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SODIUM BUTYRATE AND MONENSIN SUPPLEMENTATION TO POST-WEANED HEIFER DIETS: EFFECTS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND HEALTH

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BY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

SODIUM BUTYRATE AND MONENSIN SUPPLEMENTATION TO POST-WEANED HEIFER DIETS: EFFECTS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND HEALTH

by

Tess Catherine Stahl

University of New Hampshire, December 2019

Much of the research on sodium butyrate (**SB)** has been conducted with pre-ruminant calves. Previous research with post-weaned calves was shown to be beneficial. The objective of this study was to compare sodium butyrate to monensin (**MON)** on the growth, digestibility, and health of post-weaned heifers. Forty Holstein dairy heifers with a mean age of 84.2 d ± 1.2 d (mean ± SD) and average body weight (**BW)** of 99.78 kg ± 10.77 (mean ± SD) were housed in a naturally ventilated freestall barn. Heifers were blocked by birth date and randomly assigned to 1 of 4 treatments in a completely randomized block design: (1) 100 g of soybean meal carrier (control; **CON**); (2) 0.75 g SB/kg of BW + carrier; (3) 1 mg monensin/kg of BW + carrier; (4) monensin + 0.75 g SB per kg of BW (**MSB**). Data were statistically analyzed using single degree of freedom contrasts evaluating CON vs. all other treatments; SB vs. MON; and SB and MON vs. MSB. Treatments were top-dressed and hand-mixed into a total mixed ration (**TMR**) once daily. Heifers had free access to water. Amount of orts and feed offered to each heifer was measured daily. Feed and orts samples were frozen at -20°C for future analysis. Orts samples were taken daily and subsampled for later DM determination, while TMR samples were taken

weekly and composited monthly for later DM and nutrient analysis. Initial BW, heart girth, paunch girth, and body length were measured before the start of the study and every week thereafter during the 12 wk trial (168 d old). Blood samples were obtained, and glucose and ketone concentrations were determined prior to the start of treatment and weekly until the conclusion of the study. Fecal grab samples were taken prior to treatment and every week from each heifer for coccidia counts. Apparent total-tract nutrient digestibility samples were taken in two different phases: 21 d on study until 27 d, and again at 63 d until 69 d. Apparent total tract nutrient digestibility was taken during this time and determined through acid-insoluble ash. Additives had a positive effect, tending to increase average BW and final BW. Any additive tended to increase heart girth, while MSB tended to be greater than either SB and MON. No other effects were seen on skeletal growth. Daily dry matter intakes (**DMI**) were increased in the diets containing additives as compared to control. Dry matter intake values were 4.00, 4.47, 4.16, and 4.46 kg/d for CON, SB, MON, and MSB, respectively. Feed efficiency (**FE**) was improved in MON supplemented heifers as compared to SB. Fecal samples from each heifer indicated the presence of coccidia. Compared to control, additives decreased the number of coccidian oocysts present in feces. Monensin and SB treatments tended to have greater plasma glucose as compared to MSB. Average blood ketone concentrations were greater with any additive compared to CON, in SB vs. MON, and in MSB as compared to SB and MON. During the week 3 digestibility period, DMI tended to be greater in heifers fed SB when compared to MON, as well as heifers fed MSB when compared to SB and MON. Apparent total tract digestibility of DM, NDF, ADF, Hemicellulose, OM, and fat digestibility showed no differences among treatments. Starch digestibility was increased in heifers fed the combination diet when compared to SB and MON. During the week 9 digestibility period, DMI, along with apparent total tract

digestibility of DM, CP, ADF, Hemicellulose, Starch, OM, and fat digestibility were not different among treatments. Neutral detergent fiber digestibility tended to be greater in control diets when compared to any additive. Overall, additive supplementation offers positive results in growth performance, digestive functions, and improvement in overall health.

CHAPTER ONE

Review of Literature

INTRODUCTION

In the coming years, global agriculture faces a rising demand. It is estimated that the world population will grow from 7.6 billion to above 9.8 billion by 2050 (UN, 2017). With increasing populations comes increasing product consumption. Agriculture and all other food-related industries contributed $1.053 trillion to the gross domestic product in the U.S. in 2017 (USDA, 2019a). In 2018, the livestock cash portion of total agriculture-related sales is at $175.6 billion. Out of that amount, cattle and calf sales accounts for $66.4 billion and dairy sales accounts for $35.3 billion (CRS, 2018). Due to the possible economic gains, producers should continue to investigate ways to make their herds more efficient especially considering longevity of land and available resources.

Efficiency of the physical and financial performance in animal production derives from knowledgeable and skillful handling to optimize welfare, health, husbandry, and management (Beynon, 1991). Improving efficiency and management in heifers is vital to increase their financial performance, especially considering the cost of raising dairy replacement heifers accounts for > 12% of total dairy farm expenses and feed comprises 60% of that cost (Gabler et al., 2000). In a survey done on 44 dairy farms in Pennsylvania, Heinrichs et. al (2013) found that the total cost to raise a calf from birth until freshening averaged $1, 808.23 (± $338.62), about 73% of that amount just in feed costs. This cost in the current dairy climate is closer to $2,500 (K. Aragona, personal communication). Appropriate heifer raising is vital to improving the economic efficiency of the operation because nutrition and management of the calf have shown

to affect body weight, body condition scoring, withers height, and age at first calving (Heinrichs et al., 2005). Heinrichs et al. (2013) had shown the most efficient animals were those that calved less than 24 mo. old, and those first lactation heifers had greater than 88% of milk production compared to their mature herd mates. Research in this area by Zanton and Heinrichs (2005) has shown heifers raised over 900 g/d in the prepubertal period decreased first lactation milk production. Thus, an improvement in their productivity would affect future farm profitability.

In order to improve heifer efficiency, one needs to start with developing musculature and absorptive structures within the rumen. The development of these structures comes with the fermentation of solid feed and roughages. Once fed, the rumen microbes will begin breaking down fiber and carbohydrates and converting them to important volatile fatty acids (**VFA**). Primary VFA produced in the rumen are acetate, propionate, and butyrate. The three primary VFA will lower the pH of the rumen, making the environment even more microbe-friendly and causing rapid rumen development (Heinrichs, 2014). This rapid rumen development will come from progressing the development of the rumen mucosa via papillae on the luminal surface. Extensive papillae development is shown to increase the surface area of the rumen, and it is believed that this then increases its absorptive capacity (Dieho et. al, 2016).

When feeding VFA salts sodium acetate, sodium propionate, and sodium butyrate (**SB)** to 2 wk old calves, all caused significant development to the rumen mucosa; though, sodium butyrate showed the greatest effect on rumen papillary growth (Sander et. al, 1959). Providing sources for the rumen to increase the absorptive capacity allows heifers to digest and utilize nutrients more efficiently. An increase in efficiency may result in a decrease in feed use or an improvement in growth. Some products that can improve FE and health of cattle include antibiotic ionophores, such as monensin (**MON**) and lasalocid (Heinrichs, 1993).

In 2006, the European Union banned the use of antibiotics as growth promoters in animal feeds, including monensin sodium, salinomycin sodium, avilamycin, and flavophospholipol (European Commission, 2005). Since then, there has been more pressure on researchers to find alternative additives that can produce equal or greater efficiency and growth results than that of ionophores. Research has been done looking at the supplementation of SB and its effects when added to the diets of pre-weaned calves (Guilloteau et al., 2009; Gilloteau et al., 2010a; Górka et al., 2011a,b; Górka et al., 2014), lactating cows (Kowalski et al., 2015; Herrick et al., 2017), and recently in post-weaned heifers (Rice et. al, 2019). Sodium butyrate has shown expansive growth and concentration of rumen papillae in cattle but has also shown to increase intestinal epithelial growth in broiler chickens (Abdelqader and Al-Fataftah, 2015). Heat-stressed broiler chickens were provided butyric acid and were found to have improved intestinal health and accelerated epithelial cell recovery (Abdelqader and Al-Fataftah, 2015). By improving the recovery of damaged epithelia, butyrate provides the small intestine with improved nutrient breakdown and absorption (Guilloteau et al., 2010b). With these results in mind, the current research investigated the effects of incorporating SB into the diet of post-weaned heifers. The study evaluated SB and MON to see if they have comparable effects on growth, health, and FE.

The Preweaned Ruminant

As discussed, transitioning from abomasal digestion to ruminal fermentation of feeds is an important period in a calf's life (Erickson and Kalscheur, 2019). Proper nutrition and careful calf management during the first few weeks of life ensures this transition will occur smoothly. It is vital to the calf becoming a true ruminant that the dietary requirements and digestive processes are understood and correctly implemented. If dietary requirements are not met, it will have negative impacts on the growth of the animal and hinders the maturation of the rumen. Calves

are born with a small, nonfunctional rumen. Thus, their digestive processes initially involve gastric digestion of the liquid feed source in the abomasum. After the first day of life, following the administration of colostrum, starter grain will be provided. Starter grain is a solid feed source that is fermented in the rumen. After the fermentation process, the end products of digestion go directly to the development of ruminal epithelial tissue. Both milk and starter sources, along with water, are meant to stimulate the growth and development of a fully functioning ruminant animal (Kertz et al., 1984).

In those first few weeks of life while gastric digestion is the main source of nutrient breakdown, and the abomasum is nearly 50% of the total mass of the stomach (Warner and Flatt, 1964). During this stage, the reticulorumen is the smallest, only making up 38% of the total mass (Warner and Flatt, 1964). As discussed, the first nutrients the calf receives comes from a liquid source. These come in the form of colostrum and milk or milk replacer. In the calf, milk-based liquid feeds will pass through the esophagus where they will be shunted to the esophageal groove. The esophageal groove functions to bypass the reticulorumen and go directly to the omasum and abomasum (Hegland et al., 1957).

The transition from preweaning to ruminant digestion is a large adjustment period in the life of the calf. It is important during this time that that starter grain is fed to supplement the dietary needs of the calf and to ensure proper reticulorumen development. It is shown that at about 2 weeks old, calves will start consuming considerable amounts of starter (Williams and Frost, 1992; Khan et al., 2008). Early in life, ruminal tissue in the calf is undergoing a period of rapid growth (Davis and Drackley, 1998). This ruminal growth is occurring 4 to 8 times faster than the growth rates of the rest of the body (Davis and Drackley, 1998).

Through all research done, it is apparent that starter intake is important to the growth and development of the calf, specifically the rumen. Solid feed intake is essential in developing ruminal epithelial tissue, which supports proper fermentation of feed and absorption of nutrients (Figure 1; Heinrichs, 1993). Starter grain is an easily fermentable feed source for calves, and the end products stimulate rumen development. Physical form of starter affects intake in calves (Lesmeister and Heinrichs, 2004; Bach et al., 2007; Porter et al., 2007; ; Khan et al., 2016; Terré et al., 2016), as well as rumen growth and development (Greenwood et al., 1997b; Beharka et al., 1998; Lesmeister and Heinrichs, 2004; Laarman et al., 2012).

Figure 1

In the calf, feed particle size has been shown to significantly impact ruminal function and digestive capacity. Beharka et al. (1998) tested particle size in pre-weaned dairy bull calves to see the effect on gastric development; these calves were fed ground (1 mm particle size) or

unground diets with equal composition (25% alfalfa hay and 75% grain mix). The starter was fed and adjusted based on consumption, and all calves were fed milk at a rate of 8% of BW. Researchers slaughtered these calves at week 11 and all digestive organs were removed and weighed. They found that particle size had no effect on the weighs of the reticulorumen or abomasum. However, when examining ruminal tissue samples, they saw major differences in shape, volume, and length of papillae. Calves fed the ground diet had shorter, thicker, and advanced branching patterns in their papillae. The papillae of the unground diet calves were uniform, flattened, and tongue shaped. So, even though ground diet calves had branching, because of the distribution in the volume of papillae present they had a smaller surface area than the papillae of calves fed unground feed. We can associate that branching in the ground diet is compensation for the loss of surface area and proper nutrient absorption due to parakeratosis, which occurs in high concentrate diets or diets containing easily fermentable (small particle size) components (Bull et al., 1965).

