Methane has traditionally been assigned a carbon dioxide equivalence of 25x more potent than carbon dioxide. However, methane has a short life span in the environment (United States Environmental Protection Agency) which has an impact on its' warming potential. Methane has an atmospheric lifetime of about 12.4 years (Myhre et al. 2013), and about 80-89% of total methane in the atmosphere is broken down to CO_2 through hydroxyl oxidation (Levy, 1971; Badr et al., 1992; He et al., 2020). The CO_2 in the atmosphere is utilized by plants during photosynthesis, then the cattle consume the plants. Cattle break down the cellulose or starch from the plant, then the gases are released from the cattle back into the atmosphere, this cycle is known as the biogenic carbon cycle. Thus, the methane released from cattle is not "new" carbon, but it is recycled carbon (Liu et al., 2021). Reducing C emissions from cattle, to a level that is less than the amount of C being utilized, could allow for a cooling of the atmosphere (Liu et al., 2021).

Ruminants are able to digest cellulose, hemicellulose, starches, and sugars (carbohydrates) into high quality products that can be used for human consumption. When ruminants digest cellulose, hemicellulose, starches, and sugars, VFAs are produced in the rumen. During VFA production by-products are produced, some of the by-products are methane, carbon dioxide, and hydrogen. Through glycolysis, one 6-carbon glucose is converted into two 3-carbon pyruvates. Pyruvate can be converted into propionate without a loss of carbons because propionate is a 3-carbon VFA. When propionate is produced, there can be less of an energetic loss as propionate is a hydrogen sink that accepts hydrogen ions (Beauchemin et al., 2009). When acetate is produced there is a loss of one carbon because acetate is a 2-carbon VFA. When acetate is converted to pyruvate there is an increase in carbons and hydrogens. One carbon and four hydrogens are removed from the rumen for each molecule of methane that is formed.

Methanogenesis in the Rumen

Methanogens are able to exist in anaerobic environments that are difficult for other organisms to survive in (Hook et al., 2010). In ruminants there are 7 common methanogens (microbes) that are present in the rumen. *Methanomicrobium mobile, Methanobacterium formicicum, Methanobacterium bryantii, Methanobrevibacter ruminantium, Methanobrevibacter smithi, Methanosarcina barkeri,* and *Methanosarcina maza*i (Sirohi et al., 2010). Liu et al. (2008) noted that methanogens can only utilize substrates from organic acids. Hook et al. (2010) suggested that different species of methanogens have unique characteristics which will cause various effects within the rumen. In an anaerobic environment, H+ is the electron acceptor in the electron transport chain since oxygen is not present. The H+ accepts an electron from NADH; therefore,

producing H_2 and regenerating NAD+ (Russell, 2002). Thus, allowing the methanogens to utilize H_2 and reduce CO_2 into CH_4 is critical to maintain fermentation in the rumen (Janssen and Kirs, 2008).

When grouped by electron donors, there are four methanogenic pathways, which are described in greater detail in Welander and Metcalf (2005). In the H_2/CO_2 hydrogenotrophic pathway, gas production, as carbon dioxide, is reduced with H_2 as the electron donor. The methylotrophic pathway is similar to the hydrogenotrophic pathway as it uses H_2, however it reduces methanol to methane after a methyl group is delivered to coenzyme M. The acetoclastic pathway produced acetyl CoA because it oxidizes and reduces acetate. The methylotrophic pathway oxidizes and reduces methanol and methylamines to CO_2 and CH_4 (Welander and Metcalf, 2005).

For all of the pathways briefly described, methane is the end product. To decrease methane and carbon dioxide emissions, there must be a separate sink for H_2 or less H_2 production. When there is fermentation of glucose, that is converted to pyruvate, there will be an increase in H_2. Therefore, keeping H_2 levels low will allow for more utilization of the feed sources and there can be greater recovery of energy fermenting microorganisms (Sharp et al, 1998). Thus, decreasing the loss of energy could result in a decrease in methane; therefore, the remainder of this review of literature will focus on changes of dietary digestibility and energy to reduce methane production in the rumen.

Factors Affecting Methane

In ruminants, methane emissions can be influenced by a variety of factors such as the amount of feed consumed, the type of diet, processing method of the diet, passage

rate, the addition of lipids, oils, and changes to the microbiome (Johnson and Johnson 1995; Hook et al, 2010). Although there's a large difference between the factors affecting methane, nonetheless, all the factors influence digestibility and microbes which will affect the VFA profile in the rumen. Johnson and Johnson (1995) suggested that there would be a decrease in methane production if all the CHO's digested would be fermented to propionate and not to acetate. Wolin and Miller, (1988) indicated that the shift in A:P ratio would cause a decrease in methane by 33%.

Feed Consumption Levels

The amount of CH_4 that is produced from cattle is directly and positively correlated to the amount of feed consumed (Blaxter and Clapperton, 1965; Shibata and Terada, 2010). When the level of feed consumed increases there is an increase in CH_4 because there is an increase in fermentation which results in more methane. Blaxter and Clapperton (1965) evaluated 48 studies that feed was offered at different levels of intake. The authors reported that methane was directly influenced by the level of intake. Beauchemin and McGinn (2006) reported an increase in CH_4 on a g/d basis for cattle fed at *ad libitum* compared to cattle that were fed at 65% of *ad-libitum* intake on both a high forage diet and a high concentrate diet. Winders et al. (2020) also reported an 19% increase in CH_4 production on a g/d basis for cattle that were fed at *ad-libitum* compared to cattle that were fed at 75% of intake. Cattle fed at *ad-libitum* had 8% lower CH_4 production on a g/kg of DMI basis which suggests that there was not a biological decrease in methane production, but DMI factors into the amount of methane produced.

Quality of the Diet

The type of feed that is consumed has a great impact on time spent ruminating, digestibility (energy content), VFA concentration, and passage rate. Through glycolysis, one 6-carbon glucose is converted into two 3-carbon pyruvates. When acetate is produced there is a loss of one carbon because acetate is a 2-carbon VFA. Pyruvate can be converted into propionate without a loss of carbons because propionate is a 3-carbon VFA. When propionate is produced, there can be less of an energetic loss as propionate is a hydrogen sink that accepts hydrogen ions (Beauchemin et al., 2009). Therefore, shifting the production of VFAs from acetate to propionate could lead to a decrease in methane. Baumen et al. (1971) found 13.3 moles per day of propionate in a high forage diet compared to 31.0 moles per day in a high concentrate diet. The A:P ratio was reported as 3:1 ratio for the high forage and 1:1 for cows on a concentrate diet. Johnson and Johnson (1995) found a higher A:P when there was consumption of a high forage diet because of the increased fermentation from the cell wall CHO's in forages compared to the sugars in rapidly fermentable CHO's in a high concentrate diet.

Forage vs Concentrate Diets

Johnson and Johnson (1995) suggested that when there was an increase in fermentation of CHO, there was a reduction of methane on a per unit of gross energy (GE). When 80 – 90 % of the diet consisted of concentrates, there was a 2-3% decrease in the gross energy intake (GEI). When the diet consisted of more forages (30-40% of concentrates) the methane produced was increased to 6 to 7% of GEI (Martin et al., 2010). During the backgrounding phase, cattle are typically fed more forages to reduce acidosis. Beauchemin and McGinn (2005) reported a greater CH_4 loss per amount of GEI during the backgrounding phase, to cattle that were fed 70% barley silage or corn silage

and 30% steam rolled barley (SRB) or DRC. The finishing diet consisted of 9% barley silage and 91% SRB or DRC, and there was a 4% decrease in methane loss per unit of GEI intake. The change in the diet formation caused a shift in the A:P ratio from 2.75:1 mol for the backgrounding phase to 0.98:1 mol in the finishing phase.

Lipids and Oils

Lipids and oils are added to ruminant diets to increase the energy level of the diet. Johnson and Johnson (1995) suggested that methanogens decrease when there is an increase in lipids, due to the inhibition of protozoa, increased production of propionic acid and cellular process of biohydrogenation. Unsaturated fatty acids act as a hydrogen accepter which cause methanogens to be inhibited (Johnson and Johnson 1995; McAllister et al. 1996). During biohydrogenation, polyunsaturated fatty acids are turned into saturated fatty acids. With an increase in saturated fatty acids there is an increase in absorption, thus affecting fermentation due to the change in the microbial community (Sun et al., 2022).

When 4% canola oil was added to an 85% concentrate diet, Mathison, (1997) reported a 33% reduction in methane production. The authors reported a decrease in the digestibility of the fiber. Fat tends to decrease the digestibility of fiber, which will affect passage rate and could lead to a decrease in DMI. Dong et al. (1997) fed coconut, canola oil and cod-liver oil and reported that fiber digestion was decreased in the rumen. Nonetheless when feeding oils on a high concentrate diet, a difference in DMI was not reported. Hales et al. (2017) evaluated the effect of feeding corn oil at 0, 2, 4, and 6% DM in a diet that consisted of 0.05% urea, 10% alfalfa hay, on average 4% soybean meal, and on average 78% DRC. Soybean meal was increased as oil increased while DRC

decreased as oil increased (Hales et al., 2017). The authors reported a 34% linear decrease in methane energy loss as a proportion of GE intake was increased. Winders et al. (2020), reported a 13% decrease in CH_4 (g/d) for cattle fed corn oil (115 g/d) compared to the control (132 g/d). While methane measurements were being conducted, there were no significant differences in DMI, when cattle were fed an 80% concentrate diet.

Lipids and oils that are not ruminally protected are a methane mitigation strategy, however, there can be negative effects with the addition of lipids. When lipids comprised of over 6% (DM; NASEM, 2016) of the diet, there can be negative effects on the diet digestibility and DMI, which will hinder the efficiency of the animal (Beauchemin et al., 2009).

By-Products

The addition of by-products from the dry and wet milling industry are used to add energy and protein to the diet. By-products increase digestible energy concentration in the diet, increased performance, and by-products like Sweet Bran and RAMP can reduce acidosis.

McGinn et al. (2009) utilized 60, 381-kg Hereford steers to determine methane emissions in two treatments. Cattle were fed with 60% DM barley silage and no dried distillers with soluble (DDGS; control) or cattle were fed 60% DM barley silage and 35% DM DDGS in replacement for steam-rolled barley which was fed at 35% DM in the control diet. When methane was measured, there was no difference in DMI, but a 20% reduction in methane was reported for the cattle fed DDGS (177 g/d) compared to control (221 g/d). Although there was not a difference in DMI, there was a reduction of methane

when expressed as g/ kg of DMI for cattle fed DDGS. With the addition of DDGS, the fat content of the diet increased from 2.0 to 5.1%, so the authors suggested that the reduction of methane was from addition of fat in the diet. The NDF as a percent of DM increased from 38.5% for control to 42.4% for cattle fed DDGS. McGinn et al. (2009) reported a numerical improvement in ADG from 1.33 kg/d (control) to 1.44 kg/d for the DDGS treatment.

Terklebrhan et al. (2020) utilized 24 (10 months, 17.5 kg) Xiangdong goats to measure the effect of replacing corn meal with corn gluten feed on rumen fermentation and methane emissions. The goats were fed either 40% corn meal or 40% WCGF in combination with 40% peanut vine, 13% soybean, 3% wheat bran and the rest of the diet consisted of a supplement. The NDF of the diet, was 38.4% for the corn meal (CM) treatment and 48.4% for the WCGF treatment. Goats were adapted for 28 d and then 19 d of collection occurred, with 12 of those days for CH_4 and CO_2. No differences in DMI were reported between treatments, but the WCGF diet had lower starch intake and total-tract organic matter digestibility compared to the corn meal treatment. There was a 17% decrease in methane production measured as g/d and a 16% decrease in methane as g/kg of DMI for the WCGF treatment. The WCGF treatment also led to a 43% lower ruminal dissolved hydrogen.

In summary, there is limited literature containing by-products and the effect on methane production, nonetheless the addition of by-products in cattle diets is currently being used in the industry because of the performance benefits. Grains, lipids, oils, and by-products are all common ways to increase the digestible energy of finishing diets. The impact of reducing the carbon footprint of feedlot cattle with the use of by-products such

as DDGS, WCGF, Sweet Bran, and RAMP has not been elicited; however, by-products may have potential to reduce the carbon footprint of feedlot cattle, without impacting performance.

