

Winter 2017

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

Emily Margaret Rice

*University of New Hampshire, Durham*

Follow this and additional works at: <https://scholars.unh.edu/thesis>

---

## Recommended Citation

Rice, Emily Margaret, "SUPPLEMENTATION OF SODIUM BUTYRATE TO POST-WEANED HEIFER DIETS: EFFECTS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND HEALTH" (2017). *Master's Theses and Capstones*. 1162.  
<https://scholars.unh.edu/thesis/1162>

This Thesis is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Master's Theses and Capstones by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact [nicole.hentz@unh.edu](mailto:nicole.hentz@unh.edu).

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

BY

EMILY MARGARET RICE

B.S. Biomedical Sciences: Medical&Veterinary Sciences, University of New Hampshire, 2015

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Biological Science: Agricultural Science

December, 2017

This thesis has been examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences: Agricultural Science by:

Thesis Director, Dr. Peter S. Erickson  
Professor of Dairy Management and Extension Dairy Specialist  
University of New Hampshire, Durham, NH

Dr. Andre F. Brito  
Associate Professor of Biological Sciences  
University of New Hampshire, Durham, NH

Dr. William E. Berndtson  
Professor of Biological Sciences  
University of New Hampshire, Durham, NH

On August 24<sup>th</sup>, 2017

Original approval signatures are on file with the University of New Hampshire Graduate School.

## ACKNOWLEDGEMENTS

I would first like to thank the Department of Biological Sciences at the University of New Hampshire for allowing me the opportunity to continue my education and discover my love for research. All of the faculty members that have guided me along my journey at UNH prepared me for success and I thank you.

To my best friend and life-coach, Mom, I am forever grateful for everything you have done and continue to do for me as I follow my passion. Through all of the curveballs that life has thrown at me, you have never doubted my abilities and have supported me in every way imaginable. The love and admiration I have for you continues to grow as I become older and (questionably) wiser. I hope to someday be as great of a person as you have been to me and everyone who has the pleasure of meeting you.

To Jimmy, Izzy, Aunt Lizzy, Aunt Judy, PopPop, and the rest of my family, I am thankful for all you have done for me. Your overwhelming love and support have no-doubt helped me succeed in all my endeavors in life. As I continue to follow my dreams, I hope to make you all proud of my accomplishments.

To Pete, I am so thankful to have been blessed with you as my advisor. Not only am I grateful for you giving me this amazing opportunity but also for believing in me when I sometimes didn't believe in myself. Your whole-hearted advice and guidance have helped shape me into the person I have become today, both in research and in life. Thank you for guiding me through my master's research and supporting me every step of the way, I will forever be grateful.

To all of my friends, near and far, I have so much gratitude for all of your support. Kayla Aragona, it's a known fact that without you I would not have survived grad school! I have so

much gratitude for everything you have done for me. From teaching me how to run lab analyses to being a shoulder to cry on, you have been one of my closest friends the past few years. Thank you for everything and I hope to be as great a friend to you as you have been to me. To all of my friends I have made in grad school, Kelsey Juntwait, Andre Pereira, Colleen Chapman, and Shona Ort thank you for being my friends even if I can be a little weird sometimes. I cherish our friendships and cannot wait to see what you all accomplish in life!

To Dr. Berndtson and Dr. Brito, I so deeply appreciate all of the advice you have given me throughout my journey at UNH. Whether it be talking about how teaching lab has been or sharing a laugh over your experiences in graduate school, I have cherished all the time you have given me. I am so thankful to have had such wonderful committee members.

I would like to thank all of the undergraduate students for helping me throughout my study. I will never forget how dependable and eager to learn all of you have been, even if it meant wrangling heifers each and every week. I could not have finished my research without all of the hours of sampling and analysis you helped me with.

Finally, I want to thank the Fairchild Dairy and all of the farm staff. Even if I ask too many questions sometimes I am so thankful to have been lucky enough to know all of you. Jon Whitehouse, I know that I can annoy you to no end at times, I am so happy that you put up with me (most of the time)! And to everyone else at the barn, I will always miss our chats about the cows and how much you helped me with any issues I had during my study.

## TABLE OF CONTENTS

THESIS TITLE PAGE .....	i
ACKNOWLEDGEMENTS .....	iii
TABLE OF CONTENTS.....	v
LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
ABSTRACT.....	viii
CHAPTER ONE: REVIEW OF LITERATURE.....	1
INTRODUCTION.....	1
PREWEANING RUMINANT .....	3
WEANING.....	10
IONOPHORES.....	13
INTRODUCTION TO BUTYRATE .....	17
Production via Microbial Action .....	18
Effects on Rumen Epithelium and Absorption via Tissues and Organs.....	21
Effects of Sodium Butyrate on Growth, Digestive Ability, and Health .....	25
CONCLUSION .....	33
CHAPTER 2: SUPPLEMENTATION OF SODIUM BUTYRATE TO POSTWEANED HEIFER DIETS: EFFECTS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND HEALTH.....	36
INTRODUCTION.....	36
MATERIALS AND METHODS .....	38
Experimental Design and Treatments.....	38
Management and Feeding.....	39
Feed Analysis .....	39
Measurements and Blood Sampling and Analysis .....	40
Digestibility Measurements.....	41
Coccidia Count .....	42
STATISTICAL ANALYSIS.....	42
RESULTS.....	44
DISCUSSION .....	46