During the first few days of life, due to their sole dependence on milk-based liquid feed for nourishment, it is important to introduce that solid feed source early. Solid feed should be consumed as a portion of the diet once the ruminal tissue growth rate increases (Davis and Drackley, 1998). Since ruminal tissue develops 4 to 8 times faster than the rest of the body during the 3rd to 8th week of life for the calf, this is when dietary needs are increased (Davis and Drackley, 1998). Starter should be a considerable portion of diet during that time period to provide additional energy and protein with the milk-based liquid feed for adequate nutrient intake and gut development (Davis and Drackley, 1998; Khan et al., 2016). Providing a solid feed source with adequate physical form is vital to provide the necessary intake (Davis and Drakley, 1998; Lesmeister and Heinrichs, 2004; Bach et al., 2007; Porter et al., 2007; Khan et

al., 2016; Terré et al., 2016) which will establish the correct development of the rumen structure, specifically affecting the morphology of the rumen papillae (Bull et al., 1965; Greenwood et al., 1997b; Beharka et al., 1998; Lesmeister and Heinrichs, 2004; Laarman et al., 2012). Since starter grain is fermented in the rumen, it will produce VFA (to be discussed later) that are utilized by the epithelial tissue. Thus, with all this considered, it is important to provide calves with the correct nutrition during the first few weeks of life in order to establish a well-developed and properly functioning rumen.

Weaning

The weaning period is a crucial time in the calf's life. During this state, producers will reduce the calves' milk-based liquid feed consumption and focus on providing an increased amount of solid feed. This is a period of transition from mainly abomasal digestion to solely depending on solid feed sources, apart from water, for all needed nutrients. At this stage, there is the development of the balance between ruminal digestion and gastric/intestinal digestion. As discussed, solid feed consumption will aid in the growth of ruminal papillae. Fermenting solid feed in the rumen while in the preweaning and weaning periods will provide the calf with the energy needed to enhance growth rates in the postweaning period. The feeding program of milk replacer (**MR**) and starter grain in the preweaning period can greatly affect calves in the weaning period (Cowles et al., 2006; Eckert et al., 2015; Guindon et al., 2015; Chapman et al., 2017). However, accelerated MR fed calves can compensate in starter grain intake if they are gradually weaned (Khan et al., 2011). Each producer has their own preference on the weaning schedule for calves, mostly based on age, BW, solid feed intake, or a combination of those factors (Greenwood et al., 1997a; Kehoe et al., 2007; Sweeney et al., 2010; Eckert et al., 2015; Benetton et al., 2019). On average, U.S. producers wean their calves at 8.2 weeks, but it is not uncommon

to wean earlier or later (USDA, 2007). Those that opt for an earlier weaning time typically will do so to reduce feed and labor costs.

Ionophores

Ionophores are carboxylic polyether antibiotics only used as growth promoters, not used in human or veterinary medicine with no apparent ruminal antibiotic resistance (McGuffey, 2017). Ionophores act by disrupting the ion gradient in the cell membrane of Gram-positive bacteria. When ionophores interfere, available K^+ and ATP are reduced, which prevents bacteria from sustaining adequate cellular growth (Russell and Strobel, 1989). Gram-negative bacteria can thrive under these conditions due to their thicker cell membrane. A thicker cell membrane makes them less susceptible to ionophore caused cell destruction (Callaway et al., 2003).

Ionophores were used in the 1970s as a coccidiosis controller in broiler chickens in the United States (McGuffey et. al, 2017). In learning more about ionophores and how they act within the cell membrane of bacteria, it was discovered that feeding ionophores decreased the prevalence of Gram-positive bacteria resulting in an increase in Gram-negative bacteria (National Research Council, 2001). Seven ionophores were approved for use in different types of livestock (Feed Additive Compendium, 2000). Of these seven ionophores approved, MON is the most common, followed by lasalocid.

Understanding how ionophores affect the rumen and its microbes is connected to the concentrations of VFA in the rumen. When microbes ferment structural carbohydrates in the rumen, they produce acetate and butyrate. However, when Gram-positive bacteria are decreased, the concentrations of acetate and butyrate are reduced. The microbes also can ferment the nonstructural carbohydrates to propionate. However, when the Gram-negative bacteria thrive,

propionate increases. To comprehend how exactly adding ionophores to the diet can alter the VFA ratio in the rumen, Russell and Strobel (1987) added MON or bacitracin to rumen fluid that was incubated with hay or corn. Monensin plus hay was shown to reduce fiber digestion, leading to a decrease in acetate production. This result was also seen in bacitracin but to a lesser degree. Monensin plus corn was shown to increase propionate production and not affect any of the other VFA concentrations. Monensin addition to a 50:50 forage to concentrate diet was shown to produce more moles of VFA/ kg DMI, specifically increasing acetate and propionate (McGuffey, 2017). The ability to modify the glucogenic to non-glucogenic VFA ratio is suggestive of proper energy balance in cattle (Ellis et al., 2015).

To study the effect of MON on energy balance of heifers, Baile et al. (1982) supplemented either 0, 200, or 600 mg per day of MON to 60 Holstein heifers. These heifers were supplemented from when they weighed 196 kg until 3 days prior to calving. Both the 200 and 600 mg/d MON supplemented heifers gained an additional 0.09 kg/d as compared to control heifers ($P < 0.05$). Supplementing MON to heifers also resulted in a lower DMI. This reduced DMI led to a 12.6 (in 200 mg/d) and 13.4% (in 600 mg/d) greater FE. At calving, MON supplemented heifers were 30 to 40 kg heavier than control heifers and not associated with increased calving difficulty. This implies that this gain did not cause an increase in body condition score. These results are similar to Rouquette et al. (1980), who observed increased ADG, no effect on DMI, and improved FE on MON supplemented heifers while grazing on Bermudagrass.

Adding ionophores to the diets of heifers has shown a FE response, however, there is another primary benefit. Similar to poultry, ionophores are shown to prevent coccidiosis in ruminants (Bergen and Bates, 1984). Both MON and lasalocid are approved for the control of

coccidiosis and increased growth rate response in heifers. Quigley et al. (1997) investigated the supplementation of lasalocid in MR and starter grain in terms of coccidian oocyst shedding, fecal scores, BW, and intakes of calves. Calves were assigned to a 2x2 factorial arrangement with lasalocid supplemented in MR (0 or 80 mg/kg) from d 1 of life until d 42 and starter (3 or 44 mg/kg) from d 2 of life until d 42. On d 10, while lasalocid was being administered, calves were orally dosed 100,000 *Eimeria* oocysts. When lasalocid was added to the MR, calves had greater body weight gain (**BWG**). When compared to control calves, the lasalocid supplemented calves had fewer *Eimeria zuernii* oocysts in their feces with a lesser fecal score. There were no differences in oocysts shed or fecal scores in the low or high lasalocid starter supplemented calves when fed in addition to lasalocid MR.

Summary of Ionophore Benefits and Use

Ionophores act as fermentation modifiers in the rumen, resulting in an improvement in FE. They also function as anticoccidials. Feeding ionophores has been shown to increase energy metabolism efficiency in the animal and/or bacteria in the rumen (McGuffey, 2017). A reduction in methane production is an important result in ionophore supplemented animals (Van Vugt et al., 2005; Odongo et al., 2007). Methane and VFA are terminal acceptors for hydrogen (Hungate, 1966). Chalupa (1977) evaluated fermentation balance equations and found that an increase in propionate production must be accompanied by a reduction in methanogenesis. In increasing the amount of propionate in the rumen, hydrogen has more of an opportunity to bind to VFA, leaving less free hydrogen available to form methane (Van Nevel and Demeyer, 1977). However, it is worth noting that other research has indicated that ionophore supplementation did not decrease methane emissions (Sauer et al., 1998; Guan et al., 2006; Grainger et al., 2008). Ionophores have also been shown to reduce ruminal ammonia and microbial protein synthesis,

resulting in more protein flowing to the abomasum (McGuffey, 2017). Based on these results, it is apparent that ionophores have been beneficial to the producer and the environment. However, in 2006 the European Union banned antibiotics or any antibiotic-like substance in livestock diets (European Commission, 2005). Since then, researchers have been searching for any additives that could be considered a replacement for ionophores. One additive that has gained research interest is SB because it has shown to reduce the prevalence of scours and improve health and growth of the epithelial cells in the small intestine.

Introduction to Butyrate

Out of the three VFA (acetate, propionate, and butyrate), increasing butyrate in the rumen is the most vital for rumen development. Butyrate is a result of dietary carbohydrate (cellulose, hemicellulose, starch, and soluble sugars) fermentation in the rumen. Butyrate is typically found in the lowest concentration and is mainly metabolized by the epithelial layer of the rumen (Ash and Baird, 1973). In the rumen epithelial layer, butyrate has been shown to stimulate papillae growth, which increases the surface area for the absorption of nutrients (Tamate et al., 1962). A product that can be supplemented to increase levels of ruminal butyrate is the organic salt, SB. Sodium butyrate has been shown to positively affect the growth of papillae in older cattle (Kowalski et al., 2015).

Increasing the absorptive ability of the rumen is particularly important in younger heifers because a highly developed rumen with an increase in absorption of nutrients can lead to increased tissue and skeletal growth. Supplementation of SB in young calves' diets has been shown to increase growth rates, health, and rumen development (Górka et al., 2011a). When growth rates are improved, heifers may reach breeding size at a younger age. If they reach proper size younger, they may be able to be bred sooner, and enter the lactating herd earlier. A more

rapid addition to the lactating herd will save producers money by decreasing feed costs spent on heifers and resulting in more productive animals quicker than expected.

Production via Microbial Action

Volatile fatty acid production occurs through the anaerobic fermentation of carbohydrates. The molar ratio of the three VFA within the rumen is 65% acetate, 20% propionate, and 15% butyrate, but are dependent on diet composition (Bergman, 1990). To maintain VFA, the rumen relies on microbial action on structural carbohydrates that would otherwise be poorly digested. The rumen contains cellulolytic bacteria, hemicellulolytic bacteria, and some that will digest both. These bacteria will release cellulase and hemicellulase, capable of digesting cellulose and hemicellulose into oligosaccharides. Oligosaccharides are further broken down into a variety of hexoses and pentoses (Beever, 1993), which are then utilized by both cellulolytic and non-cellulolytic bacteria to obtain ATP to then create VFA.

These microbes are dependent on pyruvate production as a substrate to produce VFA. Glucose will be converted to pyruvate via the Embden-Myerhof pathway of glycolysis, but the fate of pyruvate depends on the microbes present in the rumen and the ruminal environment. Pyruvate can be converted to acetate through two different enzymatic pathways. The most common pathway is the pyruvate-formate lyase system that will produce formate and acetyl-coenzyme A (**acetyl-CoA**). The second pathway is the pyruvate-ferredoxin oxidoreductase pathway, which will convert pyruvate into reduced ferredoxin, carbon dioxide (**CO_2**), acetyl-CoA (Baldwin and Allison, 1983). Acetyl-CoA is now available to be converted into acetate and 1 ATP via phosphotransacetylase and acetokinase. Acetyl-CoA is a vital substrate in the production of butyrate in bacteria and protozoa.

High concentrate diets will support an environment for a greater concentration of protozoa (France and Siddons, 1993). Of these protozoa, *Dasytricha ruminantium* is necessary to convert acetyl-CoA into butyrate, lactate, and acetate. Particularly in excess soluble sugar-containing solutions, *D. ruminantium* will convert acetyl-CoA to butyryl-CoA. Once it is butyryl-CoA, phosphate butyryltransferase or butyrate kinase is needed to catalyze the conversion of butyryl-CoA into butyrate, generating 1 ATP molecule (Yarlett et al., 1985). *Butyrivibrio fibrisolvens* ferment glucose and produce hydrogen, CO_2, and butyric, formic, and lactic acids (Bryant and Small, 1955). *Butyrivibrio fibrisolvens* D1 is the specific strain of these Gram-negative bacteria that will primarily produce butyrate, especially in the presence of a high-fiber diet. The process begins with two molecules of acetyl-CoA being enzymatically transformed into crotonyl-CoA, which is then converted into butyryl-CoA. From here, the conversion of butyryl-CoA to butyrate is catalyzed by the same enzymes as *D. ruminantium* (Miller and Jenesel, 1978).