Summary of the Literature

Improving cattle performance always remains important to the producer. Reducing subacute acidosis could increase cattle performance as there may be improved DMI, or ADG. Past research shows that acidosis can depend on the length of transition diets, amount of forage in the diet, bunk management, blending feed sources with different rates of starch digestions, and utilizing direct fed microbes. Feeding DFMs, like *Megasphaera elsdenii* NCIMB 41123, can help decrease acidosis; however, there is limited research on feeding *Megasphaera elsdenii* NCIMB 41123 as a top dress in an adaptation program. A common feed source that is used in adaptation programs is RAMP. Past research has shown that RAMP can be used when adapting cattle to a high concentrate diet. RAMP can help decrease acidosis. It is well established that adapting cattle to a grain diet with RAMP will lead to an increase in HCW, because the digestible energy of the diet increased. Increasing the digestible energy in diets is commonly utilized in feedlots because of the improvement to cattle performance. However, a reduction on enteric methane emissions because of the more digestible diet is often overlooked. Limited research has been conducted on methane production from cattle fed higher energy diets. Therefore, the objective of the research presented in this thesis includes:

1. Evaluate encapsulated *Megasphaera elsdenii* NCIMB 41123 in an accelerated beef step-up program and an acidosis challenge model

2. Evaluate methane and carbon dioxide emissions from finishing steers on different diet adaptation strategies

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CHAPTER 2 - EVALUATION OF ENCAPSULATED *MEGASPHAERA ELSDENII* NCIMB 41123 IN AN ACCELERATED BEEF STEP-UP PROGRAM AND AN ACIDOSIS CHALLENGE MODEL

Abstract

A metabolism experiment was conducted to evaluate daily feeding of encapsulated Megasphaera elsdenii *(M. elsdenii)* NCIMB 41125 along with a one-time drench of 1×10^{11} CFU of *M. elsdenii* (Lactipro NXT, MS Biotec) on dry matter intake (DMI), *in-vitro* lactate utilization, volatile fatty acid (VFA) concentration, and lactate concentration. Ruminally cannulated British breed crossbred steers (n = 40, initial BW 437 ± 98 kg) were individually-fed a finishing diet consisting of 70% steam-flaked corn, 18% modified distillers grains plus solubles, and 7% alfalfa hay (DM basis). Treatments consisted of steers which were fed no *M. elsdenii* (CONTROL), steers drenched with the commercial dose 1×10^{11} CFU of *M. elsdenii* (LactiproNXT) on d 1 of the experiment and received no other *M. elsdenii* (DRENCH), and steers drenched with a commercial dose of LactiproNXT on d 1 of the experiment and received 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top dress (LOW), 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top dress (MEDIUM), and 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top dress (HIGH). CONTROL cattle were adapted to the finisher diet in 18 d while DRENCH, LOW, MEDIUM, and HIGH were adapted in 9 d. The experiment included 5 continuous periods that started with an accelerated step-up and ended with an acidosis challenge.

Data were analyzed with the MIXED procedure of SAS. No differences were observed for DMI in the step-up, finishing, or challenge period. During the recovery period, DMI increased by 4.6% for LOW, MEDIUM, and HIGH compared to DRENCH ($P = 0.07$). During the recovery period DMI, expressed as a percentage of pre-challenge intake, increased by 17.5% ($P = 0.05$) for LOW, MEDIUM, and HIGH compared to DRENCH. For *in- vitro* lactate utilization, tubes that contained 220 m/L of lactate were injected with rumen fluid from steers then incubated in a hot water bath. For *in- vitro* lactate utilization, d 90, 91, and 92 at h 12, a treatment effect was observed with steers in the LOW, MEDIUM, and HIGH treatments having 30% greater utilization than CONTROL ($P = 0.14$). During the step-up period, cattle given *M. elsdenii* had 3% greater butyrate ($P < 0.01$) and 4% less total VFA ($P = 0.06$) compared to CONTROL. During the feeding period, there tended to be a 3% increase in butyrate for M. *elsdenii* cattle compared to CONTROL ($P = 0.15$). For total VFA concentration, cattle fed *M. elsdenii* daily had a 10% increase compared to the DRENCH steers ($P = 0.08$). An accelerated step-up was possible with the DRENCH and daily feeding of *M. elsdenii*. Daily feeding of *M. elsdenii* in combination of a drenched dose of LactiproNXT may have a positive effect on DMI for cattle during and after an acidosis challenge event.

Keywords: Acidosis, Accelerated Step-Up, Megasphaera elsdenii

Introduction

Acidosis is one of the major causes of death in the feedlot industry. In 2015, 427,910 head of cattle died from digestive disorders (United States Department of Agriculture Animal and Plant Health Inspection Service). Digestive disorders ranged from bloat, scours, parasites, enterotoxemia, and acidosis. Acidosis can have negative

effects on the animals' dry matter intake (Britton and Stock, 1989) which may negatively impact profit. With increasing price of forage, longer days on feed, and the negative impacts on cattle performance, minimizing acidosis is important, especially during diet adaptation and during extreme weather events.

Streptococcus bovis is a gram-positive bacterium that produces lactic acid, which causes a drop in ruminal pH below 4.8, the PKA of a volatile fatty acid (VFA; Nagaraja and Titgemeyer, 2007). When cattle are not adequately adapted to a high starch diet or during extreme weather events there can be an accumulation of lactic acid in the rumen and cause severe acidosis (Beauchemin and Penner, 2009); however, extreme accumulation is not likely in the feedlot industry. In some cattle, a single incident of ruminal acidosis may have negative impacts throughout the entire finishing period, resulting in low feed intake and poor performance (Owens et al., 1988). Therefore, minimizing acidosis is important, especially during diet adaptation when acidosis is most prevalent. Traditionally, ruminants in the feedlot are adapted gradually over 3-4 weeks from a high forage to a high concentrate diet (Samuelson et al., 2016). A gradual increase of a high concentrate diet minimizes the accumulation of lactate in the rumen. Megasphaera elsdenii *(M. elsdenii)* is a lactate utilizing bacteria that has the potential to mitigate acidosis during the transition of feedlot cattle from a high-forage diet to a high concentrate diet. Therefore, the objective of this study was to evaluate the effects of LactiproNXT *(M. elsdenii)* drench or LactiproNXT drench and daily feeding of encapsulated *M. esldenii* at different rates during an accelerated step-up program, finishing period, and following an acidosis challenge event on dry matter intake, ruminal lactate, *in- vitro* lactate utilization, and ruminal VFA.

Materials and Methods

All procedures involving animal care and management were approved by the University of Nebraska Lincoln's Institutional Animal Care and Use Committee (IACUC #: 2076)

A metabolism study was conducted at the University of Nebraska-Lincoln Eastern Nebraska Research, Extension, and Education Center near Mead, NE, utilizing 40 ruminally cannulated British breed crossbred yearling steers (initial body weight = 437 ± 98 kg) to evaluate the effects of *Megasphaera elsdenii* in an accelerated step-up program and during an acidosis challenge event. Canulation surgeries were conducted on June 29th, 2021, to August 3rd, 2021. Upon completion of the last cannulations, steers had 18 days to recover before the start of feeding grass hay. Steers were fed ground smooth bromegrass hay *ad libitum* for 14 days, to mimic a yearling steer coming off pasture. Steers were implanted with 200 mg of trenbolone acetate and 20 mg estradiol (Revalor-200, Merck Animal Health) nine days before the start of the experiment. One week before the start of the experiment, steers were fed ground smooth bromegrass hay at 2% (DM basis) of BW for 7 days before weighing to minimize gut fill (Watson et al., 2013). Steers were weighed using a hydraulic squeeze chute (Silencer, Moly Manufacturing INC., Lorraine, KS) for 2 consecutive d to determine initial BW (Stock et al., 1983). Steers were blocked by cannulation date and stratified by BW within block and assigned randomly to one of five treatments (8 steers per treatment).

Treatments consisted of steers which were fed no *M. elsdenii* (CONTROL), steers drenched with the commercial dose 1×10^{11} CFU of *M. elsdenii* (LactiproNXT) on d 1 of the experiment and received no other *M. elsdenii* (DRENCH), and steers drenched with a

commercial dose of LactiproNXT on d 1 of the experiment and received 1×10^6 CFU of encapsulated *M. elsdenii* daily as a top dress (LOW), 1×10^7 CFU of encapsulated *M. elsdenii* daily as a top dress (MEDIUM), and 1×10^8 CFU of encapsulated *M. elsdenii* daily as a top dress (HIGH). The experiment included five phases: step-up period (d 1-19); finishing period (d 20-88); feed restriction (d 89, 24-h full feed restriction); challenge period (d 90, cattle were fed at 150% of max DMI from the finishing period); and recovery period (d 91-95). In the recovery period, DMI was also expressed as the percentage of pre-challenge intake (the average intake of the 9 days immediately before challenge).

DRENCH, LOW, MEDIUM, and HIGH treatments were stepped-up to the finishing diet over 9 days, while the CONTROL steers were stepped-up over 18 days. Steers were individually fed for 95 days in a Calan gate system (American Calan, Northwood, NH). Before initiation of the trial, steers were trained to use the Calan gate system for 14 d. CONTROL and DRENCH steers were housed in the same barn to prevent nose to nose contact with cattle fed *M. elsdenii* daily. Cattle that were fed *M. elsdenii* daily (LOW, MEDIUM, and HIGH) were housed together. There were separate water sources between barns to prevent cross contamination via water or nose to nose contact.

The starter diet consisted of 37% steam-flaked corn (SFC), 18% modified distillers grains plus solubles (MDGS), 40% alfalfa hay (AH), and 5% supplement (DM basis). Cattle were adapted using three diets, with SFC increasing as AH decreasing. A common finishing diet consisted of 70% SFC, 18% MDGS, 7% AH, and 5% supplement. CONTROL cattle were transitioned to the finisher diet over 18 days (6 d per diet) and the

cattle that received *M. elsdenii* were transitioned to the finisher diet in 9 d (3 d per diet). Diet and supplement composition are shown in Table 2.1. All supplements were formulated to include 33 mg/kg of monensin (Rumensin, Elanco Animal Health, Greenfield, IN) and 9.8 mg/kg of tylosin (Tylan, Elanco Animal Health). Steers were fed once daily at 0700 h and had *ad libitum* access to water. The amount of feed offered was adjusted daily to target *ad libitum* intake. Feed refusals were collected every 3 days during the step-up period, every 7 days during the finishing period, and every day during challenge and recovery period. Samples were collected at 0600 h, weighed, subsampled, and dried in a forced-air oven at 60°C (Model LBB2-21-1, Despatch, Minneapolis, MN) for 48 h (AOAC, 1999; Method 4.1.03), to correct for dry matter (DM). Individual feed ingredients were sampled weekly and dried in a forced-air oven at 60°C (Model LBB2-21-1, Despatch, Minneapolis, MN) for 48 h (AOAC, 1999; Method 4.1.03). Dried weekly feed samples were composited monthly for the duration of the experiment. The monthly samples were composited into one sample per feed ingredient and sent to a commercial laboratory (Ward Laboratories, Kearney, NE) to be analyzed for DM crude protein (CP; LECO Co.), neutral and acid detergent fiber (NDF and ADF, respectively; ANKOM Technology 1998; Mertens, 1992).

Collection procedures

Rumen fluid samples were collected every 3 days in the step-up period, every 7 days in the finishing period, and every day during challenge and recovery period at 1300 h (6 h post feeding) by hand through the rumen cannula. A small cup was inserted into the rumen and 5 subsamples from different parts of the rumen were collected. The 5 subsamples were combined in a large cup and strained through 4 layers of cheese cloth

into 3 different 50 mL conical tubes and flash frozen in liquid nitrogen to stop fermentation.

During the challenge and recovery period (d 88, 90, 91, 92) when rumen fluid was being collected, one tube of the strained rumen fluid was retained at room temperature instead of being flash frozen. Then 0.1 mL of the collected rumen fluid was injected into glass tubes that contained 220 m/L of a lactate culture to estimate *in-vitro* lactate utilization. A total of three tubes per day per animal were injected with rumen fluid. Tubes were incubated in a 38°C water bath for 0, 12, and 24 h for d 88 and for d 90-92 at 0, 12, and 18h. On d 90, 91, 92, samples were only incubated for 18 h compared to 24 h on d 89 because of the results reported by Wagner et al. (2022). After incubation, the tubes were frozen for analysis of *in-vitro* lactate utilization to determine the amount of lactate that was utilized.

Laboratory Procedures

For rumen lactate and VFA analysis rumen fluid samples were thawed at 4°C and centrifuged at 5,000 × g for 10 minutes. One mL of supernatant was pipetted into new centrifuge tubes in duplicates. Three mL of 0.013 N H_2SO_4 were pipetted in each tube and samples were refrigerated at 4°C for one hour. After refrigeration, tubes were centrifuged at 10,000 × g for 15 minutes. After centrifuging, the sample was poured into a 3mL luer-lock tuberculin syringe fitted with a 0.45 µm filter tip (25mm GDX Disposable filter, Polethersulfone Filter Media with Polypropylene Housing and a 0.45µm Pore Size). The sample was filtered through this tip into a second 3mL luer-lock tuberculin syringe fitted with a 0.22 µm filter tip (25mm GDX Disposable filter, Polethersulfone Filter Media with Polypropylene Housing and a 0.22µm Pore Size).