## LIST OF TABLES

Table 1. Ingredient composition of experimental diet (range of use Feb-Aug).....	57
Table 2. Ingredient composition of experimental diet (range of use Aug-May) .....	58
Table 3. Nutrient analysis of experimental diet .....	59
Table 4. Nutrient analysis of refusals by treatment .....	60
Table 5. Nutrient analysis of diet fed during digestibility period .....	61
Table 6. Intake and performance of heifers during the 14 wk trial.....	62
Table 7. Overall body weight and skeletal measurement gains.....	63
Table 8. Coccidia count and blood parameters .....	64
Table 9. Apparent total-tract nutrient digestibility .....	65

## LIST OF FIGURES

Figure 1. Comparison of calf rumen fed milk and hay vs. milk and grain .....	8
--	---



## ABSTRACT

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

by

Emily Margaret Rice

University of New Hampshire, December, 2017

The objective of this study was to determine the effect of varying levels of sodium butyrate (**SB**) on the growth, digestibility, and health of post-weaned heifers. Forty Holstein dairy heifers with a mean age of 84 d and average body weight (**BW**) of 100.88 kg 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) 0.25 g SB/kg of body weight + carrier (**0.25SB**); (3) 0.50 g SB/kg of BW + carrier (**0.50SB**); (4) 0.75 g SB/kg of BW + carrier (**0.75SB**). Treatments were top-dressed and hand-mixed into a total mixed ration (**TMR**) once daily. Heifers had free access to water. Initial BW, hip and withers heights, heart girth and body length were measured before the start of the study and every week thereafter until the 15 wk trial was over. Blood samples were obtained and plasma urea nitrogen, glucose, and  $\beta$ -hydroxybutyrate (**BHB**) concentrations were determined prior to the start of treatment and weekly after until the conclusion of the study. Fecal samples were taken prior to treatment and every other week from each heifer for coccidia counts. Apparent total-tract nutrient digestibility was determined utilizing a chromium oxide marker. Each heifer underwent this phase at 47 d on study until 54 d on study. Sodium butyrate appeared

to increase the digestibility of acid detergent fiber (**ADF**) and organic matter (**OM**) ( $P = 0.08$ ;  $P = 0.07$ , respectively). Sodium butyrate had a positive effect on average BW and overall BW gain. Sodium butyrate had no performance effects on skeletal growth or blood metabolites. Average daily gains were 1.16, 1.15, 1.17, and 1.24 kg/d ( $P = 0.12$ ) and daily dry matter intakes (**DMI**) were 5.09, 4.84, 5.19, and 4.91 kg/d ( $P = 0.85$ ) for CON, 0.25SB, 0.50SB, and 0.75SB, respectively. There was a trend towards improved feed efficiency (**FE**). Fecal samples from each heifer indicated the presence of coccidian, but the counts were variable within blocks and not consistent across heifers within each pen. There was a trend towards positive feed efficiency. Sodium butyrate supplementation offers positive results in the growth performance and feed efficiency of post-weaned heifers.

## CHAPTER ONE

### Review of Literature

#### INTRODUCTION

With a population of 325 million, the United States makes up approximately 4.4% of the world's population of 7.4 billion (US Census Bureau, 2017). Experts are projecting an increase of nearly 1 billion people over the next 13 years to reach a total world population of 8.6 billion by the year 2030. Because of this expected growth, there will be an increased worldwide demand for resources. The agricultural industry will be faced with the challenge of supplying the ever-growing populations with resources produced in an efficient manner. The United States economy depends on the agricultural industry for 5.5% of its total gross domestic product, with the dairy cattle and milk production sector contributing nearly 9% of the total agricultural sales (AG Census; USDA, 2007). Along with domestic sales, the international export of dairy products also support a large sector of the United States economy. With the projected increase in world population the USDA predicts that commercial exports of dairy products will increase from \$4.6 billion in 2016 to \$9.5 billion in 2026. This will require milk production to increase from 212.5 billion pounds in 2016 to 259.7 billion pounds by 2026 (USDA, 2017). Due to these projected challenges and the possible economic profits that coincide with them, dairy producers should begin investigating ways in which they can prepare their herds for the future.

A healthy, successful milking herd is representative of efficient management practices on the farm and especially that of the younger stock. Proper management and nutrition of replacement heifers have proven to be important factors in the profitability of the future herd. The preweaning phase of a dairy calf's life is a time to focus on transitioning the animal from

depending on abomasal digestion to optimizing ruminal fermentation for breakdown of feeds. Calves fed only milk were shown to have minor development of the reticulo-rumen while calves fed a diet of milk and hay were observed to have significant development of the reticulo-rumen and increased papillary growth (Tamate et al., 1962). Solid feed intake during the preweaning period establishes a calf's rumen for fermentation which leads to a reduction in the prevalence of scours and other harmful diseases in calves (Otterby and Rust, 1965).