Pyruvate can also be converted into lactate under conditions of low ruminal pH. Lactate production in the rumen is stimulated by a sudden drop of pH, typically caused by the consumption of high-concentrate diets which require rapid microbial fermentation. Lactate will need to be removed from the rumen, and it is done in 1 of 3 ways: passage through the lower gut, absorption from the rumen, and microbial fermentation (Counotte et al., 1981). In terms of microbial fermentation, *Megasphaera elsdenii*, ferments lactate into propionate via the acrylate pathway. *Megasphaera elsdenii* can also ferment lactate into butyrate. In order to determine percentages of lactate conversion, Counotte et al. (1981) incubated in vitro cultures of lactate. They aimed to see how much lactate was converted via the acrylate pathway into propionate and the amount fermented into butyrate. It was concluded that in ruminants fed normal diets, *M.*

elsdenii fermented 60 to 80% of lactate in the rumen, and a good portion of that percentage of lactate is either fermented into butyrate or propionate via the acrylate pathway. They plotted the effect of pH on VFA formation of *M. elsdenii* and found that as pH decreased in the rumen, more lactate was fermented into butyrate.

Many species of bacterium will obtain their energy solely from the synthesis of VFA. Acetate will be utilized when the bacteria species have limited means to obtain their own energy source. Hino et al. (1990) aimed to see how acetate would affect the proliferation of *M. elsdenii* when added to a medium of glucose and Trypticase. This bacterium was able to grow in the medium, but the addition of acetate improved growth rates. Thus, increasing acetate concentration increased the production of butyrate. Their results suggest that acetate functions as an electron acceptor for hydrogen ions produced during glucose metabolism, thus giving them the energy to put towards butyrate synthesis. Another anaerobic bacterium that obtains energy through fatty acids is *Clostridium kluyveri*, depending on acetate and ethanol to obtain benefits from complex substrates (Bornstein and Barker, 1947). This bacterium will oxidize ethanol into a 2-carbon compound, which is now referred to as “active” acetate. “Active” acetate at this stage will then be condensed with acetate to form a 4-carbon compound. The 4-carbon compound will further be reduced into butyrate.

Rumen Epithelium and Absorption Effects on Tissues and Organs

Dietary composition has a major impact on the concentration of VFA in the rumen. Diets high in fiber stimulate the production of acetate-producing microbes. Diets high in starch and/or concentrate will stimulate the production of propionate and butyrate-producing microbes, though acetate will remain the largest available VFA (France and Siddons, 1993). Volatile fatty acid production and absorption is important because the cow will derive 70 – 80% of its energy from

VFA. Specifically, this is done through propionate. Propionate is the primary VFA that will increase glucose production, though butyrate can contribute a portion (to be discussed below) if propionate production is low. Nearly 50% of the propionate produced will be available for uptake by the liver to be converted into glucose (Bergman et al., 1965).

Much of the VFA produced in the rumen is also absorbed in the rumen. From ruminal absorption, it can either be utilized by tissues of the rumen epithelial layer or transported into the bloodstream. Nearly 30% of acetate, 50% of propionate, and 90% of butyrate were absorbed in the rumen to be used by epithelial tissue instead of reaching portal circulation (Bergman and Wolff, 1971; Bergman, 1990). Using sheep, Dobson et al. (1956) were the first to determine the organization of the rumen epithelial tissue. The first mucosal layer is covered in papillae that vary in shape and size, depending on their location in the rumen. Most of the papillae are tongue-like but occasionally will appear conical. Most papillae, in the superficial layer encountering rumen digesta, are keratinized stratified squamous epithelial tissue. At the core of the papillae are dense collagen fibers, along with arterioles, venules, and lymphatic vessels. This papillary core allows the rumen to absorb VFA and transport them into the blood via diffusion.

Of the three main VFA produced in the rumen, butyrate is metabolized the most by the epithelial tissue of the rumen. Nearly 90% of the butyrate produced is absorbed and metabolized here, being converted into ketone bodies or going through oxidation to produce CO_2 (Bergman, 1990). Ketone formation in the rumen epithelium is referred to as alimentary ketogenesis, which is important because typically the production of ketones is limited to the liver. Since ruminants' ferment feeds, this creates a more acidic rumen environment and affects the survival of the microbes. The ketogenic activity of the rumen epithelium is then important to equalize that acidity, preventing ruminal acidosis. The balance in pH will also create a proper microbial

environment so the microbes can digest feeds, thus ensuring that ruminants would continue to produce adequate concentrations of VFA. Pennington (1951) incubated butyrate, acetate, and propionate with rumen epithelial cultures to determine if there were any effects on ketone body production. Results indicated that butyrate produced larger concentrations of ketones than the other VFA, and the amount of butyrate utilized to produce ketone bodies was 59 to 74%. If butyrate was supplied at a normal concentration of 100 μmoles/culture, then 70% of the butyrate available was converted into ketones. If the amount of butyrate supplied was reduced to 50%, then 65% of the available butyrate was converted into ketones. These results suggest that the rumen epithelial tissue has a high affinity for the conversion of butyrate into usable ketone bodies.

Although butyrate is mainly absorbed and utilized by the epithelial tissue of the rumen it can circulate in the blood supply, impacting the liver and lower gastrointestinal tract (**GIT**). Butyrate that is not used in the ruminal tissue is transported via the hepatic portal vein into the liver. The liver will then convert butyrate into butyryl-CoA through the enzyme butyryl-CoA synthetase. Butyryl-CoA will then be ready to transform into either acetyl-CoA, ketones, or long-chain fatty acids (Bergman, 1990). When butyrate concentration is high in the liver, it will have an inhibitory effect on propionate utilization. Demigne et al. (1986) incubated sheep hepatocyte cultures and found that 2 mM of butyrate decreased the hepatic conversion of propionate into glucose by 63%. However, since 90% of the butyrate is absorbed and metabolized by the rumen in normal conditions, the small amount of circulating butyrate would minimize the inhibitory effect it would have.

As mentioned, butyrate can aid in the forming of glucose. Gluconeogenesis is highly regulated by the secretion of insulin, which has been seen to increase with the administration of

butyrate or propionate. Sano et al. (1995) conducted an experiment to pinpoint the physiological role of butyrate and how it would affect concentrations of plasma insulin and glucagon. In sheep, butyrate was infused intravascularly via the femoral vein at rates of 0, 1, 2, 4, 8, 16, 32, and 64 μmol·kg BW^{-1}·min^{-1}. Butyrate infusion at a rate of 2 μmol·kg BW^{-1}·min^{-1} or higher was shown to increase plasma insulin. Butyrate infusion at a rate of 32 and 64 μmol·kg BW^{-1}·min^{-1} was shown to increase plasma glucose. Based on the increase in both plasma insulin and glucose, it can be inferred that insulin concentrations were not affected by glucagon since glucagon needed a greater infusion rate of butyrate to elicit a response. Therefore, it appears that, at least in sheep, butyrate is capable of stimulating insulin and glucagon secretion from the pancreas. Through insulin, glycolysis will be stimulated and can lead to an eventual increase in blood glucose levels. Through glucagon, gluconeogenesis will be stimulated and can lead to an eventual decrease in blood glucose levels.

The results seen in Sano et al. (1995) in sheep were confirmed by Herrick et al. (2017), now showing the effects of butyrate on glucose metabolism in lactating cows. They dosed SB at either 1 g/kg of BW or 2 g/kg of BW ruminally and compared the metabolic response in cows treated with 2 L of water (control) or 3.5 g/kg of BW of lactose. Plasma ketone concentration was increased ($P < 0.01$) in cows dosed with SB vs. control or lactose treated cows. Plasma insulin concentrations tended to increase ($P = 0.06$) in cows dosed with SB vs. control or lactose treated cows. This supports the hypothesis that SB supplementation increases insulin production and secretion, indirectly affecting glucose metabolism.

Along with butyrate absorption in the rumen and GIT, it can be utilized by the mammary gland. Supplementing SB in the diet of lactating cows can impact milk and components. In a 4 X 4 Latin square, each experimental period lasting two weeks, Huhtanen et al. (1993) used 4 mid-

lactation cows and infused varying amounts of butyrate intraruminally. Though milk yield was unchanged by butyrate infusions, there was an increase in components, both milk fat ($P < 0.01$) and protein ($P < 0.05$), with the increase in butyrate infusion rate. The significant increase in milk fat resulted in an increase ($P < 0.05$) in milk fat yield. Finally, in terms of components, lactose concentrations ($P < 0.01$) and lactose yield ($P < 0.10$) underwent a linear decrease when butyrate infusion was increased. Overall, the infusion of butyrate did not impact milk production, but it did impact milk composition. This increase in milk components, through the addition of butyrate, can be explained at a molecular level. Kleiber et al. (1954) injected 1-C^{14} and 2-C^{14}-labeled butyrate into the jugular veins of 4 lactating cows. Through the injection of carbon labeled butyrate, they were able to trace the transfer of carbon and estimate the rate of transfer from butyrate into lactose, casein, albumin, and milk fat. The results found, in the milk constituents, about 6% of 1-C^{14} and 22% of the 2-C^{14}-labeled butyrate. Specifically, more 1-C^{14} and 2-C^{14} were found in lactose and casein than in milk fat. Since lactose is composed of glucose and galactose and labeled butyrate was found in lactose, butyrate can be considered gluconeogenic. Likely, through the conversion of butyryl-CoA to acetyl-CoA (Bergman, 1990).

Sodium Butyrate Effects on Growth, Digestibility, and Health

As discussed, 90% of butyrate will be absorbed in the rumen epithelial tissue, thus butyrate will play a key role in maturing the epithelial layer of the rumen in dairy calves (Bergman, 1990). The epithelial tissue will greatly influence the digestive capabilities and health of cattle, along with other animals. Abdelqader and Al-Fataftah (2015) supplemented butyric acid into the diets of heat-stressed broilers to test the performance of the animals as well as any intestinal effects. Butyric acid was seen to enhance intestinal development by stimulating an increase in villi height, villi surface area, absorptive epithelial cell area, and intestinal weight.

These results indicate that butyrate stimulated epithelial cell proliferation in order to repair the intestinal damage that occurred due to heat-stress. Outward signs resulting from the repair of intestinal damage and proliferation of epithelial cells were that butyric acid supplementation increased growth rates and feed efficiency in heat-stressed broilers. Sakata and Tamate (1978) intraruminally administered SB (2g/kg BW per day) one time a day to sheep either within 10 s (rapid rate) or over 20 to 24 h (slow rate). When biopsying the rumen papillae of sheep supplemented with SB, the rapidly dosed sheep had an increase ($P < 0.01$) in the mitotic indices the day following treatment when compared to indices before administration. However, slowly dosed sheep did not show any difference in the mitotic index because only moderate butyrate production was stimulated in the rumen based on the mode of administration. These results indicated that rapid intraruminal administration of SB will stimulate the proliferation of rumen epithelial cells.

To confirm the results of intraruminal SB infusion, Moolchand et al. (2013) supplemented SB into the diets of 15 ruminally-fistulated goats at 120 d of age. They were assigned to 1 of 2 treatment groups of infusion with or without SB. Sodium butyrate treated goats were infused once daily for 28 d with 0.3 g/kg of BW of SB over 10-15 s. On d 14, just before infusion and 0.5, 1.0, 1.5, 2.5, and 3.5 hrs after infusion, researchers took rumen fluid samples for analysis of VFA. On d 26, the ruminal liquid rate of passage was estimated. At d 28, goats were slaughtered, and GIT compartments were collected, weighed, and their morphological characteristics were measured. Butyrate concentrations increased ($P <0.01$) and were able to remain elevated for 3.5 h after SB infusion. Papillae height increased ($P <0.005$), the space between papillae decreased ($P < 0.05$), and epithelial layer thickness increased ($P < 0.05$) with SB infusion. The rumen in SB infused goats was 89.09% of total stomach weight, which was

greater ($P < 0.05$) than the 86.71% in control animals. Weight of the ruminal digesta in SB infused goats was 91.72% of total stomach weight, which tended to be greater ($P < 0.06$) than the 89.81% in control animals. The results of this study indicated that SB infusion improved the absorptive capacity of the rumen by increasing papillae size and density, while also causing a longer retention time of feeds in the rumen. Combining increased papillae size and surface area with longer feed retention supports the thought of improved rumen efficiency and digestive capabilities. An improvement in rumen and overall digestive efficiency can result in the improvement of growth performance in animals.