Then, the sample was filtered into a 2 mL glass vial for lactate and VFA analysis by high-performance liquid chromatography (HPLC). For HPLC analysis, a Dionex Ultimate 3000HPLC system (Thermo Scientific), was fitted with an Aminex HPX-87H column Catalog# 1250140 (BioRad, Hercules, CA). The column was 300 x 7.5mm, 9um particle size with 8% cross linkage. The HPLC conditions were as followed: oven temp 65°C, RID 50°C, column flow was 0.6 ml/min and run time was 30 min. The mobile phase was 5mM sulfuric acid (Sigma).

The *in-vitro* lactate utilization samples were thawed at 4°C and vortexed. One mL of supernatant were pipetted into centrifuge tubes in duplicate. 3 mL of 0.013 N H_2SO_4 was pipetted in each tube and vortexed, then samples were refrigerated at 4°C for one hour. After refrigeration, the sample was poured into a 3mL luer-lock tuberculin syringe fitted with a 0.22 µm filter tip (25mm GDX Disposable filter, Polethersulfone Filter Media with Polypropylene Housing and a 0.22 µm Pore Size). Then, the sample was filtered into a 2 mL glass vial for lactate utilization analysis by HPLC. For HPLC analysis, a Dionex Ultimate 3000HPLC system (Thermo Scientific), was fitted with an Aminex HPX-87H column Catalog# 1250140 (BioRad, Hercules, CA). The column was 300 x 7.5mm, 9um particle size with 8% cross linkage. The HPLC conditions were as followed: oven temp 65°C, RID 50°C, column flow was 0.6 ml/min and run time was 30 min. The mobile phase was 5mM sulfuric acid (Sigma).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) as a complete randomized block design with animal as the experimental unit. Repeated measures were used within the step-up period (d 1-19), finishing period (d 20-

88), and recovery period (d 91-93). However, the challenge period (d 90) was not repeated because it was one day. The periods that contained multiple days were analyzed using covariant regression to evaluate linear and quadratic treatment effect over time. The model included treatment, day, day × day, treatment × day, and treatment × day × day. For the periods with only a single day (challenge period), only treatment and block were included in the model. Data were also tested for linear and quadratic effects of dose with DRENCH as the intercept. The following contrasts were reported: CONTROL versus Mega E (any cattle that received *Megasphaera elsdenii*, DRENCH, LOW, MEDIUM, HIGH*)*, DRENCH versus daily LOW (DRENCH + 1×10^6 CFU of encapsulated *M. elsdenii*), MEDIUM (DRENCH + 1×10^7 CFU of encapsulated *M. elsdenii)*, and HIGH (DRENCH + 1×10^8 CFU of encapsulated *M. elsdenii*), linear, and quadratic effects. Proc IML was used to determine contrast coefficient for unequal spacing. For rumen lactate concentration, the repeated measure structure was chosen first based off the lowest Akaike Information Criterion (AIC), then the model statement of treatment, day, day × day, treatment × day, and treatment × day × day was determined based off significance for each period of the study. For rumen VFAs, the repeated measure structure was chosen first based off the lowest AIC, then the model statement of treatment, day, day × day, treatment × day, and treatment × day × day was determined based off significance for each period of the study and for each VFA. For *in-vitro* lactate utilization, treatment × day × hour interaction was not significant, so lactate concentrations were analyzed as a covariate with h as the covariate with a mixed model with treatment × hour as a fixed effect. Day 90, 91, and 92 were analyzed as a repeated measure. Since individual repeated measure structures and model statements were selected, the means reported were

from LSMEANS and not arithmetic means. Statistical significance was declared at $P \leq 0.10$ and a tendency at $P \leq 0.15$.

Results and Discussion

One steer from both the DRENCH and HIGH treatments were removed from the experiment. The steer on the HIGH treatment died due to infection near the bladder and the steer on the DRENCH treatment lost all rumen contents twice, so it was presumed that no *M. elsdenii* remained in the rumen from the drench on d 1. On day 27 of the trial, all cattle were treated with tulathromycin (Draxxin, Zoetis Inc., Florham Park, NJ) due to respiratory concerns. Due to complications with rumen cannulas, when a steer lost a cannula and there was a subsequent decrease in DMI, that steer's intake measurement was removed from the data for 3 consecutive days after the loss of the cannula. There were 9 incidences of lost cannulas throughout the duration of the study.

Intake

During the step-up period, there were no significant differences between CONTROL and any steers that received *M. elsdenii* and no linear or quadratic effects of *M. elsdenii* dose for DMI ($P \geq 0.45$; Table 2.2). Leeue et al. (2009) and Drouillard et al. (2012) also reported no differences in DMI for cattle that received *M. elsdenii* on an accelerated step-up program. Brown et al. (2006), in a review study, reported negative impacts on DMI when cattle had *ad libitum* access to a high concentrate diet during an adaptation program that was shorter than 14 days. Miller (2013) conducted multiple studies dosing *M. elsdenii*. In one study by Miller (2013), the authors reported a decrease in DMI during the first 30 days of the feeding period after cattle fed M. *elsdenii* were adapted using an accelerated step-up ($P = 0.09$); however, there were no differences

reported for DMI over the 129 days of the study. In another study conducted by Miller (2013), the authors reported an increase in DMI after an accelerated step-up program for animals that received *M. elsdenii* compared to the CONTROL animals, which also follows the results of Henning et al. (2010a). Overall, there appears to be no negative impacts on DMI for cattle adapted using an accelerated step-up period when receiving *M. elsdenii*. When the net energy for gain intake (Mcal per d) was calculated for the step-up period, *M. elsdenii* treatments tended to have a greater amount of NEg ($P = 0.12$). The increase in energy available for gain was a result of the accelerated step-up, which resulted in an increase in SFC and decrease in alfalfa.

There were no significant differences among treatments for DMI during the finishing period ($P \geq 0.16$). Drouillard et al. (2012) reported no differences in DMI during the finishing period ($P \geq 0.24$) after cattle experienced an accelerated step- up period (8 days). In another study where cattle that experienced an accelerated step-up program and were dosed with *M. elsdenii,* there was no negative effect for DMI and ADG (Miller, 2013). There were no significant differences among treatments for DMI on challenge day ($P \geq 0.30$). On challenge day, the average DMI was 20.9 kg across all treatments. The cattle consumed 130% of the 150% of the feed they were offered, suggesting that intake was not limiting during the challenge. During the finishing period and on challenge day Wagner et al. (2022) did not report any differences in DMI between treatments.

In the recovery period, the daily dosed *M. elsdenii* treatments had a 22% greater DMI after the acidosis event compared to the DRENCH treatment ($P = 0.07$). Dry matter intake tended to increase linearly as the dose of *M. elsdenii* increased ($P = 0.11$),

primarily due to DRENCH having a lower DMI compared to LOW, MEDIUM, and HIGH. In addition, when DMI was expressed as the percentage of pre-challenge intake, the daily dosed cattle consumed a higher percentage of intake ($P = 0.05$) compared to the DRENCH treatment. When the dose of *M. elsdenii* increased, DMI increased linearly ($P = 0.06$), when recovery intake was expressed as a percentage, this was due to the low DMI percentage from the DRENCH treatment. Cattle that received *M. elsdenii* daily recovered their DMI following the acidosis event faster than the DRENCH and CONTROL treatments, which suggests that M. elsdenii from the LactiproNXT drench may not be prevalent in the rumen later in the feeding period. However, feeding cattle *M. elsdenii* daily to ensure the population of *M. elsdenii* remains established in the rumen can be beneficial during and after an acidosis event.

Rumen Lactate Concentration

In an acidosis challenge, Krehbiel et al. (1995) reported D (-) and L (+) lactate concentration levels between 10 and 110 mM for the control and for the wethers dosed with 6, 12, or 18 g/kg BW of glucose. The concentration of lactate observed in the current study were low compared to Krehbiel et al. (1995). During the step-up, cattle that received *M. elsdenii* had a 93% increase in rumen lactate concentration compared to the CONTROL treatment ($P = 0.02$; Table 2.3). An increase in rumen lactate was expected because cattle fed *M. elsdenii* went through an accelerated step-up. Additionally, the cattle fed *M. elsdenii* daily had a 78% increase in lactate concentration compared to the DRENCH treatment ($P = 0.03$) which resulted in a linear increase in the lactate concentration as the dosed amount of *M. elsdenii* increased ($P = 0.03$). No statistical differences were observed among treatments during the feeding period or on the

challenge day for rumen lactate concentration ($P \geq 0.26$). For the recovery period, DRENCH had a 78% lower concentration of rumen lactate compared to the daily dosed treatments ($P = 0.07$). In the recovery period, cattle fed *M. elsdenii* daily had a linear increase for rumen lactate concentration compared to DRENCH cattle ($P = 0.10$). Wagner et al. (2022) reported no differences in lactate concentration during an acidosis challenge day or during the recovery for the acidosis event ($P \geq 0.64$). The lactate concentrations that Wagner et al. (2022) reported were smaller than the concentrations that were observed in this study. However, the differences in rumen lactate concentrations are hard to identify unless observed in greater concentrations. Measuring rumen lactate concentration may not be an accurate representation of acute acidosis unless greater concentrations are observed.

In-Vitro Lactate utilization

For *in-vitro* lactate utilization on d 88 (pre-challenge), the daily dosed treatments tended to decrease *in-vitro* lactate utilization at 12 h of incubation by 46% compared to the DRENCH treatment with a 42% decrease in the utilization of lactate compared to the CONTROL ($P = 0.13$, Table 2.4). On d 90, 91, and 92, the daily fed *M. elsdenii* cattle had a 30% greater utilization compared to CONTROL at h 12 ($P < 0.05$; Table 2.5), with the MEDIUM treatment having the greatest utilization compared to all treatments ($P < 0.05$). At h 12, the average lactate of LOW and HIGH was 81.2 mmol compared to DRENCH at 97.0 mmol for a difference of 15.8 mmol of lactate for LOW and HIGH compared to DRENCH, while the difference between MEDIUM compared to DRENCH was 43.0 mmol of lactate. On d 90, 91, and 92, a treatment by hour interaction was observed ($P = 0.07$). As expected, there was an hour effect ($P < 0.01$) for d 88 and d 90,

91, and 92 (Table 2.4 and 2.5). The 0 h measurements across all days and treatments were similar which indicates that the tubes were accurately injected with 0.1 ml of rumen fluid. The 0 h measurements were similar to the 0 h *in-vitro* lactate utilization measurements found in Wagner et al. (2022). On pre-challenge day (-2), Wagner et al. (2022) reported 113 mmol of lactate for CONTROL (CON) at h 12, which is similar to the 127 mmol for CONTROL at h 12 observed in this study. However, for the three treatments dosed with *M. elsdenii*, at h 12, Wagner et al. (2022) reported 120, 127, 119 mmol of lactate, which are greater concentrations of lactate than we observed and are lower utilization values than our study. The daily fed *M. elsdenii* treatments had greater utilization (LOW, MEDIUM, HIGH of 98, 82, 88 mmol, respectively), at h 12 for the pre challenge day compared to Wagner et al. (2022). Feeding *M. elsdenii* daily potentially allowed *M. elsdenii* to continuously proliferate in the rumen, leading to a greater lactate utilization in the current study compared to Wagner et al. (2022), where *M. elsdenii* was only dosed once one day before at a commercial dose or, 4 days before an acidosis challenge. In the Wagner et al. (2022) study, *M. elsdenii* may not have had the opportunity to proliferate in the rumen before the acidosis challenge. Daily feeding of *M. elsdenii* before an acidosis challenge may result in greater lactate utilization from the rumen and subsequently minimize the drop in ruminal pH and overall severity of the acidotic event. As a result, daily feeding of *M. elsdenii* may allow the animal to return to pre-challenge intake at a faster rate post acidosis event.

Rumen Fluid Volatile Fatty Acid

In the step-up period, cattle that received *M. elsdenii* tended to have a lower proportion of acetate compared to CONTROL ($P = 0.12$, Table 2.6). This response would

be expected because the CONTROL cattle were stepped up over 18 days and there was a greater percentage of forage in the CONTROL steer's diet for more days compared to the cattle that received *M. elsdenii* as they were stepped-up in 9 days. The proportion of acetate for DRENCH (53.6%) was not significantly different from CONTROL (53.3%). The LOW (51.9%) and MEDIUM (52.0%) treatments were about 3% lower proportion than DRENCH and HIGH was about 4% lower proportion than DRENCH. This was intriguing considering the DRENCH cattle were on the same diet as the daily treatments. The DRENCH steers also had a lower lactate concentration compared to the daily dosed cattle; therefore, it is possible that less lactate was being converted to propionate or butyrate. As a result, the proportion of acetate in the rumen was numerically greater for the DRENCH cattle compared to the daily fed *M. elsdenii* cattle.