The cost of heifer raising is one of the highest expenses on the farm, ranging from 15 to 20% of the total milk production costs on the farm (Heinrichs, 1993). Managing heifers during their pre-productive period can significantly affect rearing expenses and future performance in the milking herd. Optimizing health and growth during the postweaning period can help heifers reach maturity and breeding age sooner. Heifers bred at a younger age can enter the lactating herd sooner, decreasing the costs of feeding the replacement heifers on the farm (Heinrichs et al., 2013). Identifying ways to improve the average daily gains (ADG), skeletal growth, and feed efficiency of heifers has become an important topic amongst dairy researchers.

The fermentation of solid feeds and roughages is important in developing the musculature and absorptive properties of the rumen. The breakdown of fiber and carbohydrates releases important volatile fatty acids (**VFA**) into the rumen. The feeding of sodium acetate, sodium propionate, and sodium butyrate (**SB**) to 2 wk old calves caused significant development of the rumen mucosa. Of the VFA salts, sodium butyrate showed the greatest effect on rumen papillary growth (Sander et al., 1959). Sodium acetate is metabolized in the rumen of calves at significantly lower rates than that of butyrate and propionate (Sutton et al., 1963). By causing extensive growth of rumen papillae, the absorptive capacity of the rumen is increased. This allows heifers to breakdown feed and utilize the nutrients in a more efficient manner. By

increasing feed efficiency, a farm will save on feed cost and also improve the growth and health of the animals. Antibiotic ionophores, such as monensin and lasalocid, have also been shown to increase the feed efficiency and health of cattle (Heinrichs et al., 1993). Calves fed lasalocid in milk replacer (MR) had higher body weight gains and shed fewer *Eimeria zuernii* oocysts than those not fed lasalocid (Quigley et al., 1997).

Since the European Union banned the use of antibiotics as growth promoters in animal feeds in 2006, there has been more pressure on researchers to find alternative additives that can produce results equal or greater than that of ionophores. An area that has shown a great deal of promise in improving the growth of dairy heifers is the supplementation of sodium butyrate. Studies have tested the effects of its addition into the diets of preweaned calves and lactating cows. Sodium butyrate not only stimulated expansive growth and concentration of rumen papillae in cattle but also has been shown to increase intestinal epithelial growth in broiler chickens (Abdelqader and Al-Fataftah, 2015). Heat-stressed broiler chickens that were fed butyric acid were found to have improved intestinal health and accelerated epithelial cell recovery. Improving the recovery of damaged epithelia can help to preserve the absorption and nutrient digestion that takes place in the small intestine. However, there is only limited research investigating the effects of its incorporation into postweaned heifer diets. With the future in mind, this study focusses on investigating the effects of sodium butyrate used as a growth enhancer and possible coccidiostat.

## PREWEANING RUMINANT

One of the most critical periods in a calf's life is the transition from abomasal digestion to ruminal fermentation of feeds. This transition is stimulated by proper nutrition and management

of the calves during the first few weeks of life. Understanding the nutritive requirements and digestive abilities of calves during this time is essential in facilitating the transition to becoming a true ruminant. The composition of the diet fed has significant impacts on not only the growth of the animal but also on the maturation of the rumen itself. Because a calf is born with a small, nonfunctional rumen it depends on the abomasum as the main site of digestion. The abomasum efficiently breaks down liquid feed sources and supplies the calf with the available nutrients. Starter grain is provided as a source of solid feed that is fermented in the rumen. The end products from this fermentation are utilized for the growth of the ruminal epithelium. Providing calves with an adequate plane of nutrition stimulates the development of a fully functioning rumen and ultimately a healthy animal.

At the earliest stage of the calf's life, the reticulo-rumen makes up 38% of the total mass of the stomach and the abomasum makes up nearly 50% (Warner and Flatt, 1965). The first nutrients the calf receives are liquids in the form of colostrum, milk, or milk replacer. These liquids will pass through the esophagus and be shunted into the abomasum via the esophageal groove. The esophageal groove is a muscular fold of tissue found at the base of the esophagus that travels to the omasum and abomasum, by-passing the reticulo-rumen (Hegland et al., 1957). Closure of the groove is caused by several stimuli, including environment and the composition of the diet fed (Orskov, 1972).

Because of their digestive restrictions, young calves receive their nourishment from liquid feed sources for about 8 weeks until weaning is complete. On some farms, calves receive milk that has been collected directly from the milking herd, either saleable whole milk from the bulk tank or waste milk to be dumped. Whole milk is a high-grade liquid feed source that provides calves with the necessary nutrients for growth. It has greater amounts of fat and protein

per gallon than commercial milk replacers (USDA, 2007). Producers will typically feed milk at 8 to 10% of the calf's body weight (Davis and Drackley, 1998). However, whole milk is the most costly liquid feed source for calves and many producers look toward other sources of liquid feed for their calves. Jaster et al. tested the effects of whole milk, whole milk with a fat supplement, milk replacer, and milk replacer with a fat supplement on the growth and health of dairy calves. Calves were fed milk sources at a rate of 9% of body weight, which adjusted for the lower energy content found in the milk replacer. Lard was the source of fat in the fat supplement. Calves fed the fat supplement had increased body weight gains, which was to be expected, and there were no significant differences detected in the body weight gains or skeletal growth of the calves fed whole milk and those fed MR (Jaster et al., 1990). These data suggest that feeding milk replacer to calves will result in similar gains to feeding whole milk.