As discussed in the preweaning ruminant section, young ruminants do not have a fully developed rumen. Due to this, most digestion occurs in the abomasum and lower gut. In the lower gut, the small intestine (**SI**) is the compartment where most nutrient absorption is occurring in the pre-ruminant. In the duodenum, chyme from the abomasum will mix with pancreatic secretions, containing different enzymes to digest the ingesta. If the volume of pancreatic juices is increased, nutrient digestibility and FE can be improved. To study if there are ways to increase pancreatic juice volume, Guilloteau et al. (2010a) conducted two separate experiments, with SB supplemented orally and duodenally to eight calves. Each study began on d 54 of life and ended at d 88.

In experiment one, Guilloteau et al (2010a) evaluated 4 calves on d 6 of three different periods (P1 to P3) and aimed to study the duodenal effects of SB infusion. Calves were fed either MR (control diet) or 3 g SB per kg of DM (butyrate diet) added to MR. Infusion of saline (control solution) occurred during P1 and infusion of the same quantity of SB as the diet (SB solution) occurred during P2 and P3. Solutions were infused from 5 to 7 h after the morning meal. They collected pancreatic juice every 5 min for 2 consecutive h via cannulas placed in 2 of

the 4 calves on study at the pancreatic duct and the duodenum. Blood samples were taken at 20 and 30 min before infusion and at 5, 15, 25, 35, 45, 60, and 90 min after infusions. The results showed that SB infusion did not have any effect on pancreatic juices, protein, or the flow rate of chymotrypsin. However, SB increased the flow rate of lipase ($P < 0.05$) compared to control. Thus, it can be concluded that duodenal infusion of SB has little effect on pancreatic secretions.

In experiment two, Guilloteau et al. (2010a) evaluated four calves and aimed to study the dietary effects of SB supplementation. During the pre-experiment period, calves were fed MR (control diet) until they were cannulated at the junction of the duodenum and pancreatic duct. In the second period calves were fed the control diet. In the third period, there was a transition between the control diet and the SB diet (3 g SB per kg of DM). In the fourth period, the calves were fed entirely SB diet. They continuously collected pancreatic juice over a 24 h period on the third day of P1, P2, and P3. During d 5 of each period when pancreatic juice collection was no longer occurring, blood samples were taken at different times. Blood was taken 30 and 60 min before the morning feeding and then 5, 15, 30, 45, 60, 90, 120, 150, 180, and 210 min after the meal. Finally, fecal samples were collected for 4 consecutive days during each of the three periods to determine apparent digestibility. Results found that, in terms of digestibility, SB fed calves tended to have a higher DM and N digestibility compared to control. Sodium butyrate fed calves also had increased ($P < 0.05$) fat, ash and calcium digestibility. In terms of pancreatic juice secretion, SB fed calves tended to increase ($P < 10$) total pancreatic juice secretion relative to BW compared to control. Sodium butyrate fed calves also had a 1.4-fold increase ($P < 0.05$) in total protein content in pancreatic juice. Total daily production of chymotrypsin ($P < 0.10$) and lipase ($P < 0.05$), relative to BW, were increased by 52% and 40% respectively. This, along with the increase in total pancreatic juice, explains the increased nutrient digestibility in calves

supplemented with SB. Sodium butyrate reduced pancreatic juice flow rate immediately after feeding, but still maintained a maximal duodenal flow of digesta at this time. This indicates SB can alter circadian digestion kinetics. In general, increasing the volume of pancreatic secretions will result in an enhancement of the digestive abilities of the SI, allowing for greater breakdown of proteins. Overall, these results support the theory that, when administered orally, SB will improve the digestibility of nutrients from MR feedings (Guilloteau et al., 2010a).

In order to establish a proper functioning ruminal environment, proliferation of epithelial cells needs to occur to stimulate the maturation of gut tissues. This epithelial cell proliferation will occur through the aid of SB. In the diets of pre-weaned dairy calves, including SB in MR positively increased growth and digestive abilities. Guilloteau et al. (2009) studied 88 dairy calves divided into 2 groups to compare the effects feeding MR with the addition of SB (3 mg/kg of DM) or flavomycin (16.5 mg/kg of DM). Calves were provided ad libitum starter grain, and both levels of MR and starter increased as age increased. Eight calves from each treatment group were chosen as the most representative of all calves and were slaughtered at 151 d of age. After slaughter, the SI was removed, and each section of the SI was measured for length and analyzed for the physical characteristics of the villi. In SB fed calves, the duodenum tended to be longer with longer villi when compared to the duodenum of flavomycin fed calves. The increase in duodenal length and villi length are indicative of maturation of the SI, thus aiding in increased absorption of nutrients. There were no differences in DMI, but calves fed SB had higher body weight gain during the first and final 2 mo of study when compared to flavomycin fed calves. Also, SB supplementation increased FE, which is explained by the improved SI development. So overall, these results support the hypothesis that SB can be a useful growth promoter in milk-fed calves.

Górka et al. (2014) studied the effects of adding SB to MR and/or starter. Twenty-eight Holstein bull calves began study at 5 d of age and were placed into 1 of 4 feeding groups: (1) MR and starter without SB (-/-); (2) MR without and starter with encapsulated SB at 0.6% as fed (-/+); (3) MR with crystalline SB at 0.3% as fed and starter without (+/-); or (4) both MR and starter supplemented with SB (+/+). The MR used in this study contained 60% soy protein concentrate to elicit a slower SI development (Seegraber and Morrill, 1986). All calves were fed twice daily MR (22% CP and 18% fat in DM) at amounts equal to 10% of their initial BW. Starter diet was offered ad libitum to all calves. Calves then remained on their respective treatments for 3 wk. At the end of wk 3, calves were slaughtered, and the GIT was removed and analyzed for structure and morphology. Results showed that DMI with MR was not different amongst the treatments, however, starter DMI was increased ($P = 0.05$) during the final week on study in calves that had SB supplemented into their starter. These results indicated that a greater amount of digesta was able to pass through the GIT into the SI, which enhanced duodenal epithelial development. In terms of the small intestine development, SB supplemented in starter tended ($P \leq 0.07$) to increase overall SI weights as well as jejunum weights. Small intestine and jejunum lengths were greater ($P \leq 0.02$) in -/- and +/+. Total ileum weight ($P = 0.04$) and length ($P \leq 0.02$) were highest in the +/+ calves. In the duodenum, crypt depth and tunica mucosa thickness ($P \leq 0.02$), as well as villus height, was highest in -/+ calves. When compared to calves not supplemented SB in the MR, starter with SB calves had lower ($P \leq 0.04$) villus height in the proximal jejunum and villus height, crypt depth, and tunica mucosa thickness in the middle jejunum. Villus height was increased ($P = 0.04$) in the distal jejunum when SB was added to starter. Overall, these results indicate that increasing villi length will increase the surface area for absorption of digesta.

Mitotic indices were increased ($P \leq 0.04$) and apoptotic indices were decreased (MR and starter interaction, $P < 0.01$) in the middle jejunum when SB was added to both MR and starter (Górka et al., 2014). In order to understand these results, a few things need clarification: mitotic indices were used when determining the rate of epithelial cell proliferation and apoptotic indices were used when determining the rate of cell death (Sakata and Tamate, 1978). Therefore, increasing the mitotic index, with the addition of SB, indicated increased epithelial cell proliferation in the jejunum. So, an increase in the mitotic index with a decrease in the apoptotic index leads to a higher mitotic: apoptotic ratio. This higher ratio indicates an accelerated enterocyte maturation, differentiation, and turnover. Even though there was an effect on the mitotic and apoptotic indices, supplementing SB in MR did not affect intestinal mucosa growth. However, a decrease in mucosa thickness means SI functions require less energy expenditure.

Complete analysis of Górka et al. (2014) generally states that the addition of SB to MR and starter can be viewed as having positive effects on SI growth and development. Since the SB was used in a high soy-protein MR, the addition of SB in MR resulted in the partial reversal of the negative effects on SI development. The SB in the starter resulted in the best duodenum mucosa development. This could be due to the encapsulated SB used in the starter, covered in a triglyceride matrix (30:70 butyrate: triglyceride) to slow the release of butyrate into the rumen leaving more to pass to the duodenum. No synergistic effects were found when adding SB to both MR and starter. Górka et al. (2014) suggest that longer-term studies (> 28 d) be conducted with the combination of SB in MR and starter.

Not only does supplementation of SB affect the absorptive abilities of the SI, but it was also shown to have major effects on rumen papillae. Overall ruminal development will lead to an increase in digestive capabilities and improvement in calf health. Górka et al. (2011b) fed bull

calves ad libitum starter grain. Calves were split into 3 different milk feeding groups: whole milk, MR, or MR with the addition of SB (MR+SB). Liquid feed was based on DM content in whole milk-fed calves to ensure liquid DMI was equal for all calves on study. Overall, 60% of the milk protein provided was replaced with soy protein in MR fed calves, thus ensuring slower SI development (Seegraber and Morrill, 1986). Milk replacer + SB tended to have greater ADG (P <0.09) during the first week on study, and greater BW (P < 0.10) during the three weeks on trial. Milk replacer + SB did not have any effect on the intake of starter grain. In terms of SI development, when compared to only MR, MR+SB increased (P = 0.01) the mitotic index and decreased (P < 0.01) the apoptotic index in the jejunum. In terms of ruminal development, MR+SB increased reticulorumen weight, reticulorumen weight as a percentage of whole stomach weight, and improvement of rumen papillae width and length (P < 0.05) compared to MR.

The benefits for SB have been understood as it pertains to the pre-weaned and weaned heifer. However, the gap that has gone unfilled in research is that if there are any derived benefits in the post-weaned heifer. Rice et. al (2019) investigated this gap in knowledge. They aimed to determine the effect of supplementing varying amounts of SB into the diets of 3 to 6 mo old heifers. Heifers entered study with a mean age of 84 d and were assigned to one of four treatments: (1) 100 g of soybean meal carrier (control); (2) 0.25 g of SB/ kg of BW plus carrier; (3) 0.50 g of SB/ kg of BW plus carrier; and (4) 0.75 g of SB/ kg of BW plus carrier. Sodium butyrate increased average BW (P = 0.04) and tended to increase final BW (P = 0.07). Overall BWG increased linearly (P = 0.02) as SB levels increased. There was a treatment by week interaction for heart girth and heart girth gain (P < 0.02), but the remaining skeletal measurements were unaffected by treatment. There was a linear trend towards FE (P = 0.08), with FE increasing by 16.67% in heifers fed 0.75SB. Fecal samples from each heifer indicated

the presence of coccidian oocysts. There was a positive quadratic response towards the reduction of these oocysts ($P = 0.03$) with 0.25SB being the most effective. Overall, these results indicate that SB supplementation increased growth rates, BWG, FE, and health of the animal. It is apparent that the effects on intestinal development and absorption improvement, ultimately leading to improved growth performance that has been seen in younger animals supplemented SB are still present in older heifers.

Coccidiosis

Coccidiosis is a significant disease in the lives of young ruminant animals, holding a very significant economic impact on producers. The economic loss due to coccidiosis is due to the health impacts from treating intestinal damage, as well as mortality in severe cases through the loss of future productive animals (Quigley et al., 1997). Coccidiosis is caused by the protozoan species *Eimeria*, and each specific *Eimeria* will infect their specific host animal. Worldwide, there have been twelve *Eimeria* species identified in cattle shown to be mild to moderately pathogenic. However, the two primary species associated with the clinical symptoms of coccidiosis are *E. bovis* and *E. zuernii* (Quigley et al., 1997; Constable, 2019).