Cattle that received *M. elsdenii* had a higher proportion of propionate compared to the CONTROL treatment ($P < 0.01$) for the step-up period which was expected due to the increase in concentrates in the diet of the cattle that went through an accelerated step-up. Numerically, the cattle fed *M. elsdenii* daily had a greater proportion of propionate compared to the DRENCH cattle; however, it was not significantly different ($P = 0.54$). A lower proportion of butyrate for the CONTROL treatment was observed compared to the cattle that received *M. elsdenii* ($P < 0.01$). The increase in butyrate has been reported in other studies for cattle that received *M. elsdenii* (Henning et al., 2010a; Weimer et al., 2015; Wagner et al., 2022).

For the total VFA concentration during the step-up period, DRENCH and CONTROL were not significantly different from each other ($P \geq 0.10$); however, LOW and MEDIUM were lower than DRENCH ($P \leq 0.10$). As a result, the daily fed cattle had

6% lower total VFA concentration compared to the DRENCH cattle in the step-up period ($P = 0.01$). The *M. elsdenii* cattle had 4% lower concentration in total VFA compared to the CONTROL treatment ($P = 0.06$). In the step-up, no differences in DMI were observed so there may have been less VFA absorption occurring in the rumen of the CONTROL cattle. Since the CONTROL cattle did not experience the accelerated step-up, CONTROL cattle may not have experienced a drop in ruminal pH. Possibly less VFAs would be in the undissociated form to be absorbed across the rumen wall (Bergman, 1990). For total VFA concentration linear and quadratic effects were observed with DRENCH having the greatest concentration ($P = 0.04$). However, DRENCH (113.2mM) and HIGH (109.7 mM) were not different from each other ($P > 0.10$), but they were greater than LOW (105.0 mM) and MEDIUM (104.9 mM; $P \leq 0.10$). No significant differences were observed for the acetate to propionate ratio (A:P), however DRENCH tended to have a greater A:P concentration during step-up compared to the daily fed cattle ($P = 0.14$; Table 2.7).

During the feeding period, the proportion of acetate for the CONTROL treatment was 3% lower compared to the cattle that received *M. elsdenii* ($P = 0.07$). During the feeding period, the CONTROL treatment had a 4% increase in the propionate proportion compared to the cattle that received *M. elsdenii* ($P < 0.01$). The LOW treatment (45.0%) was greater than HIGH (42.7%; $P \leq 0.10$), however LOW was not different then DRENCH, (43.9%) or MEDIUM (44.2%, $P > 0.10$) which resulted in a quadratic effect ($P < 0.01$). *M. elsdenii* cattle tended to increase the proportion of butyrate ($P = 0.15$) compared to CONTROL. For total VFA concentration, a quadratic effect was observed ($P = 0.10$) with DRENCH having the lowest VFA concentration followed by LOW, and

HIGH, with MEDIUM having the greatest concentration of total VFA. The CONTROL treatment had a 6% lower A:P compared to cattle that received *M. elsdenii (P* = 0.02). The MEDIUM and HIGH treatments were significantly different from CONTROL ($P \leq 0.10$), but not different from DRENCH or LOW. As a result, no differences were observed between the daily fed cattle and DRENCH cattle ($P = 0.60$). However, there was a quadratic effect ($P = 0.05$) with LOW having the lowest A:P of the *M. elsdenii* cattle during the feeding period.

On challenge day, no significant differences were observed between acetate, propionate, total VFA concentration, or the A:P ($P \geq 0.20$). However, there tended to be a quadratic effect for butyrate during the challenge day ($P = 0.11$). The LOW treatment had the lowest proportion of butyrate (4.9%) which was similar to CONTROL (5.3%), then followed by MEDIUM, DRENCH, and HIGH. The numerical increase in butyrate for DRENCH, MEDIUM, and HIGH follows the results of other studies that have used *M. elsdenii* NCIMB 41125 (Henning et al., 2010; Weimer et al., 2015; Wagner et al., 2022).

During the recovery period, CONTROL had a greater proportion of acetate compared to the *M. elsdenii* cattle ($P = 0.05$). The greater proportion of acetate was not biologically meaningful as the average proportion for CONTROL was 45.8% and 45.7% for the *M. elsdenii*. The CONTROL cattle had a tendency for a 19% increase in the proportion of propionate compared to the *M. elsdenii* cattle ($P = 0.11$). No significant differences were observed for butyrate between any treatments ($P \geq 0.36$). For total VFA concentration, approximately a 10% increase for the cattle fed *M. elsdenii* daily compared to DRENCH ($P = 0.08$). This may suggest that the LactiproNXT drench given on d 1 was not active in the rumen 90 days later. However, other studies did not report any

significant differences in total VFA concentrations. The DRENCH cattle tended to have a greater A:P compared to the daily fed cattle ($P = 0.12$).

Conclusion

Acidosis is a major concern in feedlot cattle; thus it is important to find ways to mitigate or decrease the severity of acidosis. One of the traditional ways to mitigate acidosis is transitioning cattle slowly from a high forage to a high concentrate diet. However, this requires feeding more forages for a longer period, which increases the price of production. Feeding Megasphaera elsdenii NCIMB 41125 daily as a top dress in combination of drenching with LactiproNXT or just drenching with LactiproNXT may allow producers to minimize the amount of forage needed for transitions by implementing an accelerated step-up program. No negative effects were observed for cattle that received *M. elsdenii* on a 9-day accelerated step-up program compared to cattle that were stepped up using a traditional 18-day transition. The accelerated step- up did not affect DMI. Transitioning feedlot cattle in 9 days would significantly reduce the amount of forage that a feedlot would have to purchase, which would decrease the cost of feeding cattle. Differences in DMI were not observed during the feeding period for cattle fed *M. elsdenii* compared to the CONTROL cattle. Acidosis can occur on a per head basis so there is potential to still decrease the severity of acidosis without noticing when cattle are on a finishing diet. Feeding *M. elsdenii* daily may be beneficial during a winter storm, reimplant day, or any event that causes changes or delays in the cattle's feeding schedule. After an acidosis challenge event, cattle that were fed *M. elsdenii* daily had a faster recovery to pre-challenge dry matter intake compared to the DRENCH cattle. The daily fed *M. elsdenii* treatments also had greater utilization of lactate compared to the

DRENCH and CONTROL which results in the daily fed *M. elsdenii* treatments to utilize the lactate sooner after an acidosis event, allowing the cattle's DMI to return to normal. These data may suggest that LactiproNXT drench alone may not remain active in the rumen throughout the entire feeding period and thus cattle experiencing an acidosis challenge late in the feeding period may benefit from the *M. elsdenii* top dress fed daily in addition to the drench. Overall, there is a benefit to feeding *M. elsdenii* with a drench to reduce acidosis and an accelerated step-up is possible with *M. elsdenii* as a drench or as a drench plus top dress.

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Table 2.1. Dietary composition (% of DM) from step 1 through the finishing diet

Ingredient	Step 1	Step 2	Step 3	Finisher
Steam-flaked corn	37	52	62	70
Modified dried distillers grains plus solubles	18	18	18	18
Alfalfa Hay	40	25	15	7
Supplement[1]				
Fine ground corn	2.202	2.202	2.202	2.202
Limestone	1.680	1.680	1.680	1.680
Urea	0.600	0.600	0.600	0.600
Salt	0.300	0.300	0.300	0.300
Tallow	0.125	0.125	0.125	0.125
Beef Trace mineral premix[2]	0.050	0.050	0.050	0.050
Rumensin- 90 premix[3]	0.165	0.165	0.165	0.165
Vitamin A-D-E[4]	0.015	0.015	0.015	0.015
Tylan- 40 premix[5]	0.011	0.011	0.011	0.011
NEg, Mcals,/d[6]	61.20	68.50	73.40	77.40
Chemical Composition, %[7]				
Neutral detergent fiber	30.9	24.2	19.7	16.1
Acid detergent fiber	20.0	14.2	10.4	7.3
Crude protein	16.5	15.2	14.3	13.6

[1] Supplement fed at 5% of dietary DM for all treatments.
[2]Premix contained 6.0% Zn, 5.0% Fe, 4.0% Mn, 2.0% Cu, 0.29% Mg, 0.2% I, 0.05% Co.
[3] Supplement formulated to provide 33 mg/kg of monensin (Rumensin ®, Elanco Animal Health, DM Basis).
[4]Premix contained 30,000 IU vitamin A, 6,000 IU vitamin D, 7.5 IU vitamin per gram.
[5] Supplement formulated to provide 9.8 mg/kg of tylosin (Tylan ®, Elanco Animal Health, DM Basis)
[6] Based on the 2016 revised NASEM.
[7] Based on monthly composites, analyzed for each ingredient. Sample analysis was conducted at Ward Laboratories (Kearney, NE). All values are presented on a DM basis.

Table 2.2 Dry matter intake of ruminally cannulated steers dosed with *Megasphaera Elsdenii*

Item	Treatments[7]						*P*-value			
	Control	Drench	Low	Medium	High	SEM	Control vs Mega E[8]	Drench vs Daily[9]	Linear[10]	Quadratic[11]
Step-up DMI, kg[1]	9.6	9.4	9.5	9.4	9.2	0.26	0.51	0.84	0.71	0.45
Step-up DMI, NEg Mcals /d[2]	6.9	7.0	7.4	6.8	7.0	0.28	0.12	0.32	0.42	0.42
Finishing DMI, kg[3]	13.1	12.1	12.7	12.8	12.5	0.54	0.36	0.48	0.69	0.16
Challenge DMI, kg[4]	20.6	19.8	21.5	20.9	21.8	1.35	0.76	0.30	0.30	0.99
Recovery DMI, kg[5]	10.4	8.8	12.0	10.8	10.9	1.12	0.85	0.07	0.11	0.26
Recovery DMI, % of pre-challenge intake[6]	78.3	68.8	88.7	83.2	86.9	7.7	0.64	0.05	0.06	0.57

[1] DMI for day 1-18
[2] DMI for day 1-18 expressed as Mcal per day of net energy for gain intake, based on the 2016 revised NASEM
[3] DMI for day 20-88
[4] DMI for day 90
[5] DMI for day 91, 92, and 93
[6] Recovery DMI, % of pre-challenge intake, is expressed as % of the average intake of the 9 d immediately before to challenge
[7] Control, received no *M. elsdenii*; Drench, received 1×10^{11} CFU of *M. elsdenii* as a drench on d 1; Low, received drench on d 1 + 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top-dress; Medium, received drench on d 1 + 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top-dress; High, received drench on d 1 + 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top-dress
[8] Control versus Drench, Low, Medium, and High
[9] Drench versus Low, Medium, and High
[10] Linear effect of dose (Low, Medium, and High) with Drench as the intercept
[11] Quadratic effect of dose (Low, Medium, and High) with Drench as the intercept

Table 2.3. Rumen lactate concentration (mM) of ruminally cannulated steers dosed with *Megasphaera Elsdenii* during an accelerated step-up and acidosis challenge[1]

Item	Treatment[6]					SEM	*P*-value			
	Control	Drench	Low	Medium	High		Control vs Mega E[7]	Drench vs Daily[8]	Linear[9]	Quadratic[10]
Period										
Step-Up[2]	0.0	0.1	0.4	0.4	0.4	0.12	0.02	0.03	0.03	0.63
Feeding[3]	0.3	0.3	0.4	0.3	0.4	0.09	0.99	0.38	0.43	0.87
Challenge[4]	0.6	0.0	3.9	0.5	1.2	1.91	0.71	0.40	0.56	0.26
Recovery[5]	0.5	0.2	1.0	0.7	0.6	0.21	0.59	0.07	0.10	0.36

[1] Means are reported from repeated measures estimates and may not match arithmetic means for each period
[2] day 1-18
[3] day 19-88
[4] day 90
[5] day 91-94
[6] Control, received no *M. elsdenii*; Drench, received 1×10^{11} CFU of *M. elsdenii* as a drench on d 1; Low, received drench on d 1 + 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top-dress; Medium, received drench on d 1 + 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top-dress; High, received drench on d 1 + 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top-dress
[7] Control versus Drench, Low, Medium, and High
[8] Drench versus Low, Medium, and High
[9] Linear effect of dose (Low, Medium, and High) with Drench as the intercept
[10] Quadratic effect of dose (Low, Medium, and High) with Drench as the intercept

Table 2.4. Utilization of lactate[1] over time from rumen fluid collected on d 88

	Treatments[2]						*P*-value		
Incubation time, h	Control	Drench	Low	Medium	High	SEM	Treatment	Hour	Treatment x Hour
0	146.6	148.2	145.0	143.7	145.3	10.66	0.13	<0.01	0.18
12	126.8[a]	130.8[a]	98.3[b]	82.2[b]	87.8[b]	10.66			
24	0.0	0.0	0.0	0.0	0.0	10.66			

[a,b] Means within a row that lack a common superscript differ ($P \leq 0.05$) when F-test is significant.