Depending on the economic situation of the producer, commercially-produced milk replacers have proven to be a money-saving alternative to feeding saleable milk. A survey of U.S. dairy producers conducted in 2007 by the National Animal Health Monitoring System (NAHMS) found that 69% of dairy heifers are fed milk replacer during the preweaning period (USDA, 2007). Milk replacers are easily stored and can be formulated with varying fat and protein concentrations based on nutritive requirements and farm management practices. Conventional milk replacers are typically fed in limited amounts to encourage intake of starter grain prior to weaning. Adjusting calves to solid feed intake during the preweaning period will not only decrease the amount of money spent on raising the animal but will also lessen the stress experienced during the weaning period (Huber et al., 1984; Arthington et al., 2003).

Recently, researchers have begun to investigate the effects of feeding calves higher protein milk replacers to stimulate more rapid growth rates. Calves fed an intensified or

accelerated milk replacer (30.6% CP, 16.1% fat) had greater overall body weight gains and skeletal growth than calves fed conventional milk replacer (21.5% CP, 21.5% fat). Milk replacers were formulated with different nutrient contents due to the larger volume of intensified MR fed. However, the calves on the intensified diet had lower starter grain and experienced reduced growth rates during the weaning period (Davis Rincker et al., 2011). Along with providing calves with milk or milk replacer it is important that they are given solid feed to stimulate the development of the rumen.

A study conducted by Stamey et al. (2012) determined the effects of starter grain CP content on the growth of calves and to compare a conventional MR to an accelerated MR for dairy calves. Calves that were fed conventional MR (20% CP, 20% fat) had higher starter dry matter intakes than calves provided with accelerated MR (28.5% CP, 15% fat). Nutrient composition of the MR were different for treatments for either a low or high plane of nutrition. The accelerated milk replacer stimulated more rapid gains in body weight, withers height, body length, and heart girth than the conventional milk replacer. Calves that were fed the accelerated milk replacer were supplied with either conventional starter grain (19.6% CP, DM basis) or enhanced starter grain (25.5% CP, DM basis). There was no significant difference in growth rate between the two different starters. However, during the weaning period on wk 6 calves that were fed the accelerated MR and conventional starter had higher dry matter intakes and feed efficiency than those that were fed the enhanced starter grain. After weaning in wk 7 calves fed the conventional MR and conventional starter had greater feed efficiency than calves fed accelerated MR and conventional or enhanced starter grain. These data indicate that calves provided with accelerated MR have increased growth rates and lower dry matter intakes. These



results support the findings of Cowles et al. (2006) that determined calves fed accelerated MR had lower dry matter intakes prior to weaning when compared with calves fed conventional MR.

In addition to the content of the milk or milk replacer, the amount of liquid fed has a major impact on dry matter intake of calves. Huber et al. (1984) tested the effects of feeding calves either a set amount (4.1 kg/d) of whole milk or a gradually increasing amount of milk (4.1 kg/d to 7.0 kg/d). Feeding calves higher quantities of milk resulted in greater weight gains, but lower starter grain intakes than the calves that were fed set amounts of milk. The low starter intakes explain why calves fed high amounts of milk experience slower body weight gains during the weaning and postweaning periods. Starter intake is the main driving force behind the development of a functioning rumen, which may be delayed if intake is inadequate. During weeks 3-8 of the calf's life they undergo rapid growth of the ruminal tissue, nearly four to eight times faster than the growth rates of the rest of the body (Davis and Drackley, 1998).

Solid feed intake is essential in establishing proper growth and development of the rumen epithelium which ultimately provides the calf with the ability to effectively ferment and absorb nutrients from the feed (Figure 1; Heinrichs, 1993. Penn State Extension).

Figure 1. Comparison of calf rumen fed milk and hay vs. milk and grain



Starter grain is easily fermented by calves and the end products are utilized for rumen growth. The physical form of the grain has been shown to affect the growth rates of the animals and the development of the rumen. Calves fed a pelleted starter had lower dry matter intakes than calves fed a multiparticle starter with the same ingredients and nutrient composition (Bach et al., 2007). During the preweaning period, calves on the multiparticle starter grew at numerically faster rates which suggests they may experience less stress during weaning. Not only will the physical form of starter affect the growth rates of the calf but will also impact the ruminal environment.

Feed particle size can significantly influence the ruminal function and digestive ability of the calf. Beharka et al. (1998) tested the effects of feeding preweaned, dairy bull calves ground (1 mm particle size) or unground diets equal in composition (25% alfalfa hay and 75% grain mix). Calves were fed the starter amounts based on consumption, while also being given milk at a rate of 8% of body weight (**BW**). At 11 wk of age calves were slaughtered and digestive organs were removed and weighed. The form of the diet had no effect on the weights of the reticulorumen or abomasum. However, examination of ruminal tissue samples revealed major differences in the shape, distribution, and length of papillae among calves fed different diets. Measurements of the papillae indicated that the calves fed the ground diet had shorter, thicker, and more branched papillae, while the papillae of calves on the unground diet were uniform, flattened, and tongue-shaped. Even though the papillae in calves on the ground diet showed signs of branching, they had less surface area than the papillae of calves fed the unground diet. Branching may have occurred to compensate for the loss of surface area and absorptive ability due to parakeratosis which occurs in diets high in concentrates or easily fermentable components (Bull et al., 1965).