Time from initial ingestion, to the stage of detectible parasitic infection, is 15-17 d in *E. zuernii* and 15-20 d in *E. bovis* (Farm Health Online). The life cycle (Figure 2, Farm Health Online) is as follows: (1) sporulated protozoan oocysts (eggs) are shed in the feces of infected animals, able to survive on the ground for up to a year; (2) sporulated oocysts are now readily available to infect other cattle through fecal-oral transmission. When the oocyst becomes exposed to CO_2 and digestive enzymes in the host GIT, it will split open and release 8 sporozoites; (3) each sporozoite will travel to the SI; (4) after ingestion, it takes sporozoites 3 to 7 days to finally enter the SI, settle into the epithelial layer, and asexually reproduce (beginning

at d 5 and completed at d 10) into up to 120,000 first-generation merozoites; (5) merozoites are released when the host cell ruptures; (6) in the lower SI and upper LI, this group of first generation-merozoites will then asexually divide, producing up to 30 second-generation merozoites; (7) the second-generation merozoites will settle into the LI, distinguishing themselves as male or female to now undergo sexual reproduction; (8) the zygote formed through sexual reproduction will form a protective wall around itself, thus becoming an oocyst and causing the host cells to rupture. One single oocyst can produce up to 23 million oocysts in the next life cycle; (9) the oocyst will now, along with tissue and fluids from ruptured host cells, travel through the lower GIT into the feces (at this stage, the oocyst is unsporulated); finally, (10) in the presence of oxygen, the oocyst will take 2 to 4 d to sporulate, now capable of infecting cattle (Dedrickson, 2019).

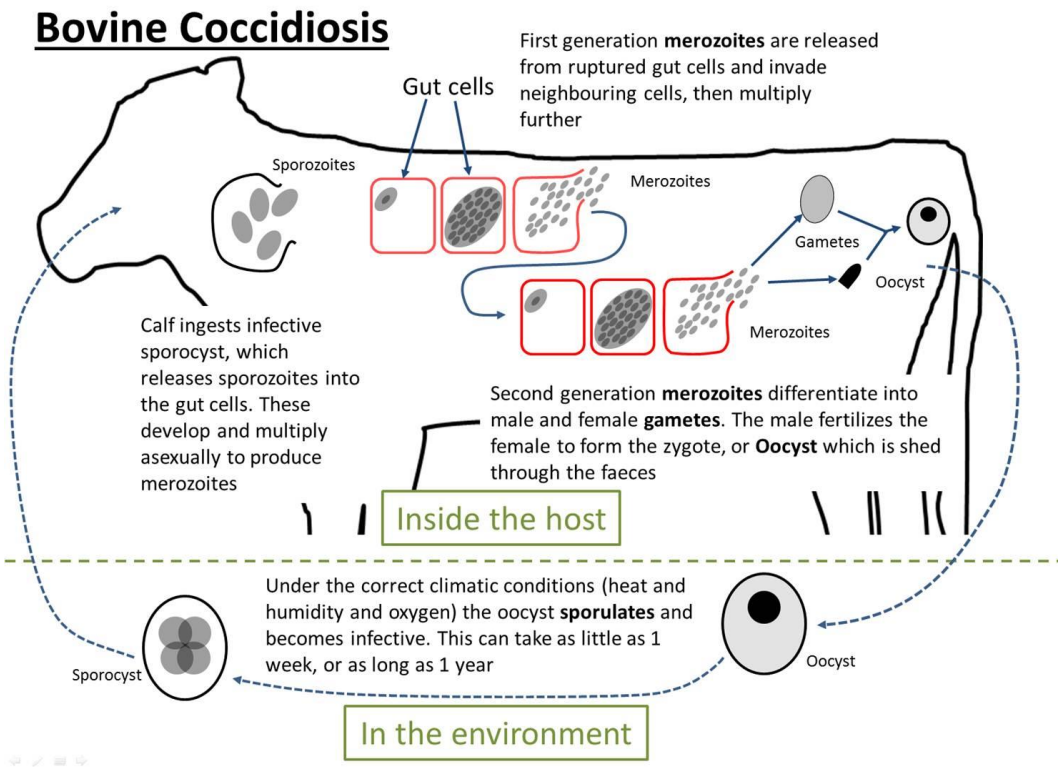

Figure 2

Due to the release of oocytes in the feces, they become overly abundant in the environment, thus making the disease hard to prevent. The disease is known to be sporadic throughout the seasons, and presence in the environment can depend on many factors that can cause stress: wet weather, severe temperature fluctuations, overcrowding, and pen changes (Rice et al., 2019; Constable, 2019) Young animals, between 1—2 mo to 1 yr of age, are the most susceptible to coccidiosis due to their lessened immune system function (Constable, 2019). Most species of *Eimeria* will present as subclinical coccidiosis, never being diagnosed as coccidiosis (Cornelissen et al., 1995). However, there are cases that will present with clinical symptoms. Clinical symptoms can include watery scours, bloody scours, and posterior fecal staining. Upon infection, the onset of diarrhea will occur within 16—23 days in *E. bovis* and *E. zuernii* (Constable, 2019). Development of clinical coccidiosis is dependent on: (1) the species of *Eimeria* involved; (2) the age of the infected animal; (3) number of oocysts ingested; (4) the presence of a simultaneous microbial infection; and (5) the farm-specific management practices implemented (Cornelissen et al., 1995).

There are occurrences of minor infections, where cattle will appear healthy, though FE is reduced, and oocysts will be present in formed feces. Severe infections, though rare, can be developed by thin, bloody scours continuing for more than 1 wk. Alternatively, it can present as thin feces with some small amount of blood, shreds of epithelial tissue, and mucus. In severe infections, calves could have elevated body temperature, experience weight loss, suffer from a depressed appetite and can be dehydrated. In coccidiosis, inflammation would be present in the LI and pathogenic coccidian protozoa can cause damage to the mucosa layer of the lower SI, cecum, and colon (Constable, 2019). Any damage done to the intestinal epithelium will decrease the absorption of nutrients. This ultimately leads to a decrease in DMI, thus reducing growth

rates. Coccidiosis is damaging to the development of the calf, potentially leading to a less efficient mature animal.

Conclusion

It is ideal for farmers to produce healthy, productive heifers while improving FE and potentially decrease feed cost. Alternatively, the producer may also see an increase in BW with the same feed intake expected for heifers in their respective ages. Monensin has proven to be a beneficial feed additive in heifer diets. However, with the reduction in ionophore use in Europe, researchers have studied alternative feed additives to replace these antibiotics. An example of a feed additive that could replace ionophores is SB. Rice et al. (2019) were the first researchers to investigate SB on growth and health performance of post-weaned heifers. The current study is a continuation of that work evaluating SB and MON.

CHAPTER 2: SODIUM BUTYRATE AND MONENSIN SUPPLEMENTATION TO POST-WEANED HEIFER DIETS: EFFECTS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND HEALTH

INTRODUCTION

Raising replacement heifers is one of the largest expenses of the farm (Gabler et al., 2000; Heinrichs et al., 2013). It is important to closely manage youngstock, along with providing adequate nutrition, to ensure those animals will reach developmental maturity at an earlier age. Through diet manipulation, performance can be enhanced, such as through changes in VFA. For example, feeding ionophores will reduce acetate and butyrate production, thus increasing propionate production (Russell and Strobel, 1987; McGuffey, 2017). When adding ionophores to the diet of youngstock, an increase in FE results in increased nutrient absorption (Rouquette et al., 1980; Baile et al., 1982). Ionophores have reduced coccidian oocyst shedding in the feces, leading to an improvement in the health of the animal (Quigley et al., 1997). Monensin has been shown to enhance performance in dairy cattle (McGuffey, 2017) and is one of the two most commonly used ionophores. However, in 2006 the European Union put a ban on antibiotic-like growth promoters (European Commission, 2005).

An example of a feed additive that could replace ionophores is SB. Butyrate is utilized by ruminal epithelial tissues to increase proliferation of rumen papillae (Górka et al., 2011a,b). Increasing the length and volume of rumen papillae will result in an increase in the absorptive capabilities of the rumen (Górka et al., 2011a,b). With absorptive capacity increased, the heifer can utilize more nutrients for growth. Other than ruminal tissue, SI epithelial tissue can be enhanced by SB supplementation (Guilloteau et al., 2009; Górka et al., 2014). Inclusion of SB in the starter grain increased the mitotic and decreased the apoptotic indices of SI enterocytes

(Górka et al., 2014). This suggests that SB can maintain the growth of SI epithelial cells, which aids in the absorptive function of the lower GIT. Sodium butyrate has also been shown to increase the secretion of pancreatic juices that aid in the digestion of feeds (Guilloteau et al., 2010a). It is apparent that the effects on intestinal development and absorption improvement, ultimately leading to improved growth performance that has been seen in younger animals supplemented SB are still present in older heifers.

Rice et al. (2019) investigated SB on growth and health performance of post-weaned heifers and found increased BW, tended to have greater final BW and FE as SB increased from 0 to 0.75g/ kg, along with a reduction in coccidian oocysts at 0.25g SB/kg. The objective of this study was to compare MON, SB, or the combination, on growth and health performance of post-weaned heifers.

MATERIALS AND METHODS

Experimental Design and Treatments

This experiment was reviewed and approved by the University of New Hampshire Animal Care and Use Committee (Protocol No. 170903).

Forty Holstein heifers with a mean age of 84.2 ± 1.2 d (mean ± SD) and average initial BW of 99.78 kg ± 10.77 kg (mean ± SD) were blocked by date of birth and randomly assigned to 1 of 4 treatments in a complete randomized block design. Treatments were: (1) carrier (control; **CON**); (2) 0.75 g of SB/kg of BW + carrier (**SB**); (3) 1 mg of monensin/kg of BW + carrier (**MON**); (4) monensin + 0.75 g SB per kg of BW (**MSB**). All heifers were given 100 g of carrier (soybean meal) per day and their respective treatments were adjusted weekly according to individual BW. Sodium

butyrate provided was unprotected and was a 90% SB product with 68-69% butyric acid and ~21-22% Na+, which also included ~10% maltodextrin (Ultramix GF, Nutriad Inc. USA, Hampshire, IL). Heifers entered the pen to train to use Calan doors (American Calan Inc., Northwood, NH) at 12 wk of life, entered study on the first Tuesday of 13 wk of life and remained on the study for 12 wk. Heifers were individually fed a total mixed ration (**TMR**) with treatments hand-mixed at approximately 1100 h daily.

Management and Feeding

Heifers were group-housed in a naturally ventilated freestall barn with mattresses bedded with kiln-dried sawdust. Two adjacent pens (pen 1: 5.46 x 4.75 m; pen 2: 5.54 × 4.88 m) were utilized, pen 1 having the capacity to hold 6 heifers and pen 2 having the capacity to hold 8 heifers. Heifers had unlimited access to water through automatically refilling water troughs and no competition for stall space. Each heifer was allotted a 1 wk training period to train to use their assigned Calan feeding doors (American Calan Inc., Northwood, NH).

Heifers were fed the formulated TMR (Table 1) at approximately 1100 h daily in individual feed tubs to allow for daily feed intake measurements. Feed was mixed and distributed using a motorized feeding vehicle (Super Data Ranger; American Calan Inc.). The ration was fed to obtain feed refusals amounting 10% or less, and the amount fed was adjusted daily according to individual intakes. Treatments were hand-mixed into each heifer's feed.

Feed Analysis

Feed refused by and feed offered to each heifer was measured daily at 1030 h and 1100 h respectively to determine dry matter intake (**DMI**). Samples of TMR were taken once weekly on Mondays to get a representative sample of the diet fed out to the animals, and feed refusal samples were obtained daily from each heifer. Both TMR and refusal samples were frozen at -20°C for future analysis. Samples were thawed and placed in a forced hot air convection oven (Binder, Bohemia, NY) to dry at 55°C for 48 h to determine DM concentration.