[1] Lactate values are reported in mmol of lactate.

[2] Control, received no *M. elsdenii*; Drench, received 1×10^{11} CFU of *M. elsdenii* as a drench on d 1; Low, received drench on d 1 + 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top-dress; Medium, received drench on d 1 + 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top-dress; High, received drench on d 1 + 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top-dress

Table 2.5. Utilization of lactate[1] over time from rumen fluid collected on d 90, 91, and 92

	Treatments[3]						*P*-value[2]		
Incubation time, h	Control	Drench	Low	Medium	High	SEM	Treatment	Hour	Treatment x Hour
0	146.8	145.9	146.2	144.8	144.8	8.64	0.14	<0.01	0.07
12	102.6[a]	97.0[ab]	83.8[b]	54.0[c]	78.7[b]	8.64			
18	10.4	23.2	29.1	13.7	9.2	8.64			

[a,b] Means within a row that lack a common superscript differ ($P \leq 0.05$) when F-test is significant.

[1] Lactate values are reported in mmol of lactate.

[2] The model included day as the repeated measure animal as the subject, and compound symmetry as the covariance structure the treatment × day × hour interaction was tested before selecting the repeated model ($P = 1.00$).

[3] Control, received no *M. elsdenii*; Drench, received 1×10^{11} CFU of *M. elsdenii* as a drench on d 1; Low, received drench on d 1 + 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top-dress; Medium, received drench on d 1 + 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top-dress; High, received drench on d 1 + 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top-dress

Table 2.6. Rumen concentration of volatile fatty acids (molar proportions) from ruminally cannulated steers dosed with *Megasphaera Elsdenii* during an accelerated step-up and acidosis challenge[1]

Item	Treatment[6]					SEM	P-Value			
	Control	Drench	Low	Medium	High		Control vs Mega E[7]	Drench vs Daily[8]	Linear[9]	Quadratic[10]
Acetate										
Step-Up[2]	53.3[b]	53.6[b]	51.9[a]	52.0[a]	51.6[a]	0.588	0.12	0.84	0.97	0.56
Feeding[3]	45.5[a]	46.8[ab]	45.9[ab]	47.2[c]	46.9[b]	0.615	0.07	0.89	0.88	0.25
Challenge[4]	51.4	54.1	52.9	52.8	54.2	2.069	0.33	0.73	0.82	0.57
Recovery[5]	45.8	47.2	45.4	44.3	45.7	1.480	0.05	0.19	0.22	0.56
Propionate										
Step-Up	37.1[a]	37.1[a]	39.4[b]	38.2[a]	38.2[a]	0.724	<0.01	0.54	0.48	0.70
Feeding	45.9[c]	43.9[ab]	45.0[bc]	44.2[b]	42.7[a]	0.560	<0.01	0.90	0.57	<0.01
Challenge	43.4	39.1	42.2	40.5	38.8	2.209	0.17	0.59	0.78	0.23
Recovery	47.2	44.3	44.8	45.5	44.7	1.156	0.11	0.50	0.61	0.41
Butyrate										
Step-Up	9.4[a]	9.3[a]	8.7[a]	10.3[b]	10.2[b]	0.553	<0.01	0.31	0.41	0.43
Feeding	8.8	9.7	9.3	9.1	9.6	0.446	0.15	0.49	0.57	0.51
Challenge	5.3	6.8	4.9	6.7	7.0	0.927	0.27	0.61	0.89	0.11
Recovery	8.4[a]	8.2[a]	8.0[a]	10.2[b]	9.1[a]	0.978	0.65	0.45	0.36	0.60
Total, mM										
Step-Up	112.8[b]	113.2[b]	105.0[a]	104.9[a]	109.7[ab]	2.377	0.06	0.01	0.04	0.04
Feeding	130.1	125.8	131.4	135.4	132.3	4.835	0.39	0.93	0.65	0.10
Challenge	135.1	135.9	142.8	140.2	141.4	7.353	0.55	0.54	0.60	0.81
Recovery	118.8[a]	118.7[a]	134.1[b]	134.1[b]	126.5[ab]	6.247	0.16	0.08	0.13	0.20

[abc] Means without common superscripts are different ($P \leq 0.10$) when F-test is significant.

[1] Means are reported from repeated measures estimates and may not match arithmetic means for each period

[2] day 1-18

[3] day 19-88

[4] day 90

[5] day 91-94

[6] Control, received no *M. elsdenii*; Drench, received 1×10^{11} CFU of *M. elsdenii* as a drench on d 1; Low, received drench on d 1 + 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top-dress; Medium, received drench on d 1 + 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top-dress; High, received drench on d 1 + 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top-dress

[7] Control versus Drench, Low, Medium, and High

[8] Drench versus Low, Medium, and High

[9] Linear effect of dose (Low, Medium, and High) with Drench as the intercept

[10] Quadratic effect of dose (Low, Medium, and High) with Drench as the intercept

Table 2.7. Rumen concentration of acetate to propionate ratio (mM) from ruminally cannulated steers dosed with *Megasphaera Elsdenii* during an accelerated step-up and acidosis challenge[1]

Item	Treatment[6]					SEM	*P*-value			
	Control	Drench	Low	Medium	High		Control vs Mega E[7]	Drench vs Daily[8]	Linear[9]	Quadratic[10]
Period[5]										
Step-up[1]	1.9[ab]	1.9[b]	1.8[a]	1.8[a]	1.8[a]	0.041	0.33	0.14	0.18	0.42
Feeding[2]	1.0[a]	1.1[abc]	1.0[ab]	1.1[bc]	1.1[c]	0.025	0.02	0.60	0.32	0.05
Challenge[3]	1.2	1.4	1.3	1.4	1.5	0.137	0.25	0.79	0.99	0.22
Recovery[4]	1.0[ab]	1.1[b]	1.0[ab]	1.0[a]	1.0[ab]	0.056	0.74	0.12	0.21	0.13

[abc] Means without common superscripts are different ($P \leq 0.10$) when F-test is significant.

[1] Means are reported from repeated measures estimates and may not match arithmetic means for each period

[2] day 1-18

[3] day 19-88

[4] day 90

[5] day 91-94

[6] Control, received no *M. elsdenii*; Drench, received 1×10^{11} CFU of *M. elsdenii* as a drench on d 1; Low, received drench on d 1 + 1×10^{6} CFU of encapsulated *M. elsdenii* daily as a top-dress; Medium, received drench on d 1 + 1×10^{7} CFU of encapsulated *M. elsdenii* daily as a top-dress; High, received drench on d 1 + 1×10^{8} CFU of encapsulated *M. elsdenii* daily as a top-dress

[7] Control versus Drench, Low, Medium, and High

[8] Drench versus Low, Medium, and High

[9] Linear effect of dose (Low, Medium, and High) with Drench as the intercept

[10] Quadratic effect of dose (Low, Medium, and High) with Drench as the intercept

CHAPTER 3 - USING RAMP VERSUS A TRADITIONAL FORAGE GRAIN ADAPTATION STRATEGY ON METHANE AND RESPIRED CARBON DIOXIDE

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Abstract

An experiment was conducted to evaluate RAMP (Cargill Corn Milling, Blair, NE) and a traditional forage adaptation program on methane (CH_4) and carbon dioxide (CO_2) emissions, performance, and carcass characteristics of finishing beef cattle. British × Continental crossbred steers (n = 64 initial BW 347 ±7 kg) were used to evaluate 2 adaptation treatments, using RAMP or a traditional forage diet. Cattle were fed 100% RAMP or 43% forage during step 1. All cattle were adapted over 22 d to a finisher diet consisting of 65.5% steam flaked corn (SFC), 22.5% Sweet Bran, 8% wheat straw, and 4% supplement (DM basis). Steers were fed 4 step-up diets, step 1 (7 d) and step 2, 3, 4 (5 d each). There were 4 paired replications that were adapted together and rotated through a two-chamber emissions barn in 5-day cycles to measure CH_4 and CO_2 at 3 time points: step one of grain adaptation, 1 week after starting the finishing diet, and 13 weeks after starting the finishing diet. All data were analyzed using the MIXED procedure of SAS. Treatment and BW block were fixed effects for performance and emissions data. Rumination data were analyzed as a repeated measure. Feeding RAMP during step 1 led

to a 12% decrease in CH_4, in g/d ($P = 0.03$) and decreased CH_4:CO_2 ($P = 0.02$). Steers fed RAMP during step 1 had an 8% increase in CO_2 in g/day ($P = 0.03$) due to greater digestibility of the RAMP diet compared to forage as intakes were similar ($P = 0.95$). Steers fed RAMP spent 45% less time ruminating and eating compared to CONTROL ($P = 0.01$) during step 1. For emissions while on the common finishing diet, steers that had been adapted using RAMP had 9 % lower CH_4 emissions as g/d ($P < 0.01$), 8% lower CH_4 emissions as g/kg DMI ($P = 0.03$) and a lower CH_4:CO_2 ($P <0.01$), suggesting a carryover effect from adaptation. For cattle performance, steers fed RAMP tended ($P = 0.10$) to have a greater HCW and final BW. Feeding RAMP to cattle during the grain adaptation phase resulted in a 12% decrease in methane emission (g/d) which carried over to 9% less CH_4 during the finishing phase while improving productivity.

Keywords: RAMP, feedlot cattle, GHG emissions

Introduction

Reducing greenhouse gas emissions from the agriculture sector has become important to some consumers. This means producers must try to decrease enteric methane emissions from ruminant animals without negatively impacting beef production. The livestock industry produces 14.5% of global greenhouse gas emissions (Gerber et al., 2013). Beef cattle were responsible for 41% of the global (Gerber et al., 2013) and 3.9% of U.S. (Kebreab et al., 2021) greenhouse gas emissions produced by the livestock industry (Gerber et al., 2013). Although the methane that is produced from ruminants will be converted to carbon dioxide approximately 12.4 years after entering the atmosphere (Myhre et al., 2013), the beef industry has been pressured to reduce methane,

because methane is more potent than carbon dioxide for global warming potential. Therefore, CH_4 is the target due to methane's short life span and increased potency.

When ruminants digest cellulose, hemicellulose, starches, and sugars, volatile fatty acid (VFA) are produced. Through glycolysis, one 6-carbon glucose is converted into two 3-carbon pyruvates. Pyruvate can be converted into propionate without a loss of carbons as propionate is a 3-carbon VFA. When acetate is produced there is a loss of one carbon as acetate is a 2-carbon VFA. During VFA production by-products are produced, such as methane, carbon dioxide, and hydrogen. When acetate is produced it leads to an increase in methane production. Therefore, feeding forages leads to an increase in methane production due to increased acetate production from the digestion of forages. Thus, increasing the amount of propionate that is produced by feeding concentrates could result in a decrease of methane.

As cattle transition from forage to concentrate based diets, the amount of forage in the diet is gradually decreased to reduce the risk of acidosis. Forages have a decreased energy value compared to corn or even by-products so limiting their inclusion in the diet is beneficial to improving efficiency. Stock et al. (2000) suggested that Sweet Bran, a branded product from the wet milling industry (Cargill Corn Milling. Blair, NE), has a feeding value that is 109%-112% the feeding value of dry rolled corn but still provides fiber. Cattle fed RAMP (Cargill Corn Milling, Blair, NE), a complete starter feed that contains high levels of Sweet Bran, low levels of cottonseed hulls, alfalfa hay, minerals, and vitamins, during the adaptation period compared to using forages have improved gains and carcass weight, due to the added energy intake and increased digestible fiber

(MacDonald and Luebbe, 2012; Buttrey et al., 2012; Schneider, 2013). Since by-products have an increased energy value compared to forages, replacing forages during grain adaptation could lead to less methane production. Therefore, the objective of this study was to determine the effects of utilizing RAMP compared to a traditional diet adaptation program on methane emission and respired carbon dioxide from finishing steers. The effects on performance and carcass characteristics during the entire finishing period were also evaluated but as a secondary objective due to the limited number of cattle.