Based on the information presented above, the following may be concluded about management factors known to impact the preweaning ruminant. With sole dependence on liquid feed for nourishment during the first few days of life, calves must be slowly introduced to solid feeds to establish a functional rumen environment. Feeding calves an increasing amount of conventional milk replacer as they grow older will result in higher body weights and lower starter intakes than for calves fed a set amount of milk replacer. Milk replacers can be formulated with varying amounts of crude protein and fat that impact the growth rates of the calf. Higher protein milk replacers have shown to increase the rate of body weight gain and skeletal growth of

calves during the preweaning period. Due to decreased starter grain intakes, the growth rates of calves on an accelerated MR feeding program decline and level out with the calves fed conventional MR. Introducing solid feed is important in establishing proper development of the rumen. Calves that had lower intakes of starter grain had a more challenging time adapting to a new diet during the weaning period than calves that had adequate starter intake. Starter grain particle size can impact the morphology of the rumen papillae. Finely ground starter produces papillae that are shorter and branching, suggesting a negative impact on absorptive ability. In comparison, an unground grain diet resulted in papillae that were longer and more evenly distributed. Starter grain is fermented in the rumen producing VFA that are utilized by the rumen epithelium. Providing calves with proper nutrition during the first weeks of life is essential in establishing a functional and well-developed rumen.

## WEANING

The weaning process is a pivotal moment in a calf's life. They are transitioned from abomasal digestion of liquid feed sources along with fermentation of small amounts of starter grain to strictly depending on solid feed sources, with the exception of water, for all nutrients. Depending on the management practices on the farm, producers will decide when to wean calves based on age, body weight, solid feed intake, or a combination of factors. The average age of heifers at weaning is 8.2 weeks, however it is not uncommon for farms to wean calves at older or younger ages (USDA, 2007). Earlier weaning can result in reduced feed and labor costs. Consuming solid feed establishes the growth of rumen papillae and development of the rumen. Fermenting solid feed allows heifers to derive large amounts of energy from their feed which optimizes growth rates during the postweaning period.

Milk replacers with higher levels of crude protein increase weight gain prior to the weaning period, however these effects may be nullified if calves experience a challenging weaning period due to lower starter intakes than those of calves fed in a conventional way. Calves were fed an intensified milk replacer (26% CP, 16% fat) and weaned at either 6 or 8 wk of age. Starter intake was minimal across treatments, however calves weaned at 8 wk had greater solid feed intakes throughout the study than calves weaned at 6 wk. Calves weaned later spent more time ruminating and eating straw than calves weaned earlier. Due to a decrease in metabolizable energy during the weaning period, ADG decreased for calves on both treatments, although the decrease was more drastic in calves weaned at 6 wk vs. 8 wk. Calves weaned later were able to better cope with the stresses of weaning than calves weaned earlier in life. Calves weaned at 8 wk had greater nutrient intakes, growth rates, and less signs of weaning stress than calves weaned at 6 wk (Eckert et al., 2015). Weaning calves based on age has been common practice amongst producers, however weaning methods based on solid feed intake have also been utilized.

Greenwood et al. (1997) tested the applicability of using dry feed intake as a percentage of initial body weight (**IBW**) to determine the optimal weaning age of calves. Thirty-two heifers and 12 bulls were assigned to one of three treatments, weaning when dry feed intake (**DFI**) is 1, 1.5, or 2% of **IBW** for 3 consecutive days before weaning. Calves on treatment 1 consumed greater amounts of dry feed postweaning than calves on the two other treatments. There were no differences among treatments in body weights during the postweaning period. Because calves weaned when **DFI** was 1% of **IBW** were weaned earlier than calves on the other two treatments, this method would be the most economical and efficient.

Weaning age impacts on postweaning growth and the health of heifers. In a study by Hopkins (1997) 56 Holstein calves were fed 3.8 L of whole milk in the same way and fed starter mix through either a specially-designed nipple bottle or through an open container. Calves were then weaned at either 28 or 56 d of age to test the effects on starter intake on growth. Calves that were weaned at an earlier age had significantly greater starter intakes ( $P < 0.0001$ ) after the weaning period and more total starter than the calves weaned at the later stage. Wither height was greater for calves weaned at 56 d vs. 28 d. The weaning age of calves has been shown to have a relationship with the development of the rumen. Calves with increased feed consumption prior to and during weaning were shown to have increased digestive ability of the rumen and a more stable rumen environment. Anderson et al. (1987) weaned 8 Holstein bull calves at either 4 or 6 wk of age. Calves weaned at 4 wk were fed a highly palatable starter grain at varying levels depending on intakes and the calves in the second group were fed starter grain ad libitum. Calves on the early weaning program had greater feed intakes and molar proportions of butyrate. This study suggests that decreasing the age at weaning increases ruminal activity and rumen development which are indicated by lower pH and increased VFA concentration.