Samples were ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and sent to a commercial laboratory for nutrient analysis (Rock River Laboratories, Watertown, WI). Feed samples were analyzed for ADF (method 5 in an Ankom Fiber Analyzer A2000; Ankom Technology; method 973.18, AOAC International, 1998), NDF (method 6 in an Ankom Fiber Analyzer A2000 with α-amylase and sodium sulfite; Ankom Technology, Fairpoint, NY; solutions as in Van Soest et al., 1991), starch (YSI 2700 SELECT Biochemistry Analyzer; YSI Incorporated Life Sciences, Yellow Springs, OH), crude fat (ether extraction; AOAC 2003.05; AOAC International, 2006), ash (AOAC Method 942.05; AOAC International, 2006), and CP (AOAC method 990.03; AOAC International, 2006).

Measurements and Blood Sampling and Analysis

Each heifer was weighed, and skeletal measurements were taken before feeding and receiving treatments every Tuesday at 0800 h throughout the 12 wk on study. Heifers were measured for body length, heart girth, and paunch girth. All length and girth measurements were

determined using a weigh tape. Heifers were weighed on a portable scale system (Tru-Test^{TM} EziWeigh5i, Uniontown, PA).

Blood samples were obtained from the jugular vein using a 20-gauge needle prior to the administration of treatments. Once each heifer was assigned to their respective treatments, blood samples were collected every Tuesday at 0800 h for the duration of the study. Samples were collected in 2 10 mL vacutainer tubes, the first containing anticoagulant EDTA and the second without anticoagulant (Monoject, Covidien Ilc., Mansfield, MA). Blood ketone concentrations were obtained using a hand-held electronic blood glucose and ketone monitoring device (Nova Max Plus, Nova Biomedical, Waltham, MA; Deelen et al., 2016). A whole blood sample, not containing EDTA, was transferred to the sensor of the test strip using a disposable pipette.

Samples with EDTA were placed on ice until they were centrifuged at 1,278 × g at 4°C for 20 min (5430R, Eppendorf, Hamburg, Germany). Plasma was stored in 2 aliquots and frozen at –20°C until further analysis of plasma urea nitrogen (**PUN**) and glucose. Urea concentrations were measured in duplicate using the diacetyl-monoxime method and measured colorimetrically using a UV-visible spectrophotometer (Beckman Coulter Inc., Brea, CA) set at a wavelength of 540 nm. Plasma glucose concentrations were measured in duplicate via Wako Autokit for Glucose (Wako Diagnostics, Mountain View, CA) and read on a UV-visible spectrophotometer at a wavelength of 505 nm.

Digestibility Measurements

Each of the 40 heifers underwent apparent total-tract nutrient digestibility phases at 21 d on study until 27 d, and again at 63 d until 69 d. Total mixed ration samples were taken Thursday through Saturday and individual ort samples were collected Friday through Sunday. Orts and TMR samples were then frozen at -20°C for future analysis. Samples were thawed and placed in

a forced hot air convection oven to dry at 55°C for 48 h to determine DMI. Both Orts and TMR samples were then composited over the sampling days.

Fecal grab samples were collected on Saturday, Sunday, and Monday every 6 h to represent a 24-h period (d 5: 0000, 0600, 1200, and 1800 h; d 6: 0200, 0800, 1400, and 2000 h; d 7: 0400, 1000, 1600, and 2200 h) by stimulating defecation or collecting feces directly from the rectum. Samples over the 3-d period were combined to obtain a single composite and frozen at −20°C. Fecal samples were thawed at room temperature and emptied into aluminum trays to be dried in a forced-air oven at 55°C for approximately 72 h until completely dried. The dried TMR, orts, and fecal samples were ground through a 1-mm screen Wiley mill (Thomas Scientific, Swedesboro, NJ). Ground samples were sent to Rock River Laboratories (Watertown, WI) for analysis. Feed, orts, and fecal samples were analyzed for acid detergent insoluble ash (ADIA; according to Van Keulen and Young (1977)), CP, NDF, ADF, starch, ash, and fat as described for feed samples.

The equation used to estimate digestibility was:

100- [100 x (% ADIA in DM consumed / % ADIA in feces) x (% nutrient in feces / nutrient consumed DM)].

Coccidia Count

Fecal samples were obtained from each heifer prior to the start of treatment, and then weekly from each heifer on Tuesday at 0800 h. Samples were analyzed for *Coccidian* oocysts following the modified Wisconsin sugar fecal worm egg flotation method (Bliss and Kvasnicka, 1997). Heifers were observed daily for indications of illness.

Statistical Analysis

Initial BW, skeletal measurements, serum glucose, PUN, ketone, and coccidia counts served as covariates for their respective variables of interest. Weekly DMI, ADG, ME intake, FE (ADG/DMI), BW, skeletal measurements, average coccidia counts, and blood metabolites (whole blood ketones, plasma glucose, and PUN) were analyzed as a randomized complete block design with repeated measures using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) according to the following model: $Y_{ijkl} = \mu + B_i + Trt_j + W_k + \beta X_{ij} + TrtW_{jk} + E_{ijkl}$, where Y_{ijkl} = the dependent variable; μ = the overall mean; B_i = the random effect of block i (i = 1,…,10); Trt_j = the fixed effect of the jth treatment (j = control, 0.75 g/kg SB, 1 mg/kg MON, combination (MSB)); W_k = the fixed effect of the kth week on study (k = 1 – 12); β = the regression (covariate coefficient); X_{ij} = the covariate measurement; $TrtW_{jk}$ = the fixed interaction between the jth treatment and the kth week; and E_{ijkl} = the residual error ~ N (0, $\sigma^2 e$). In this model, the random effect of heifer within block subclass was used as the error term for the effect of treatment. The residual errors are errors within heifer across time and represent errors for repeated measurements in the experimental units (heifers). For most variable analyzed, first-order autoregressive resulted in the smallest Bayesian information criteria of the 5 covariate structures tested: first order-autoregressive, Toeplitz, compound symmetry, variance components, and unstructured. All variables, except length gain, paunch girth, paunch girth gain, BW, and average coccidia were modeled using a first-order autoregressive covariance spatial structure. Paunch girth, paunch girth gain, and average BW were modeled using a Toeplitz covariance spatial structure as it resulted in the smallest Bayesian information criterion. Body length gain was modeled using compound symmetry covariance spatial structure as it resulted in the smallest Bayesian information criterion. Average coccidia count was modeled using an

unstructured covariance spatial structure as it resulted in the smallest Bayesian information criterion. Degrees of freedom were calculated using the Kenward-Roger approximation option of the MIXED procedure of SAS. Covariate *P*-values for heart girth gain, coccidia count, average plasma glucose concentration, and ADG were > 0.25; therefore, they were removed from the model. Single degree of freedom contrasts for CON vs. Add (control vs. additive), SB vs MON, and Add vs MSB (single additives vs. MSB) effects were determined for all variables.

Paunch girth, heart girth, and body length were analyzed as a randomized complete block design using the MIXED procedure of SAS 9.4 (SAS Institute Inc.) according to the following model: $Y_{ij} = \mu + B_i + Trt_j + \beta X_{ij} + E_{ij}$, where Y_{ij} = the dependent variable; μ = the overall mean; B_i = the random effect of block i (i = 1,…,10); Trt_j = the fixed effect of the jth treatment (j = control, 0.75 g/kg SB, 1 mg/kg MON, combination (MSB)); β = the regression (covariate coefficient); X_{ij} = the covariate measurement; and E_{ijkl} = the residual error ~ N (0, σ^2e). Degrees of freedom were calculated using the Keward-Roger approximation option of the MIXED procedure. Single degree of freedom contrasts for CON vs. Add (control vs. additive), SB vs MON, and Add vs MSB (single additives vs. MSB) effects were determined.

Apparent total-tract nutrient digestibility, initial measurements, and overall skeletal measurement gains were analyzed as a randomized complete block design using the MIXED procedure of SAS 9.4 (SAS Institute Inc.) according to the following model: $Y_{ij} = \mu + B_i + Trt_j + E_{ij}$, where Y_{ij} = the dependent variable; μ = the overall mean; B_i = the random effect of block i (i = 1,…,10); Trt_j = the fixed effect of the jth treatment (j = control, 0.75 g/kg SB, 1 mg/kg MON, combination (MSB)); and E_{ijkl} = the residual error ~ N (0, σ^2e). Degrees of freedom were calculated using the Kenward-Roger approximation option of the MIXED procedure. Single

degree of freedom contrasts for CON vs. Add (control vs. additive), SB vs MON, and Add vs. MSB (single additives vs. MSB) effects were determined.

For all variables, significant treatment and interaction effects were noted at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. Any data points with values greater or lesser than 2.5 SD away from the mean were considered outliers and removed from the dataset.

RESULTS

The nutrient analysis of the TMR is presented in Table 2. Ingredient composition varied due to changes in the feeds used over the 13 mo trial. Dry matter intake, FE, ADG, BW, and skeletal measurements are presented in Table 3.

There were 5 instances on study where heifers were treated with antibiotics to treat fevers (body temperature > 39.17°C). Out of the five: one heifer on SB was treated from d 95 to d 97 of life (d 11 to d 13 on study); two were from MON, with one treated d 92 to d 94 of life (d 10 to d 12 on study) and the other from d 90 to d 92 of life (d 6 to d 8 on study); and two were from MSB, with one treated from d 97 to d 99 of age (d 13 to d 15 on study) and the other from d 95 to d 97 of life (d 11 to d 13 on study). The heifer that was treated for fever from the SB group was later treated from d 126 to d 128 of life (d 42 to d 44 on study) for an abscess on her leg. Six heifers on study were treated with Amprolium (Corid®, Huvepharma, Sofia, Bulgaria) from 113 to 117 d of age (d 29 to d 33 on study). Out of the six, two heifers were from the control group, two were from SB, and two were from MON. All were treated for varying amounts of severity of coccidia.

Average BW tended ($P = 0.10$) to be greater for heifers fed any additive as compared to control. Average daily gain was similar for all treatments. Final BW tended ($P = 0.09$) to be

greater for heifers fed any additive as compared to control. Dry matter intake was greater (P = 0.03) in heifers fed any additive as compared to control. Feed efficiency was increased (P = 0.04) in heifers supplemented with MON when compared to heifers supplemented with SB. However, there were no differences between CON heifers and those receiving any additive.

Average heart girth tended (P = 0.10) to be greater in heifers fed any additive compared to control, and tended (P = 0.07) to be greater in heifers fed the MSB diet compared to either SB or MON. There were no differences among all treatments in heart girth gain, final heart girth, average paunch girth, paunch girth gain, final paunch girth, average body length, body length gain, and final body length. Overall gains are presented in Table 4, and all overall measurements (BW, Heart girth gain, paunch girth gain, and body length gain) showed no differences among treatments.

Fecal coccidia oocyst counts and blood parameters are presented in Table 5. The number of coccidian oocysts present in fecal samples was reduced (P = 0.03) in heifers provided any additive as compared to control. Plasma concentrations of glucose tended (P = 0.09) to increase with either SB and MON compared to MSB. There were no differences in concentrations of final plasma glucose among all treatments. Average ketone concentrations, with any additive, resulted in greater (P = 0.002) concentrations of ketone when compared to control. There was also an increase (P = 0.0001) in average ketone concentrations in heifers supplemented SB as compared to MON. Finally, there was an increase (P = 0.03) in average ketone concentrations in MSB heifers when compared to SB and MON. Final ketones tended (P = 0.09) to be greater in heifers fed SB when compared heifers fed MON. Final ketones also were increased (P = 0.04) in MSB heifers when compared to SB or MON.

Data collected during the first digestibility measurement period (week 3) are shown in Table 6. Dry matter intake during the digestibility period tended ($P = 0.10$) to be greater in heifers fed SB when compared to MON, as well as heifers fed MSB when compared to SB and MON. Apparent total tract digestibility of DM, NDF, ADF, Hemicellulose, OM, and fat digestibility showed no differences among treatments. Starch digestibility was increased ($P = 0.03$) in heifers fed the combination diet when compared to SB and MON.