Materials and Methods

All procedures involving animal care and management were approved by the University of Nebraska Lincoln's Institutional Animal Care and Use Committee (IACUC #: 2226)

A 173-d beef finishing experiment was conducted at the Eastern Nebraska Research, Extension, and Education Center (ENREEC) feedlot near Mead, NE. Sixty-four yearling British x Continental crossbred steers (initial BW =347 kg: ± 7 kg) were utilized to evaluate feeding RAMP during diet adaptation instead of forage on CH_4 emissions and respired CO_2, cattle performance, carcass characteristics and time spent ruminating and eating. Steers were received at ENREEC in October of 2021. Steers grazed corn residue until March, then grazed a smooth bromegrass pasture until trial initiation in June of 2022. Once cattle arrived at ENREEC feedlot, they received a modified live vaccine for prevention of infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), parainfluenza-3 (PI3), bovine respiratory syncytial virus (BRSV), Mannheimia haemolytica, 73 and Pasteurella multocida (Vista Once, Merck Animal

Health, Summit, NJ), a killed vaccine for clostridial toxoids and Histophilus somnus (Ultrabac 7/Somubac, Zoetis Inc, Florham Park, NJ), and an injectable solution for the treatment and control of gastrointestinal and external parasite control (Dectomax, Zoetis Inc.). Steers were revaccinated 14 to 21 d after being received at the ENREEC feedlot.

Steers were limit-fed a common diet of 50% grass hay and 50% Sweet Bran on a DM basis at 2% of body weight (BW) for 5 d to equalize for gut fill and achieve accurate initial body weights (Watson et al., 2013). Individual weights were taken on a hydraulic squeeze chute (Silencer, Moly Manufacturing Inc., Loraine, KS) for two consecutive days prior to being fed to establish an initial BW (Stock et al., 1983). Steers were blocked by BW into four weight blocks (4 paired replications), body weight range was 341 kg to 354 kg. Then cattle were stratified within BW, and assigned randomly to pens (n=8; 8 steers/pen).

On d -1 of the trial, steers were given an oral drench (Safeguard, Merck Animal Health) for internal parasite control. On d 1 of the trial, steers were implanted with 80 mg of trenbolone acetate and 16 mg of estradiol (Revalor-IS, Merck Animal Health). On d 77 of the trial, steers were re-implanted with 200 mg of trenbolone acetate and 20 mg estradiol (Revalor-200, Merck Animal Health).

The four paired replications consisted of two treatments (1 RAMP and 1 CONTROL per replication). The RAMP treatment consisted of cattle fed 100% RAMP during step 1 and then adapted to a common finishing diet consisting of 65.5% steam-flaked corn (SFC), 22.5% Sweet Bran, 8% wheat straw, and 4% supplement (DM basis). The second treatment was control (CONTROL), and cattle were fed 30.5% SFC, 22.5%

Sweet Bran, 8% wheat straw 35% alfalfa hay, and 4% supplement (DM basis) during step one and then adapted to the common finisher diet (Table 3.1). Steers were fed 4 step-up diets; step 1 was 7 days while steps 2, 3, and 4 were 5 days each. The RAMP treatment step 2 diet consisted of 75% RAMP, 16.5% SFC, 5.5% Sweet Bran, 2% wheat straw, and 1% supplement (DM basis). The step 3 diet for the RAMP treatment consisted of 50% RAMP, 32.5% SFC, 11.5% Sweet Bran, 4% wheat straw, and 2% supplement (DM basis). Step 4 consisted of 25% RAMP, 49% SFC, 17% Sweet Bran, 6% wheat straw, and 3% supplement (DM basis). For CONTROL treatment in step 2-4, SFC increased (40.5, 50.5, 58%) and alfalfa hay decreased (25, 15, 7.5%), while Sweet Bran, wheat straw, and supplement remained constant at 22.5, 8, 4%, respectively (DM basis). All supplements were formulated to include 33 mg/kg DM of monensin (Rumensin, Elanco Animal Health, Greenfield, IN) and to provide 9.8 mg/kg DM of tylosin (Tylan, Elanco Animal Health). The RAMP received for this experiment did not include monensin or tylosin. Urea was included in the supplement at 0.8% of the finishing diet (DM basis; Table 3.1). On d 144, ractopamine hydrochloride (Optaflexx, Elanco, Animal Health) was fed for the last 28 days at 300 mg/steer daily. Optaflexx was removed for 2 d before slaughter.

Each of the four paired replications started step 1 of the step-up diet 7 days apart, starting with the heavy weight block (replication 1) until the lightest weight block (replication 4) for a total of 21 days between the start of replication 1 and replication 4. Replications were limit-fed 3.6 kg of smooth bromegrass hay and 3.6 kg of Sweet Bran on a DM basis, until 5 days before starting step 1 of the step- up diet. Five d before starting step 1, cattle were fed the grass hay/Sweet Bran at ad-libitum to ensure the cattle were full when starting step 1. Steers were fed once daily around 0700h with a Roto-Mix

truck (Dodge City, KS). Cattle had ad libitum access to water and bunks were managed for ad libitum intake. Feed refusals were collected in home pens, when cattle entered the emissions barn, when cattle left the emission barn to return to their assigned home pen, and as needed while on the finishing diet. Feed refusals were collected, weighed, subsampled, and dried in a forced-air oven at 60°C (Model LBB2-21-1, Despatch, Minneapolis, MN) for 48 h (AOAC, 1999; Method 4.1.03), to determine dry matter intake (DMI). Individual feed ingredients were sampled weekly and dried in a forced-air oven at 60°C for 48 h (AOAC, 1999; Method 4.1.03). Dried weekly feed samples were composited monthly for the duration of the experiment. The monthly composited samples were sent to a commercial laboratory (Ward Laboratories, Kearney, NE) to be analyzed for total digestible nutrients (TDN), crude protein (CP; LECO Co.), neutral and acid detergent fiber (NDF and ADF, respectively; ANKOM Technology 1998; Mertens, 1992; Table 3.2).

Cattle were harvested on d 173 at a commercial abattoir (Greater Omaha, Omaha, NE). On the day of harvest, liver abscesses and hot carcass weight (HCW) were recorded. Longissimus muscle (LM) area, 12th rib back fat, and USDA marbling scores were recorded after a 48-h chill. Yield grade was calculated using the USDA YG equation (2016) 2.50 + (0.98425 × 12th rib fat, cm) + (0.2 × 2.5 KPH%) + 0.00837 × HCW, kg) – (0.0496 × LM area, cm^2) (USDA, 1997), where KPH fat was assumed to average 2.5%. Carcass adjusted final BW was calculated from HCW divided by a common dressing percent of 63%.

Gas Emissions Collection

Using the ENREEC emissions barn, CH_4 and CO_2 emissions were measured for three 5-d phases: step one of step-up phase, early feeding phase (one week after starting the finishing diet), and late finishing phase (13 weeks after starting the finishing diet). For the step-up phase, cattle were fed their assigned step 1 diet for 1 before entering the emissions barn. For the early feeding phase, cattle were on the common finishing diet for 1 week before entering the emissions barn and cattle were on the common finishing diet for 13 weeks before entering the emissions barn for the late finishing phase.

The emissions barn uses a negative air pressure system equipped with LI-COR 7700 and LI-COR 7500 analyzers (LI-COR, Lincoln, NE) that quantify real time levels of CH_4 and CO_2, respectively. Winders et al. (2020) described the emissions barn as two airtight chambers (pens) that had no emission crossover between chambers. Each pen is 15.2 m long (east to west) x 13.3 m wide (north to south) with a 4.4 m wide alley running east to west on the north end of the pen. The two feed bunks within each pen are each 3.7 m long. On the south end of the barn, air was pulled through an air inlet and pulled towards the north end of the barn, where there were exhaust fans. To ensure the air was being effectively mixed, there were two ceiling fans in the center of the barn. The negative pressure system pulled the mixed air to the north side, where the air sampling inlets were located in each chamber, before exiting the barn. The air was sampled in one inlet at a time to make a complete cycle in twenty minutes: two minutes of ambient (outside) air, six minutes of the west chamber, six minutes ambient air, and six minutes of the east chamber.

Paired BW replications were monitored and rotated through the emissions barn together. Sixteen steers were in each paired replication; 8 steers from each block of the CONTROL treatment and 8 steers from each block of the RAMP treatment. The heaviest weight block was replication 1, the medium two weight blocks were replication 2 and 3, and the lightest weight block was replication 4. All replications were rotated through the barn to make one complete phase. Each paired replication remained paired through the duration of the experiment. Cattle entered the chambers at 0700 on d 1 (Wednesday) and remained in the chamber until d 5 (Monday) at 0700, then returned to their respective home pen. Each day was approximately 24 hours, from feeding to feeding. Methane and carbon dioxide from manure emissions, from the previous 5 d, were measured from 0700 h on d 5 (Monday) to 0700 h on day 6 (Tuesday). After 24 h of manure collection, the manure was removed via skid steer at 0700 h on d 6 (Tuesday). After the manure was removed, the next 24 hours were measured to get a baseline measurement; this was considered d 7, which was the final day in one rotation through the emissions barn. Manure emission levels of CO_2 and CH_4 were subtracted from baseline emission levels of CO_2 and CH_4 to determine the baseline value. Then values were divided over 5 days and 8 steers, to estimate daily animal emissions.

Time Spent Ruminating and Eating

Cattle were fitted with a sensor ear tag in the left ear on d 1 (Cow Manager SensOor, Agis Automatisering BV, Harmelen, the Netherlands), which measured the number of minutes ruminating and minutes eating per day, based on the ear movement via a three- dimensional accelerometer. Activity, temperature, rumination, and eating

were monitored continuously, with one reading every 1 h for 173 d. Data were recorded through the ear tag and could be stored for 7 d. With internet connection, data were continuously transmitted to the Cow Manager SensOor software on a computer. The recorded data were analyzed for each step-up diet and for 99 d when all replications were on the finishing diet. Due to the lack of accuracy of the tags to distinguish between rumination and eating while on a high concentrate diet, time spent ruminating and eating were combined for each h of the experiment (Wolfger et al. 2015).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) as a randomized complete block design. Pen was the experimental unit. For the performance data, treatment and BW block were fixed effects. For the emissions data when fed step 1 of the step-up diet, treatment and BW block were set as fixed effects. The early and late finishing periods had treatment, BW block, cycle, and barn as fixed effects. Rumination data were analyzed as a repeated measure within step as d was repeated; step 1, step 2, step 3, step 4, and 99 d average of the finishing period (the average number of days all pens were on finisher diet that the tags recorded data). The repeated measure structures were chosen first based on the lowest AIC, then the model statement of treatment, day, day × day, treatment × day, and treatment × day × day were determined based off significance for each period of the study. The LSMEANS are reported for treatment averages for the rumination data. Significance was declared at $P \leq 0.05$ and a tendency at $P \leq 0.10$.

Results and Discussion

Gas Emissions

Grain Adaptation Phase

For gas emissions, during the grain adaptation phase, one of the replications of data was removed, due to a loss of data. The loss of data was from the failure of a diaphragm pump (Thomas 2107 Series, Gardner Denver, Milwaukee, WI) which pulls air to the gas emission sensors. This was repaired and subsequent three replications were measured on step 1 of their adaptation as outlined.

In step one of grain adaptation, there were no differences in DMI ($P = 0.95$: Table 3.3), while measuring CH_4 emissions in the barn. But methane production (g/d) decreased by 12% for the RAMP treatment compared to the CONTROL treatment ($P = 0.03$). Numerically RAMP decreased CH_4 g/ kg of DMI by 12%; however, due to variation in DMI across replications, it was not significantly different ($P = 0.25$). It is well understood that replacing forage with concentrates results in a reduction in methane (Lovett et al., 2003; Beauchemin and McGinn, 2005; Martin et al., 2007, Martin et al., 2010). Martin et al. (2007) suggested when 80 to 90% of the diet consisted of concentrates, the loss of CH_4 was 2 to 3% of gross energy intake (GEI). When compared to a diet that consisted of 30 to 40% concentrates, the loss of methane was 6 to 7% of GEI (Martin et al., 2007). The CONTROL diet contained 43% forage for step 1 while RAMP consists of mainly Sweet Bran and contained small amounts of forages. The fiber components of RAMP are a more digestible fiber compared to traditional forages such as alfalfa because RAMP is mostly comprised of Sweet Bran. MacDonald and Luebbe (2012) fed high amounts of Sweet Bran or Sweet Bran fed with three different inclusion

levels of cottonseed hulls (low, medium, high) compared to a traditional grain adaptation diet. The high amounts of Sweet Bran treatment had the greatest NEg intake per kg of DM. The three complete starter diets had similar amounts of NEg intake per kg, while control had the lowest NEg intake per kg. Because RAMP is comprised of large amounts of Sweet Bran, which is greater in net energy compared to forages, adapting cattle with RAMP led to a decrease in methane emissions.