Chapman et al. (2017) investigated effects of feeding milk replacer at varying levels and protein concentrations. Twenty-four heifers were assigned to 1 of 3 treatments: 466 g DM of conventional MR (20% CP, 20% fat); 669 g DM of a moderately high protein MR (26% CP, 18% fat); or 892 g DM of a moderately high protein MR (26% CP, 18% fat). Calves fed greater amounts of high protein MR had the greatest fat intake ( $P < 0.05$ ) and calves fed conventional MR had the lowest ADG and feed efficiency expressed as ADG/DMI ( $P < 0.05$ ). Body weight and hip height gain increased in calves fed high protein vs. conventional MR. Calves fed high protein MR had nearly 50% less starter grain consumption when compared to calves fed

conventional MR. Feeding high amount of nutrient dense MR will fulfill most, if not all, of the nutrient requirements of the calf. Therefore, calves fed high protein MR typically have low starter dry matter intake. Low DMI during the preweaning period can result in a more stressful weaning transition and can have negative impacts on postweaning growth performance.

In summary, weaning at the appropriate time will save producers money spent on feed and will ensure heifers have productive growth rates during the postweaning period. Weaning at an earlier age will decrease the amount of finances allocated to purchasing milk replacer and increase the amount of milk available for sale. Transitioning to consumption of solid feed only will further establish the rumen environment and allow for efficient digestion and absorption of nutrients. Calves fed conventional milk replacer experience less stress during the challenging weaning phase than do calves fed a high protein milk replacer. This is due, in part, to the fact that they are consuming greater amounts of solid feed during the preweaning period. Calves fed a high protein milk replacer benefit from being weaned later in life (8 wk), allowing for their body to adjust to solid feed intake for a longer period of time. Increased starter grain intake, which contributes to younger ages at weaning, results in lower ruminal pH and greater concentrations of VFA, specifically butyrate. Increased butyrate levels stimulate the proliferation of rumen papillae, thus increasing the surface area available for absorption of nutrients.

## IONOPHORES

Ionophores were introduced to the United States cattle industry in the 1970's. By the end of the 20<sup>th</sup> century seven ionophores were approved for use in several livestock classes (Feed Additive Compendium, 2001). Monensin is the most extensively used followed by lasalocid. These additives are used primarily to increase feed efficiency in cattle, but also show benefits in

increasing nitrogen metabolism by rumen bacteria and amino acid uptake in the small intestine (Bergen and Bates, 1984). Ionophores act by disrupting the ionic equilibrium within certain types of bacterial cells, which ultimately results in their destruction. This action mainly effects Gram-positive bacterial strains, nullifying their effects within the gut and allowing for competitive microbes to flourish. Ionophores also reduce methane production (McGuffey et al., 2001). Ionophores can significantly impact feed efficiency and overall health of cattle, while also decreasing levels of methane released into the environment.

To test the effects of monensin supplementation, researchers have utilized in vitro incubation of ionophores with ruminal fluid. Microbial digestion of forages results in an increased concentration of acetate in the rumen because the microbes use the fiber to produce acetate. In the presence of corn, starch-digesting microbes readily convert the nutrients into propionate. Russell and Strobel (1988) conducted an in vitro study testing the effects of adding monensin or bacitracin to ruminal fluid incubated with hay or corn. In the presence of hay and monensin, fiber digestion was significantly reduced which was relative to the decrease in acetate production seen. Bacitracin also decreased fiber digestion, but to a lesser extent than monensin. When corn was the provided substrate, monensin caused an increase in propionate production without an effect on concentrations of other VFA. This indicates that the ionophore had no effect on the digestion of corn. Monensin increased the propionate to acetate ratio in the presence of hay and corn. However, with hay as a substrate the ratio increased due to a decrease in acetate. In the presence of corn, the ratio was increased because propionate production increased. Monensin increases the ratio of propionate to acetate in the rumen, which can result in a reduction of methanogenesis. Introducing monensin to ruminal fluid reduced methane production, however the results were not statistically significant in all cases. With the addition of hydrogen gas to the



cultures, methane production returned. It would appear that the ionophore is not inhibiting the actions of methanogens, but is increasing the amount of propionate which binds the hydrogens that would otherwise be available to form methane (Van Nevel and Demeyer, 1977).

Understanding the effects of ionophores on the rumen microbial population is important in explaining how concentrations of VFA are altered.

Ionophores are an important addition to dairy feeds, decreasing the prevalence of Gram-positive bacteria and allowing the population of Gram-negative bacteria to proliferate (National Research Council, 2001). Ionophores accomplish this by disrupting the ion gradient located in the cell membrane of bacteria. This interference causes a reduction in available ATP and  $K^+$  which prevents the bacterium from maintaining adequate rates of cellular growth to sustain survival (Russell and Strobel, 1989). Due to a thick outer cell membrane, Gram-negative bacteria are typically less susceptible to destruction via ionophores than Gram-positive bacteria (Callaway et al., 2003). Decreasing the population of Gram-positive bacteria will lower concentrations of non-glucogenic VFA's, acetate and butyrate. Which allows for Gram-negative bacteria to flourish and increase levels of glucogenic propionate. The ratio of glucogenic to non-glucogenic VFA is indicative of energy balance in cattle (Ellis et al., 2015).