Data collected during the second digestibility measurement period (week 9) are shown in Table 7. Dry matter intake during the digestibility period, along apparent total tract digestibility of DM, CP, ADF, Hemicellulose, Starch, OM, and fat digestibility were not different among treatments. Neutral detergent fiber digestibility tended ($P = 0.08$) to be greater in control diets when compared to any additive.

Discussion

Our results are consistent with findings from previous studies on the impact SB and MON supplementation have on BW. We observed, as compared to CON, that the addition of any feed additive tended to improve BW. For MON, these results are supported by Goodrich et al. (1984), who found that, when compared to control diets, feedlot cattle fed diets supplemented with MON gained weight 1.6% faster, ingested 6.4% less feed, and required 7.5% less feed per 100 kg of gain. In heifers, typically research indicates for improved ADG with MON supplementation (Males et al., 1979; Roquette et al., 1980; Baile et al., 1982). For SB, these results are supported by Rice et al. (2019) who observed that as SB increased, average BW increased, and final BW tended to increase.

An increase was seen in DMI in calves fed any additive when compared to CON. With both SB and MON supplementation, research does not indicate an increased response in DMI. Typically, research has shown that DMI in MON supplemented heifers typically would be decreased (Dyer et al., 1980; Baile et al., 1982; Wood et al., 2016) and Goodrich et al. (1984) demonstrates the same trend in feedlot cattle. Though, there are some studies that indicate that DMI would not be affected by MON supplementation (Roquette et al., 1980; Wood et al., 2016; Chapman et al., 2017). Research has shown that DMI in SB supplemented animals was not affected (Guilloteau et al., 2009; Górka et al., 2014; Kowalski et al., 2015; Rice et al., 2019). Though an increase in DMI was not generally seen in both SB and MON supplementation, we may be able to attribute the increase in DMI to the increase Na^+ provided in the diet.

Mineral ion content in feed has been shown to influence water intake in cattle (Murphy 1992), specifically increasing 50 ± 23 ml in cows and 54 ± 4 mL in calves for each additional gram of sodium provided (Murphy et al., 1983). The additional Na^+ provided leads to an increase in water consumption, which ultimately leads to an increased rate of passage and increased DMI. In calves, it is believed that DMI is related to water intake because calves require 4 times more water than feed (DM) (Quigley et al., 2006; Kertz, 2014; Kononoff et al., 2017). Leibholz et al. (1980) provided 60 male Friesian calves from 3 to 11 wk of age with diets supplemented with NaCl at 0.3, 1.1, 1.9, or 2.8% of the diet content or $NaHCO_3$ at 1.1 or 1.9% of the diet content. Feed intake in calves fed 1.1 and 1.9% Na from $NaHCO_3$ was 8 and 15% greater than the feed intake of calves fed 0.3% Na. Since the calves used by Leibholz et al. (1980) were close in age to calves used in the current study, we can assume that 54 ± 4 mL in calves for each additional gram of sodium provided (Murphy et al., 1983) is an appropriate estimate of the resulting water intake and subsequent increase in DMI.

Sodium butyrate was driving the increase in DMI. For example, initial BW for SB calves averaged to 94.97 kg and final BW averaged to 192.82 kg. So, average initial SB (0.75 g/ kg of BW) provided to heifers would have been around 71.25 g and average final SB (0.75 g/ kg of BW) provided to heifers would have been around 144.75 g. Sodium butyrate used in this study was 21% sodium, so SB provided an additional 15 to 30.4 g additional Na^+ over the duration of the study. Using the amount of additional water (mL) calves would need to consume per g of Na^+ (Murphy et al., 1983), heifers on this study would be consuming an additional 808 to 1,641.5 mL of water. Finally, putting that into perspective with DMI, with the 4:1 water: feed ratio provided by Kononoff et al. (2017), SB heifers would have consumed 202 to 410.4 additional g of DM.

No difference among treatments for paunch girth indicated that heifers did not get fat with the increase in DMI. This post-weaned age is a time when growth is mainly bone and muscle development, so it can be assumed that this is the type of growth being supported by the increase in DMI. An increase in DMI can also be supported by the FE response. We saw an increase in FE for heifers fed MON as compared to SB. In this study, MON supplemented heifers had 12% greater FE as compared to SB heifers.

In addition to growth benefits, SB and MON have also been shown to affect the overall health of the animal through the prevention of coccidiosis. We saw that, as compared to CON, any additive resulted in the reduction of coccidian oocysts present in the feces.

Monensin is a recognized anticoccidial, and the responses seen in this study are supported by the modes of action of MON to specifically target the *Eimeria* parasite (Chapman et al., 2010). Monensin affects the sporozoite step of the coccidian lifecycle, causing an increase in available Na^+ ions to stimulate the Na^+-K^+-ATPase to pump excess Na^+ ions out of the sporozoite (Smith and Galloway, 1983). The excess Na^+ ions are suggested to cause water to

enter the sporozoite via osmosis, the parasite will swell, and the cell eventually bursts (Smith and Strout, 1979). Monensin can also affect the merozoite step of the coccidian lifecycle. After the first-generation merozoites rupture their host sporozoite, they then will encounter the drug before they are able to sexually reproduce (Melhorn et al., 1983). Without sexual reproduction of merozoites, they cannot create the oocysts that would be shed into the feces to potentially affect the next animal. With the mode of action in mind, daily feeding of MON is necessary for continued health response.

Regarding SB, much is known about how butyrate will work in the epithelial layer of the rumen (Górka et al., 2011a,b) and lower gastrointestinal tract (Guilloteau et al., 2009; Guilloteau et al., 2010b; Górka et al., 2014). However, data are lacking regarding how much available SB is not absorbed by the rumen and available in the SI, and how SB is able to decrease the prevalence of coccidian oocysts in the feces. How much available SB will be available for the small intestine could be answered by Rice (unpublished). The researcher conducted an in situ degradability study, determining SB contains 90 ± 5% butyrate and was 99% degradable in the rumen. After 4 h of incubation, SB had a 98% disappearance rate. However, this study does not account for SB absorption.

The responses with SB and reduction of coccidian oocysts is supported by Rice et al. (2019). The researchers fed 0, 0.25, 0.5, and 0.75 g/kg BW of SB in the diets of post-weaned heifers and found a positive quadratic effect of SB on reducing the prevalence of coccidian oocysts in the feces. There are, however, a few inferences that can be hypothesized towards how this response was seen. Since SB contains approximately 21% Na^+, it is possible that Na^+ dissociates from the butyrate and that is what moves on to the lower gastrointestinal tract. In doing this, at the lower gastrointestinal tract, the response observed could possibly be due to a

disruption of the Na^+-K^+-ATPase to pump excess Na^+ ions out of the sporozoite (Smith and Galloway, 1983). Alternatively, it is known that SB is very soluble, it is possible that SB will be available to flow with the fluid phase-out of the rumen. What can be hypothesized is that some of this would bypass the rumen and be used by the small intestine and large intestine.

In the lower gastrointestinal tract, butyrate supplementation has been shown to improve epithelial cell proliferation, epithelial tissue repair, pathogen control, and defense system mechanisms such as barrier function, antimicrobial, and anti-inflammatory responses (Guilloteau et al., 2010b). Górka et al., (2011a) observed SB supplemented calves experienced fewer scour days and tended to be treated with electrolytes less often. Górka et al., (2011b) found that calves supplemented SB in MR had increased mitotic indices and decreased apoptotic indices, which supports the findings of SB supplemented calves suffering from a lesser incidence of scours. Elevated mitotic indices of intestinal epithelial cells are indicative of an increase in cell proliferation, which provides the intestinal mucosa the ability to rapidly mature and heal after injury related to scours (Guilloteau et al., 2010b). Coccidiosis is known to cause inflammation in the large intestine along with damage to the mucosa layer of the lower small intestine, cecum, and colon (Guilloteau et al., 2010b; Constable, 2019). It can be inferred that SB supplementation would reduce inflammation in the large intestine (Guilloteau et al., 2010b). Sodium butyrate could heal the intestinal mucosa, and in repairing tissue due to scours, remove the second-generation merozoites that settle into the large intestine for sexual reproduction. If the second-generation merozoites are removed, they are not able to produce oocysts that would be shed into the feces, thus reducing the prevalence of coccidian protozoa and reducing the effects of coccidiosis.

A tendency to decrease plasma glucose was seen in MSB as compared to SB and MON. Monensin supplemented heifers expressed the greatest (87.7 mg/dL) average plasma glucose concentration. Monensin supplementation results in a decrease in Gram-positive bacteria in the rumen, which will lower the concentration of acetate and butyrate, the two non-glucogenic VFA. Since Gram-positive bacteria are decreased, this then results in an increase in Gram-negative bacteria. When Gram-negative bacteria thrive, glucogenic propionate will increase (Ellis et al., 2015). Ruminal propionate uptake is converted into glucose in the liver. In lactating dairy cows, hepatic propionate uptake will make up over 55% of total hepatic glucose output (Reynolds et. al, 1988). Thus, supplementing MON increases ruminal propionate, which will increase available propionate for hepatic conversion to increase circulating glucose concentrations. Sodium butyrate supplemented heifers expressed lower average glucose concentrations as compared to MON. Aiello et al. (1989) incorporated 2.5 mM of propionate into glucose in the presence of either 0, 1.25, and 2.5 mM of butyrate. They found that butyrate inhibited propionate metabolism. The inhibition of propionate metabolism would mean less is available for conversion to glucose in the liver, thus resulting in the slightly decreased average plasma glucose concentration in SB heifers and the trend in MSB having the lowest reported average plasma glucose concentration. Additionally, between pre-ruminant to ruminant digestion, there is a shift of absorption from glucose in the intestine to gluconeogenesis in the liver (Baldwin et al., 2004). Due to this increase in hepatic enzyme activity, as fermentation becomes more important for the heifer, less carbohydrate is available for post-ruminal digestion and results in decreased absorption of glucose (Rice et al., 2019).

Average ketone concentrations increased in any additive vs. control, SB when compared to MON, and in MSB vs. the average of SB and MON. The values for average ketone

concentrations were as follows: CON = 0.44, SB = 0.50, MON = 0.44, and MSB = 0.50 mmol/L. Final ketone concentrations tended to increase in SB (SB = 0.50 mmol/L) when compared to MON (M = 0.44 mmol/L), and increased in MSB (MSB = 0.54 mmol/L) vs. the average of SB and MON (average of SB and M = 0.47 mmol/L). These results are supported by data indicating that rumen epithelium rapidly convert butyrate to ketone bodies through alimentary ketogenesis (Holtenius and Holtenius, 1996; Müller et al., 2002; Herrick et al., 2017; Rice et al., 2019).

In the week 3 digestibility period, DMI tended to be higher in heifers fed SB when compared to MON, as well as heifers fed MSB when compared to SB and MON. This tendency to increase DMI can be supported by Guilloteau et al. (2010a) for SB. They observed an increase in pancreatic juice secretion ($P < 0.10$) and a 40% increase in lipase production and 52% increase in chymotrypsin production. Pancreatic juices are vital for digestion in the SI, using enzymes and bicarbonate to proceed with the breakdown and absorption of feed. We may also be able to attribute the increase in DMI to the increase Na^+ provided in the diet (Murphy et al., 1983). The additional Na^+ provided leads to an increase in water consumption, along with the increased SI digestibility, which ultimately leads to an increased rate of passage and increased DMI. In the week 9 digestibility period, neutral detergent fiber tended to be increased in CON diets when compared to any additive. This tendency can be supported by the non-significant DMI response, possibly due to the slight increase in intake in additive diets when compared to control. An increase in DMI results in a higher rate of passage, and thus low NDF digestibility.