McGinn et al. (2009) fed 35% DM dried distillers with solubles (DDGS) to steers in replacement of steam-rolled barley that were fed a 60% DM barley silage diet. The authors noted a 20% reduction in methane (g/d basis) for the cattle fed DDGS. With the addition of DDGS, the fat content of the diet increased from 2.0 to 5.1%. The authors suggested that the reduction of methane was from the addition of fat in the diet, as feeding corn oil has been shown to decrease methane production (Hales et al., 2017; Winders et al., 2020). McGinn et al. (2009) also reported an increase in the NDF of the diet for cattle that were fed DDGS, which would typically cause an increase in methane production (Boadi et al., 2004). But, the addition of DDGS increased the gross energy of the diet or could have decreased the amount of fermentation in the rumen, as the rumen undegradable protein and oil in DDGS would have been digested in the small intestine. The NDF as a percent of DM increased from 38.5% for control to 42.4% for cattle fed DDGS for a difference of 3.9% (McGinn et al., 2009). In the current study, the CONTROL diet consisted of 35.7% NDF (Table 3.2) whereas RAMP contained 39.3% NDF, for a difference of 3.6%. Although there was an increase in NDF for both studies due to by-products, methane emission was reduced, suggesting that NDF of DDGS diet and RAMP diet were more digestible compared to the NDF found in forages or silages.

RAMP is comprised of large amounts of Sweet Bran. Sjostrand (2022) suggested that as Sweet Bran increased in the diet, NDF digestibility will increase due to the addition of the bran and solvent extracted germ meal component of Sweet Bran.

Limited studies have been conducted on gas emissions with by-products from the wet milling industry. Terklebrhan et al. (2020) replaced corn meal (CM) with WCGF in diets fed to Xiangdong goats. Twenty-four goats (17.5 kg) were fed either 40% corn meal or 40% WCGF. Goats were adapted for 28 d followed by 12 d of collection for CH_4 and CO_2. No differences in DMI were reported between treatments. The NDF of the diet for the corn meal treatment was 38.4% and 48.4% for the WCGF treatment. There was a 17% decrease in methane production as g/d and a 16% decrease in methane as g/kg of DMI for the WCGF treatment. Terklebrhan et al. (2020) reported a 10% increase in the NDF of the diet, which is greater than 3.6% increase in NDF for the RAMP treatment, nonetheless both studies reported an increase in NDF and a decrease in methane. Therefore, a reduction in methane may be possible when feeding Sweet Bran, WCGF, or RAMP.

Carbon dioxide measured in our study was greater for the RAMP treatment (7960 g/d) compared to the CONTROL treatment (8692 g/d; $P = 0.03$; Table 3.3). The RAMP steers could have respired more CO_2, instead of being released as methane from enteric fermentation because of the increased digestibility of the RAMP diet compared to CONTROL. The CO_2 released from respiration was not new carbon entering the atmosphere, but it was being utilized, from digestible organic matter, so that CO_2 is in the biogenic carbon cycle. There were no significant differences between treatments when

CO_2 was expressed per kg of DMI ($P = 0.31$). Steers fed RAMP had a decrease in the CH_4:CO_2 ratio ($P < 0.01$) compared to CONTROL. The CH_4:CO_2 ratio indicates that the RAMP treatment produced less methane in proportion to CO_2 compared to CONTROL.

Finishing Period

While cattle were in the emissions barn during the finishing period, there were no differences in DMI ($P = 0.49$; Table 3.4). Reductions in CH_4 on a g/d basis ($P < 0.01$) and as g/kg of DMI ($P = 0.03$) were observed for the RAMP treatment compared to CONTROL. There was a decrease in the CH_4:CO_2 ratio for the RAMP treatment ($P < 0.01$), which was primarily driven by the decrease in CH_4. The decrease in methane emissions during the finishing phase was a carryover effect from the grain adaptation phase as both treatments were fed a common finishing diet for 1 and 13 weeks before measurement. No significant differences were observed in CO_2, when expressed as a g/d basis ($P = 0.85$) or as g/kg of DMI ($P = 0.39$). Nonetheless, these data suggest that diet adaptation can have a lasting effect on the microbes in the rumen.

Time Spent Ruminating and Eating

During step 1 of the grain adaptation diets, steers fed RAMP spent 238 min/d ruminating and eating, which was less time than CONTROL 435 min/d (P = 0.01; Table 3.5). The average dry matter offered (DMO) for the RAMP treatment during step 1 was 10.9 kg/d and 11.0 kg/d for CONTROL. The increase in time spent ruminating and eating for the CONTROL treatment, follows other research for diets that contained high amounts of forage. Adin et al. (2009) fed lactating dairy cows a control diet that consisted of 40% forage (corn silage, whole cottonseed, vetch and wheat hay) and 54%

concentrates (solvent extracted germ meal, ground corn, ground barley, CGF, DDG, wheat bran, sunflower and rapeseed meal). The experimental diet consisted of 30% forage, 51% concentrate, and 14.5% soybean hulls (DM basis). The control treatment spent 482.6 min/d ruminating and the experimental TMR treatment spent around 428 min/d ruminating. Adin et al. (2009) had a 40% forage diet which was similar to the 43% forage diet used in step 1 diet of the CONTROL treatment in the current study. Adin et al. (2009) reported 482.6 min/d for the control treatment which was 48 more minutes per day ruminating than the CONTROL (435 min/ d) treatment in the current study. The difference in time spent ruminating could be due to the difference in DMI, Adin et al. (2009) reported a DMI for the control treatment of 25.1 kg/d, while the CONTROL treatment in the current study during step 1 only had a DMI of 10.3 kg/d. Therefore, these data suggest that the CowManager SensOor Tags accurately measured time spent ruminating and eating on a high forage diet compared to beef cattle on a high concentration diet.

Although the time spent ruminating and eating for the CONTROL treatment matches other studies, the time spent ruminating and eating for the RAMP treatment (238 min/d) in step 1 was lower than expected. The decrease could have been due to the decrease in particle size found in the RAMP diet compared to alfalfa hay and wheat straw in the CONTROL treatment. Poppi et al. (1980) suggested that particles that are longer than 1.18 mm, according to the critical size theory, have the most resistance to passage and can stimulate chewing and rumination. Since RAMP had low levels of alfalfa compared to the CONTROL diet, there was a decrease in particle size, allowing for a higher passage rate. In dairy cattle, Yansari et al. (2004) reported that a decrease in alfalfa

particle size resulted in a decrease in time spent eating and ruminating, in a total mixed ration containing 20, 20, 35, 7, 7.5, and 10% of alfalfa, corn silage, barley, soybean meal, beetpulp and wheat bran, on a DM basis, respectively. The alfalfa was included at either a large, medium, or fine particle size. In the fine particle size alfalfa diet, the cows spent 445.5 min/d ruminating and eating and the cows on the large particle size alfalfa spent 596.7 min/d. The RAMP treatment had lesser amounts of alfalfa at a smaller particle size, compared to the alfalfa hay and wheat straw found in step 1 of the CONTROL diet. Therefore, RAMP could have decreased in time spent ruminating and eating due to the decrease in particle size, lower concentration of forages, and the more digestible diet.

During step 2, the DMO for the RAMP treatment was 10.8 kg/d and 10.5 kg/d for CONTROL. The CONTROL treatment had a numerical increase in time spent ruminating and eating compared to RAMP (452 min/d and 364 min/d, respectively), however this was not significantly different ($P = 0.23$) because of a high standard error (SEM = 44.5). During step 3, the DMO for the RAMP treatment was 12.7 kg/d and 12.3 kg/d for CONTROL. No significant differences were found between the treatments during step 3 ($P = 0.31$). The numerical decrease in time spent ruminating and eating for the CONTROL cattle on step 3 compared to step 2 was expected as forage concentration decreased in the diet. During step 4, the DMO for the RAMP treatment was 12.4 kg/d and 11.8 kg/d for CONTROL. In step 4, RAMP tended ($P = 0.09$) to spend less time ruminating and eating, 407 min/d, compared to CONTROL 465 min/d. For the RAMP treatment, the increase in time spent ruminating and eating from step 2 to step 4 was not expected as there was mainly an increase in Sweet Brand and SFC, however the small increase in wheat straw may have led to the increase in time spent ruminating and eating.

A study conducted by Spowart at el. (2022) reported time spent ruminating for finishing steers. The diets in Spowart at el. (2022), were 76.6% flaked corn grain, 8% ground corn stalks, and 6% cottonseed meal (control; DM basis). The second diet included less flaked corn grain, 20% WDGS, and no cottonseed meal (WDGS20). The SB20 treatment was similar to WDGS20 but contained 20% Sweet Bran and no WDGS. The fourth treatment (COMBO) was a combination of WDGS20 and SB20, COMBO contained 53.2% flaked corn grain, 20% Sweet Bran, 10% WDGS, and 8% corn stalks. For time spent ruminating and eating, Spowart at el. (2022) reported a numerical increase in diets containing WDGS20, SB20, and COMBO compared to the control treatment. Although there was not a significant difference, it is intriguing that all three treatments that included products from the wet and dry milling industry had increased time spent ruminating and eating, specifically when included at 20% DM of the diet. The reason for the increase in time spent ruminating and eating for the RAMP treatment from step 2 to 4 remains unclear, as the increase in wheat straw could have led to the increase in time spent ruminating and eating.

For the 99 d finishing period, cattle adapted with RAMP spent more time ruminating and eating with 436 min/d compared to CONTROL at 408 min/d ($P < 0.01$) even though all cattle were on the same finishing diet. Although there was only a 28 min/d difference between the CONTROL and RAMP treatments, there was a large data set being factored into the 99-d finishing period with a low standard error (SEM = 8.6) which led to a significant difference. It was unclear why RAMP continued to increase time spent ruminating and eating and surpassed the CONTROL treatment when on the same diet. This suggest the adaption period is important in establishing eating and

ruminating patterns that persist throughout the feeding period. Nonetheless, when steers were on a high concentrate diet, they spent around 6.5 to 7 h ruminating and eating which suggests that the CowManager SensOor Tags might be accurate when combining time spent ruminating and eating for cattle on a high concentrate diet.

Performance and Carcass Characteristics

There were no significant differences in initial BW between treatments (P = 0.30). During the entire 163 d trial, DMI did not differ among treatments (P = 0.84). Cattle fed RAMP during adaptation had a 2% numerical improvement in average daily gain (ADG) from 2.15 kg for CONTROL to 2.20 kg for RAMP (P = 0.16). In terms of G:F, no significant differences were observed between CONTROL (0.1723) and RAMP (0.1735; P = 0.77). Cattle adapted with RAMP tended to have a greater HCW and carcass adjusted final BW (715 kg) compared to the CONTROL treatment (705 kg; P = 0.10). The HCW increased by 6 kg for RAMP (450 kg) compared to CONTROL (444 kg; P = 0.10). There were no significant differences between treatments for marbling (P = 0.40), 12th rib back fat (P = 0.80), calculated yield grade (P = 0.78) or LM area (P = 0.46).

During the entire 163 d trial, no differences in DMI were observed (P = 0.84). No significant differences in DMI were seen in Schneider (2013) when feeding RAMP in a 22 d diet adaptation either as 1 diet- system, fed twice a d (RAMP-1RS) or as 2 different diets delivered separately, as RAMP and the finishing diet (RAMP-2RS), compared to a traditional finishing diet that contained alfalfa, HMC, DRC, and Sweet Bran. Schneider (2013) reported numerical differences between treatments after diet adaptation for DMI with the control treatment having a DMI of 13.3 kg/d, RAMP-1RS at 13.1 kg/d and

RAMP-2RS had a DMI of 13.0 kg/d. The amounts of DMI are similar to what was observed in this study with CONTROL at 12.7 kg per d and RAMP at 12.7 kg per d.

For ADG no differences were observed between treatments (P = 0.16). Cattle adapted with RAMP (2.20 kg) had a 2% numerical improvement in ADG compared to CONTROL (2.15 kg). In past research there have been significant differences in ADG. Schneider (2013) saw a 7% increase in ADG for RAMP-1RS (1.87 kg) compared to the control treatment (1.74 kg). MacDonald and Luebbe, (2012) and Buttrey et al. (2012) also reported a significant difference in ADG. In the study of MacDonald and Luebbe, (2012) the treatment consisting of high levels of Sweet Bran and medium inclusion of cottonseed hulls had a significant increase in ADG (1.52 kg control, 1.74 kg high level of Sweet Bran, 1.51 kg medium level of cottonseed hulls). In the current study, the ADG for control was 2.15 kg and RAMP was 2.20 kg. Even though there was a numerical improvement in ADG, it was not surprising that it was not statistically different since this study had limited replications. However, the numerical increase of ADG still shows the positive improvements of adapting cattle with RAMP.