Weiss and Amiet (1990) tested the effects of supplementing lasalocid to mid-lactation dairy cows at two different times during the year. The first block extended from February to May and the second from June to September. During the first period, supplemental lasalocid decreased dry matter intake ( $P < 0.09$  and  $P < 0.11$ ) and increased weight gain ( $P < 0.09$ ) compared to the same diet without lasalocid. The cows supplemented with lasalocid were also 16% more efficient than control cows during the first week of treatment and were about 20% more efficient during the second week. In the second block, cows fed lasalocid were 20% more

energy efficient than control cows during the second week of treatment. However, no treatment effects on efficiency were observed for the remainder of the study. The energy efficiency for cows in the second block was nearly 17% lower than for cows in the first block, this may reflect greater energy costs due to heat stress. No differences in milk composition or production were detected between treatments. Sixty Holstein heifers weighing 196 kg were assigned to receive either 0, 200, or 600 mg of monensin per animal per day until 3 days prior to calving (Baile et al., 1982). Heifers fed monensin at 200 and 600 mg/d levels gained an additional 0.09 kg/d when compared to animals on the control diet. The heifers on treatments also had lower dry matter intakes, leading to 12.6 and 13.4% greater efficiency in converting feed to body weight gain than control heifers. At calving, heifers supplemented with monensin were 30 to 40 kg heavier than control animals. There was no treatment effect on milk composition or production.

Ionophores have coccidiostatic effects on dairy cattle, specifically in younger stock. Quigley et al. (1997) supplemented lasalocid into milk replacer and starter grain and studied effects on coccidian oocyst shedding, fecal scores, body weight, and intakes. Calves were fed milk replacer either with lasalocid or without it and starter grain with a low or high level of lasalocid. Calves that were fed milk replacer with lasalocid excreted significantly fewer fecal oocysts than those that were not supplemented with lasalocid in the milk replacer. Calves with lasalocid also had a lower prevalence of scouring and lower fecal scores. There were no differences in oocyst shedding, fecal scores, or prevalence of scouring between low and high level of lasalocid in starter grain of the calves supplemented lasalocid in milk replacer. Calves fed lasalocid in milk replacer had greater body weight gain, lower fecal scores, and less shedding of coccidia oocysts than calves not fed lasalocid. Supplementing ionophores at an early age has proven to have positive impacts on growth and reduction of coccidian oocyst shedding.

In conclusion, the supplementation of ionophores with dairy cattle increases feed efficiency, allowing producers to spend less money on feed while still observing positive growth rates. Monensin decreases feed intake while allowing cattle to maintain similar body weight gains, making them more efficient. Cattle are able to break down and absorb nutrients more efficiently, while decreasing the cost of feed. Ionophores also decrease the production of acetate and butyrate and allow for increased production of propionate. This alteration of the VFA profile reduces emissions of methane which has been an important movement in the world today. Feeding young heifers lasalocid in MR improves body weight gain and reduces the prevalence of coccidian oocyst shedding in the feces. Ionophores have proven to increase growth rates and health and are a useful addition to the diets of dairy cattle. However, the European Union recently put a ban on any substances considered to be antibiotic, including ionophores. Researchers have begun to search for new additives that may have similar effects on dairy cattle. One such additive that has gained a great deal of interest over the past few years is sodium butyrate, which has shown to reduce the prevalence of scours and improve health and growth of small intestinal epithelial cells.

## INTRODUCTION TO BUTYRATE

Butyric acid is a naturally forming volatile fatty acid produced from the ruminal fermentation of dietary carbohydrates such as cellulose, hemicellulose, starch, and soluble sugars. Of the three main VFA produced in the rumen, butyrate is typically found in the lowest concentrations and is mainly metabolized in the rumen epithelium (Ash and Baird, 1973). Butyrate is a major stimulator of ruminal papillae growth which increases the surface area and

absorptive ability of the rumen epithelium (Tamate et al., 1962). Young heifers that have a highly developed rumen are able to more efficiently absorb nutrients to be utilized for extensive tissue and skeletal growth. Sodium butyrate (**SB**) is an organic acid salt that is commonly used in place of butyric acid itself because it is solid and less odorous. Supplementation of SB to the diets of young calves increases growth rates, health, and rumen development (Górka et al., 2011a). By improving growth, heifers may reach breeding size at a younger age which allows them to enter the lactating herd sooner. This will save producers money and resources spent on animals not producing profits. Sodium butyrate positively affects the growth of rumen papillae in older cattle, preparing the rumen to adapt to increased concentrate levels in the postpartum diet (Kowalski et al., 2015). Butyrate is very important in the stimulation of the development of a functioning rumen and has a cascade of effects on the digestibility, growth, and health of the animals.

### ***Production via Microbial Action***

Production of VFA is primarily through anaerobic fermentation of carbohydrates, which are otherwise poorly digested without the help of microbes. The molar ratio of acetate to propionate to butyrate within the rumen is typically 65:20:15 which can vary depending on the diet composition (Bergman, 1990). Cellulolytic bacteria release the enzyme cellulase which is capable of digesting cellulose and hemicellulose into oligosaccharides. These complex carbohydrates are further broken down into a variety of hexoses and pentoses (Beever, 1993). Both cellulolytic and non-cellulolytic bacteria will utilize the end products of cellulase digestion to derive energy in the form of adenosine-triphosphate (**ATP**) and create VFA as end products. Glucose will be converted into pyruvate via the Embden-Myerhof pathway of glycolysis. The

fate of pyruvate, which is a very important substrate in the production of VFA, varies depending on the microbes present and the rumen environment.