Butyrate is the primary VFA utilized by the rumen epithelial tissue, it will be absorbed here and used to improve the structure and volume of papillae. Improvements in papillae result in an increase in surface area for absorption of feed. Some butyrate is assumed to be able to pass into the lower gastrointestinal tract, improving the structure and volume of intestinal villi along

with improving and repairing the mucosal layer. Overall gastrointestinal tract nutrient absorption increase will allow the heifer to more effectively absorb and utilize nutrients obtained from fermentation. Based on the results of this study, it can be inferred that the addition of any additive improved the absorptive capabilities of the gastrointestinal tract through BW. Specifically pertaining to the lower gastrointestinal tract, the effect of additives here has been shown to increase the health of the animal, either by possibly repairing epithelial tissue or directly affecting the coccidia that reside. This study, and the work that preceded it (Rice et al., 2019), were the first instances to see the prevention of coccidiosis with SB supplementation. Some of the effects of SB have been inferred through research pertaining to butyrate. Moving forward, there should be more investigation into SB rate of passage in the rumen to determine where specifically SB is used. Understanding if SB is utilized mostly by the rumen, moving through the fluid phase into the small intestine, or some percentage of both is important. Further understanding of SB function would help determine the inferred effects that SB potentially has on the lower gastrointestinal tract development, as well as its potential anticoccidial benefits, in older ruminant animals.

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Table 1. Ingredient composition (% of DM ± SD) of experimental diet

Item	DM (%)
Hay Crop Silage	37.46 ± 1.83
Corn Silage	33.87 ± 3.88
Energy Mix[1]	12.53 ± 4.80
Soy/Urea Mix[2]	11.89 ± 2.54
Provail[3]	2.26 ± 0.009
Mineral/Vitamin Mix[4]	1.99 ± 0.005

[1] Energy Mix contains 5% molasses, 45.80% corn meal, 15.20% steam flaked corn, and 34% whole beet pulp

[2] Soy/Urea Mix contains 7.28% distillers grain, 69.14% soy bean meal, 21.83% canola meal, and 1.75% urea

[3]Provail is a rumen undegradeable protein (**RUP**) mix that contains blood meal and methionine at 3.9% CP

[4]Mineral/Vitamin Mix contains 19.05% Ca; 6.01% P; 3.51% Mg; 20.00% Salt; 7.80% Na; 0.29% Fe; 0.26% Zn; 0.26% Mn; 12.3% Cl; 602.00 mg/kg Cu; 15.00 mg/kg Co; 25.09 mg/kg Se; 15.00 mg/kg I; 267,800 IU/kg Vitamin A; 111,071 IU/kg Vitamin D; and 2,207 IU/kg Vitamin E.

Table 2. Nutrient analysis (% of DM ± SD) of experimental diet

Item	DM (%)
CP	15.73 ± 1.43
ADF	27.83 ± 3.04
NDF	42.92 ± 3.25
Starch	14.39 ± 2.06
NFC[1]	32.48 ± 3.33
Fat	2.71 ± 0.46
Ash	7.88 ± 0.40
ME[2], Mcal	2.51 ± 0.02

[1]NFC = 100 – [CP% + (NDF% – NDICP%) + fat% + ash%].

[2]Estimated from NRC (2001).

Table 3. Intake and performance of heifers fed 0 mg/kg additive, 0.75 mg/kg sodium butyrate, 1 mg/kg monensin, and the combination of sodium butyrate and monensin from 12 to 24 wk of age

	Treatment[1]					*P*-value[2]			
Item	CON	SB	MON	MSB	SEM[3]	TRT ×WK[4]	CON vs. Add[5]	SB vs MON[6]	Add vs MSB[7]
Initial BW, kg	105.51	94.97	99.18	99.48	3.28	-	0.05	0.37	0.55
Average BW, kg	144.88	146.08	149.23	149.73	1.77	0.77	0.10	0.20	0.33
ADG, kg/d	1.11	1.12	1.13	1.14	0.03	0.60	0.42	0.87	0.55
Final BW, kg	189.55	192.82	194.19	197.08	2.49	-	0.09	0.69	0.23
DMI, kg/d	4.00	4.47	4.16	4.46	0.14	0.72	0.03	0.11	0.35
Feed efficiency, ADG/DMI	0.27	0.25	0.28	0.27	0.01	0.66	0.70	0.04	0.85
Heart girth initial, cm	107.50	104.31	105.25	105.70	1.08	-	0.06	0.54	0.49
Heart girth, cm	117.10	117.42	117.97	118.76	0.48	0.84	0.10	0.40	0.07
Heart girth gain, cm/d	0.27	0.28	0.27	0.29	0.01	0.33	0.29	0.75	0.27
Heart girth final, cm	128.09	128.73	128.73	129.49	0.71	-	0.29	1.00	0.37
Paunch girth initial, cm	131.10	125.20	126.50	127.70	1.97	-	0.05	0.65	0.45
Paunch girth, cm	145.70	146.07	146.03	146.83	1.19	0.86	0.67	0.98	0.58
Paunch girth gain, cm/d	0.36	0.37	0.37	0.39	0.02	0.93	0.40	0.93	0.24
Paunch girth final, cm	158.96	159.50	158.69	160.70	1.67	-	0.74	0.72	0.42
Body length initial, cm	87.30	84.18	87.20	87.80	1.09	-	0.48	0.06	0.13
Body length, cm	96.83	96.74	96.93	96.48	0.57	0.47	0.86	0.81	0.60
Body length gain, cm/d	0.21	0.23	0.22	0.21	0.01	0.74	0.42	0.61	0.30
Body length final, cm	105.39	106.12	106.25	105.78	0.72	-	0.40	0.90	0.63

[1]Treatment CON = 0g/d additive, SB = 0.75 g Na-butyrate/kg BW, MON = monensin sodium 1 mg/kg BW, and MSB = Na-butyrate and monensin sodium

[2]*P*-value significant if < 0.05; trend if < 0.10

[3]Standard error of the mean

[4] Treatment by week interaction

[5]Single df contrast- control vs. additive

[6]Single df contrast- Na butyrate vs. monensin sodium.

[7]Single df contrast- Additives vs. combination.

Table 4. Overall body weight and skeletal measurement gains of heifers fed 0 mg/kg additive, 0.75 mg/kg sodium butyrate, 1 mg/kg monensin, and the combination of sodium butyrate and monensin from 12 to 24 wk of age.

	Treatment[1]					*P*-value[2]		
Item	CON	SB	MON	MSB	SEM[3]	CON vs. add[4]	SB vs MON[5]	Add vs MSB[6]
BW, kg	90.54	92.38	94.33	97.26	2.36	0.14	0.56	0.19
Heart girth, cm	22.30	23.12	23.06	23.80	0.67	0.20	0.95	0.39
Paunch girth, cm	31.34	31.87	31.06	33.08	1.67	0.74	0.72	0.42
Body length, cm	18.77	19.51	19.63	19.16	0.72	0.39	0.91	0.63

[1]Treatment CON = 0g/d additive, SB = 0.75 g Na-butyrate/kg BW, MON = monensin sodium 1 mg/kg BW, and MSB = Na-butyrate and monensin sodium

[2]*P*-value significant if < 0.05; trend if < 0.10

[3]Standard error of the mean

[4]Single df contrast- control vs. additive

[5]Single df contrast- Na butyrate vs. monensin sodium.

[6]Single df contrast- Additives vs. combination.

Table 5. Coccidia count, plasma glucose, and whole-blood ketones of heifers fed 0 mg/kg additive, 0.75 mg/kg sodium butyrate, 1 mg/kg monensin, and the combination of sodium butyrate and monensin from 12 to 24 wk of age.

	Treatment[1]					*P*-value[2]			
Item	CON	SB	M	MSB	SEM[3]	TRT ×WK[4]	CON vs. Add[5]	SB vs MON[6]	Add vs MSB[7]
Initial coccidia/ kg of feces	567.0	4567.0	333.0	2930.0	302.5	-	< 0.0001	< 0.0001	0.20
Coccidia/ kg of feces	1248.9	697.9	762.5	781.8	201.0	0.98	0.03	0.81	0.83
Initial glucose, mg/dL	81.3	81.4	86.7	76.3	2.56	-	0.98	0.16	0.02
Glucose, mg/dL	84.5	85.0	87.7	83.3	1.43	0.94	0.64	0.18	0.09
Final glucose, mg/dL	89.8	89.1	88.7	85.6	1.95	-	0.36	0.89	0.17
Initial ketones, mmol/L	0.46	0.45	0.46	0.39	0.05	-	0.72	0.97	0.29
Ketones, mmol/L	0.44	0.50	0.44	0.50	0.01	0.77	0.002	0.0001	0.03
Final ketones, mmol/L	0.46	0.50	0.44	0.54	0.02	-	0.26	0.09	0.04

[1]Treatment CON = 0g/d additive, SB = 0.75 g Na-butyrate/kg BW, MON = monensin sodium 1 mg/kg BW, and MSB = Na-butyrate and monensin sodium

[2]*P*-value significant if < 0.05; trend if < 0.10

[3]Standard error of the mean

[4] Treatment by week interaction

[5]Single df contrast- control vs. additive

[6]Single df contrast- Na butyrate vs. monensin sodium.

[7]Single df contrast- Additives vs. combination.

Table 6. Apparent total-tract nutrient digestibility (%), week 3

	Treatment[1]					*P*-value[2]		
Item	CON	SB	MON	MSB	SEM[3]	CON vs. Add[4]	SB vs MON[5]	Add vs MSB[6]
DMI, kg/d	3.31	3.70	3.26	3.84	0.19	0.19	0.10	0.10
Digestibility %								
DM	58.6	62.6	63.5	65.6	3.19	0.16	0.85	0.51
CP	51.2	54.0	58.0	58.4	3.82	0.21	0.44	0.58
ADF	44.7	51.2	48.6	50.8	4.72	0.29	0.69	0.88
NDF	50.2	56.1	52.7	56.3	4.05	0.28	0.54	0.70
Hemicellulose	58.9	65.6	62.8	66.4	3.78	0.16	0.61	0.63
Starch	99.2	99.0	99.1	99.4	0.12	0.83	0.65	0.03
Organic Matter	61.0	65.0	65.7	67.5	3.06	0.17	0.87	0.57
Fat	56.5	62.9	60.1	62.2	5.23	0.38	0.69	0.92

[1]Treatment CON = 0g/d additive, SB = 0.75 g Na-butyrate/kg BW, MON = monensin sodium 1 mg/kg BW, and MSB = Na-butyrate and monensin sodium

[2]*P*-value significant if < 0.05; trend if < 0.10

[3]Standard error of the mean

[4]Single df contrast- control vs. additive

[5]Single df contrast- Na butyrate vs. monensin sodium.

[6]Single df contrast- Additives vs. combination.

Table 7. Apparent total-tract nutrient digestibility (%), week 9

	Treatment[1]					*P*-value[2]		
Item	CON	SB	MON	MSB	SEM[3]	CON vs. Add[4]	SB vs MON[5]	Add vs MSB[6]
DMI, kg/d	4.89	5.36	5.15	4.92	0.19	0.25	0.42	0.12
Digestibility %								
DM	65.3	61.1	62.3	59.4	2.58	0.13	0.73	0.46
CP	56.9	51.6	54.2	51.8	3.66	0.31	0.60	0.80
ADF	51.5	43.5	46.9	42.1	4.48	0.12	0.55	0.55
NDF	55.9	50.2	50.2	49.8	3.07	0.08	1.00	0.91
Hemicellulose	63.0	60.7	64.3	62.9	3.42	0.94	0.44	0.92
Starch	99.1	98.8	98.9	98.6	0.20	0.13	0.71	0.29
Organic Matter	67.2	62.7	64.1	61.5	2.49	0.12	0.67	0.53
Fat	64.0	60.7	66.2	60.9	3.35	0.73	0.24	0.53

[1]Treatment CON = 0g/d additive, SB = 0.75 g Na-butyrate/kg BW, MON = monensin sodium 1 mg/kg BW, and MSB = Na-butyrate and monensin sodium

[2]*P*-value significant if < 0.05; trend if < 0.10

[3]Standard error of the mean

[4]Single df contrast- control vs. additive

[5]Single df contrast- Na butyrate vs. monensin sodium.

[6]Single df contrast- Additives vs. combination.

PS 11/17/17

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16-Nov-2017

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IACUC #: 170903
Project: Sodium Butyrate and Monensin Supplementation to Postweaned Dairy Calves
Approval Date: 19-Oct-2017

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under pain or distress category C - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.*

1

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File