For G:F there were no significant differences between CONTROL (0.1723) and cattle adapted with RAMP (0.1735; P = 0.77). In past research, there has been mixed results for G:F from feeding RAMP during grain adaptation. Schneider (2013) reported a significant improvement in G:F for RAMP-1RS and RAMP-2RS compared to control, but there were no differences between RAMP treatments. Huls et al. (2016) also reported an improvement in G:F when adapting cattle to a finishing diet with Sweet Bran compared to a traditional step up diet. Steers were stepped-up to the finisher in 4 diets

that were 5, 7, 7, and 7 d. For the Sweet Bran treatment, Sweet Bran decreased from 85% to 35% DM and in the control treatment, alfalfa decreased from 45% to 7.5% DM while maintaining a 1:1 ratio of HMC and DRC. Huls et al. (2016) suggested that the improvement in G:F was from the added energy from the Sweet Bran compared to forage used during grain adaptation in the CONTROL treatment. However, studies by MacDonald and Luebbe, (2012) and Buttrey et al. (2012) did not report a significant difference in G:F. Buttery et al. (2012) adapted the control treatment to a finishing diet in 22 d (5, 5, 6 and 6 d), and 5 RAMP treatments that were stepped up in either 14, 18, 22, 26, or 30 d. All treatments were on a common finisher diet that consisted of 65.7 % SFC, 20% Sweet Bran, 8% alfalfa hay, the remainder, 6.3%, of the diet was made up of yellow grease, limestone, urea, and supplement. MacDonald and Luebbe, (2012) and Buttrey et al. (2012) utilized SFC in the finishing diets, while Huls et al. (2016) and Schneider (2013) utilized a 1:1 ratio of DRC and HMC. In the current study SFC was utilized in the finishing diet which follows the same results (MacDonald and Luebbe, 2012 and Buttrey et al. 2012) of having no significant differences in G:F.

Cattle adapted with RAMP tended to have a greater HCW and carcass adjusted final BW (715 kg) compared to the CONTROL treatment (705 kg; P = 0.10). The increase HCW for RAMP (450 kg) compared to CONTROL (444 kg; P = 0.10) was 6 kg. Numerous studies have reported a greater HCW and final BW for cattle stepped up with RAMP or Sweet Bran. Schneider et al. (2013) reported a numerical increase of 9 kg for RAMP-1RS compared to control and 5 kg increase for RAMP- 2RS compared to control, however it was not significantly different. MacDonald and Luebbe (2012), reported a significant increase in HCW by 8 kg for high Sweet Bran compared to control and a 3 kg

increase for medium level of cottonseed hulls compared to control. Buttrey et al. (2012) also saw an increase in HCW of 2 kg for treatment stepped up in 14 d and saw the greatest increase in HCW for the treatment adapted in 18 d with an increase of 16 kg. The average increase of HCW across all RAMP treatments was 8 kg greater than the control treatment (Buttrey et al., 2012). Although there was only a tendency for an increase in HCW in the current study, due to the low replication, it is consistent from cattle adapted with RAMP to increase HCW by 6-9 kg compared to cattle adapted by a traditional system of decreasing forage.

There were no significant differences between treatments for marbling ($P = 0.40$), 12th rib back fat ($P = 0.80$), calculated yield grade ($P = 0.78$) or in LM area ($P = 0.46$). Schneider (2013) and Huls et al. (2016) also did not report any differences in 12th rib back fat, calculated YG, or LM area. However, MacDonald and Luebbe (2012) and Buttrey et al. (2012) did report an increase in 12th rib back fat when adapting cattle with RAMP or Sweet Bran. MacDonald and Luebbe (2012) and Buttrey et al. (2012) suggested that the increase in back fat could be attributed to the added caloric intake.

Conclusion

The complete starter diet, RAMP, was a more digestible diet compared to a traditional forage adaptation diet used during grain adaptation. Adapting cattle with RAMP resulted in a 12% reduction in methane (g/d) during grain adaptation and a 12% expressed as g/kg of DMI. Methane was reduced by 9% (g/d) while cattle were on a common finishing diet due to carryover effects from adapting cattle with RAMP. Using RAMP instead of forages for grain adaptation could be a strategy to reduce methane

emissions. The performance benefits from RAMP would further decrease methane per kg of gain.

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Table 3.1. Dietary composition (% of DM) for treatments

	RAMP Diet Treatment[7]				
Ingredient	RAMP-1	RAMP-2	RAMP-3	RAMP-4	Finishing
RAMP[1]	100	75	50	25	-
Steam flaked corn	-	16.5	32.5	49	65.5
Sweet Bran[2]	-	5.5	11.5	17	22.5
Wheat straw	-	2	4	6	8
Alfalfa hay	-	-	-	-	-
Supplement [2]	-	1	2	3	4
Fine ground corn	-	0.264	0.529	0.793	1.057
Limestone	-	0.413	0.825	1.238	1.650
Tallow	-	0.025	0.050	0.075	0.100
Urea	-	0.200	0.400	0.600	0.800
Salt	-	0.075	0.150	0.225	0.300
Beef Trace premix[3]	-	0.013	0.025	0.038	0.059
Vitamin A-D-E premix[4]	-	0.004	0.008	0.011	0.015
Rumensin-90 premix[5]	-	0.004	0.008	0.012	0.017
Tylan-40 premix[6]	-	0.003	0.006	0.008	0.011
	CON Diet Treatment[7]				
Ingredient	CON-1	CON-2	CON-3	CON-4	Finishing
Steam flaked corn	30.5	40.5	50.5	58	65.5
Sweet Bran	22.5	22.5	22.5	22.5	22.5
Wheat straw	8	8	8	8	8
Alfalfa hay	35	25	15	7.5	-
Supplement [2]	4	4	4	4	4
Fine ground corn	1.057	1.057	1.057	1.057	1.057
Limestone	1.650	1.650	1.650	1.650	1.650
Tallow	0.100	0.100	0.100	0.100	0.100
Urea	0.800	0.800	0.800	0.800	0.800
Salt	0.300	0.300	0.300	0.300	0.300
Beef trace premix[3]	0.059	0.059	0.059	0.059	0.059
Vitamin A-D-E premix[4]	0.015	0.015	0.015	0.015	0.015
Rumensin-90 premix[5]	0.017	0.017	0.017	0.017	0.017
Tylan-40 premix[6]	0.011	0.011	0.011	0.011	0.011

[1]RAMP, Cargill Corn Milling, Blair, NE
[2]Sweet Bran, Cargill Corn Milling, Blair, NE
[3] Premix contained 6.0% Zn, 5.0% Fe, 4.0% Mn, 2.0% Cu, 0.29% Mg, 0.2% I, 0.05% Co
[4] Premix contained 30,000 IU vitamin A, 6,000 IU vitamin D, 7.5 IU vitamin per gram
[5] Supplement formulated to provide 33 mg/kg of Rumensin (Elanco Animal Health, DM Basis)
[6] Supplement formulated to provide 9.8 mg/kg of Tylan (Elanco Animal Health, DM Basis)
[7] Steers were on step 1 for 7 days and on step 2, 3, and 4 for 5 days each

Table 3.2. Chemical composition of treatment diets

	RAMP[3] Treatment Diets[2]				
Chemical composition[1]	RAMP-1	RAMP-2	RAMP-3	RAMP-4	Finisher
Neutral detergent fiber, %	39.3	34.3	29.8	24.9	20.1
Acid detergent fiber, %	17.9	15.9	14.0	12.0	10.1
Crude Protein, %	21.9	19.6	17.3	14.9	12.6
	CONTROL Treatment Diets[2]				
Chemical composition	CON-1	CON-2	CON-3	CON-4	Finisher
Neutral detergent fiber, %	35.7	31.3	26.8	23.5	20.1
Acid detergent fiber, %	23.6	19.8	15.9	13.0	10.1
Crude Protein, %	15.8	14.9	14.0	13.3	12.6

[1]Based on monthly samples, for each ingredient. Sample analyses were conducted at Ward Laboratories (Kearney, NE). All values are presented on a DM basis

[2] Steers were on step 1 for 7 days and on step 2, 3, and 4 for 5 days each

[3] RAMP is a complete starter feed (Cargill Corn Milling, Blair, NE)

Table 3.3. Effects of RAMP versus a traditional starter feedlot diet on gas emissions of steers during step 1[1]

	Treatments[3]			
	CONTROL	RAMP[4]	SEM	*P*-value
Gas emissions				
DMI, kg/d[2]	10.3	10.3	0.34	0.95
CH_4, g/d	174	153	2.5	0.03
CH_4, g/kg of DMI	17.5	15.3	0.99	0.25
CO_2, g/d	7960	8692	83.6	0.03
CO_2, g/kg of DMI	798.5	874.2	40.0	0.31
CH_4:CO_2	0.0217	0.0177	0.0004	0.02

[1]Emission were measured during step 1 of step-up diets

[2]Dry matter intake (DMI) was used to unitize reported emissions and was averaged from the weekly intakes of each treatment during rotation through the respective emission chambers

[3] Treatments included cattle adapted with a traditional forage diet or with RAMP and then fed the same common finisher diet

[4] RAMP is a complete starter feed (Cargill Corn Milling, Blair, NE)

Table 3.4. Effects of adaptation program on gas emission of steers during finishing period[1]

	Treatments[3]			
	CONTROL	RAMP[4]	SEM	*P*-value
Gas emissions				
DMI, kg/d[2]	12.2	11.8	0.21	0.34
CH_4, g/d	175	159	3.50	0.009
CH_4, g/kg of DMI	14.5	13.4	0.29	0.03
CO_2, g/d	10312	10338	96.1	0.85
CO_2, g/kg of DMI	852.2	873.8	16.67	0.39
CH_4:CO_2	0.0170	0.0153	0.0003	0.003

[1]Emissions were measured after 1 week on finishing diets and at 13 weeks on finishing diets

[2]Dry matter intake (DMI) was used to unitize reported emissions and was averaged from the weekly intakes of each treatment during rotation through the respective emission chambers

[3] Treatments included cattle adapted with a traditional forage diet or with RAMP and then fed the same common finisher diet

[4]RAMP is a complete starter feed (Cargill Corn Milling, Blair, NE)

Table 3.5. Effects of adaptation program on time spend eating and ruminating[1]

	Treatment[3]			
Item	CONTROL	RAMP[4]	SEM	*P*-Value
Ruminating and Eating, min/day				
Step 1	435	238	29.8	0.01
Step 2	452	364	44.5	0.23
Step 3	412	376	25.8	0.31
Step 4	465	407	18.7	0.09
Finishing Period[2]	408	436	8.63	<0.001

[1] Means were reported from repeated measures estimate and may not match the arithmetic means for each period

[2] 99 days were measured when all treatments were on finishing diets

[3] Treatments included cattle adapted with a traditional forage diet or with RAMP and then fed the same common finisher diet

[4] RAMP is a complete starter feed (Cargill Corn Milling, Blair, NE)

Table 3.6. Effects of diet adaptation on performance and carcass characteristics on fattening steers

	Treatments[4]			
	CONTROL	RAMP[5]	SEM	*P*-value
Performance				
Initial BW, kg	356	357	0.5	0.30
Carcass Adjusted Final BW, kg[1]	705	715	2.9	0.10
ADG[2], kg	2.15	2.20	0.018	0.16
DMI, kg/d	12.7	12.7	0.10	0.84
Gain:Feed	0.1723	0.1735	0.0059	0.77
Carcass characteristics				
HCW, kg	444	450	1.8	0.10
Marbling[3]	608	592	11.1	0.40
LM area, cm^2	98.1	95.5	2.19	0.46
12^{th} rib fat, cm	1.90	1.82	0.168	0.75
Calculated yield grade	3.78	3.74	0.679	0.78
Liver Abscesses, %	47	47	-	-
0, %	53	53	-	-
A-, %	44	47	-	-
A, %	3	0	-	-

[1]Carcass adjusted final BW was determined from hot carcass weight (HCW) divided by common dressing percentage of 63%

[2]The average days on feed 162 days

[3]Marbling score: 400=small[00], 500 = Modest[00], 600 = Moderate[00], minimum required for U.S. Low Choice

[4] Treatments included cattle adapted with a traditional forage diet or with RAMP and then fed the same common finisher diet

[5] RAMP is a complete starter feed (Cargill Corn Milling, Blair, NE)