Pyruvate can be converted into acetate via two enzymatic pathways. The most common pathway is the pyruvate-formate lyase system that produces formate and acetyl-CoA. The second mechanism is the pyruvate-ferredoxin oxidoreductase pathway that converts pyruvate into reduced ferredoxin, carbon dioxide (CO<sub>2</sub>), and acetyl coenzyme A (**acetyl-CoA**) (Baldwin and Allison, 1983). Acetyl-CoA can then be transformed into acetate and 1 ATP by phosphotransacetylase and acetokinase. Acetyl-CoA is a versatile substrate that can be utilized in the production of other compounds. One compound of importance is butyrate.

Acetyl-CoA can be utilized as the intermediate in the production of butyrate by protozoa or bacteria. Different diets elicit variable responses in the rumen environment, which leads to fluctuating concentrations of substrates and microbial concentrations. Certain high concentrate diets create a rumen environment that supports a large population of protozoa (France and Siddons, 1993). Of these, the ciliate protozoon *Dasytricha ruminantium* readily converts acetyl-CoA into butyrate, lactate, and acetate. In solutions with excess soluble sugars, *D. ruminantium* converts acetyl-CoA to butyryl-CoA using various enzymes. The conversion of butyryl-CoA to butyrate is catalyzed by phosphate butyryltransferase or butyrate kinase. This method of butyrate production will result in the generation of 1 ATP molecule (Yarlett et al., 1985). Another microbial species that uses pyruvate as the substrate to produce butyrate and ATP is *Butyrivibrio fibrisolvens*. This Gram-negative bacterial species will ferment glucose and produce hydrogen, CO<sub>2</sub>, and butyric, formic, and lactic acids (Bryant and Small, 1955). Of the many strains, *B. fibrisolvens* D1 is a strain that primarily produces butyrate, especially when a high-fiber diet is fed. Two molecules of acetyl-CoA are transformed through enzymatic reactions to produce

crotonyl-CoA which is then used to form butyryl-CoA. The conversion of butyryl-CoA to butyrate is catalyzed by the same enzymes as was the case with *D. ruminantium*. The final products of this complicated process are 1 molecule of butyrate and 1 molecule of ATP (Miller and Jenesel, 1978). Pyruvate is important in the formation of acetyl-CoA and is also converted into lactate under low ruminal pH conditions.

Lactate production in the rumen is stimulated by a sudden drop in pH, typically caused from the ingestion of high-concentrate diets which allow for rapid microbial fermentation. Lactate is removed from the rumen in three methods: passage through the lower gut, absorption from the rumen, and microbial fermentation. A common lactate-fermenting organism is *Megasphaera elsdenii*, which is the only bacterium known to ferment lactate into propionate via the acrylate pathway. *M. elsdenii* also ferments lactate in small amounts to produce butyrate. Counotte et al. (1981) incubated in vitro cultures of lactate to determine the percentage of lactate converted via the acrylate pathway into propionate and the amount fermented to butyrate. They concluded that as pH decreased more lactate was fermented into butyrate. In a second study, the effects of acetate on the proliferation of *M. elsdenii* in a glucose and Trypticase medium were investigated. *M. elsdenii* were able to grow in a medium without the presence of acetate but, with addition of acetate growth was improved. As acetate concentrations increased, butyrate production was increased. These results suggest that acetate worked as an electron acceptor for the hydrogens produced during glucose metabolism (Hino et al., 1990). Acetate is also involved in the synthesis of butyrate by a bacterial species with limited means to obtain energy.

The anaerobic bacterium *Clostridium kluveri* obtains its energy solely from the synthesis of fatty acids. It is unable to utilize complex substrates and depends on ethanol and acetate (Bornstein and Barker, 1947). *C. kluveri* oxidizes ethanol into a 2-carbon compound, referred to

as “active” acetate. The “active” acetate is then condensed with acetate to form a 4-carbon compound which is then reduced to butyrate. Another molecule of “active” acetate may also condense with the butyrate molecule and form the acid, caproate (Stadtman and Barker, 1949). The concentrations of VFA in the rumen can vary considerably and can affect the rate and mode of absorption.

### ***Effects on Rumen Epithelium and Absorption via Tissues and Organs***

The composition of the diet and the time after feeding both have major impacts on ruminal VFA concentrations. Under normal conditions, acetate makes up the highest percentage of volatile fatty acids in the rumen, followed by propionate and butyrate. High-fiber diets stimulate increased populations of acetate-producing microbes while starch and concentrate diets promote populations of propionate and butyrate-producing organisms, although acetate will usually remain the most abundant of the VFA (France and Siddons, 1993). Average pH levels in the rumen are typically between 5.8 and 6.8. Diets with high concentrations of rapidly fermentable feeds will cause a drop in pH, stimulating the production of propionate and lactate. Propionate is the only VFA that significantly increases glucose production; nearly 50% of propionate produced is readily absorbed by the liver and converted into glucose (Bergman et al., 1965). Volatile fatty acid absorption is important in stimulating the secretion, production, and inhibition of various substances in the body.

Large amounts of VFA produced in the rumen are absorbed by the tissues of the rumen epithelium or transported into the bloodstream. Nearly 30, 50, and 90% of acetate, propionate, and butyrate, respectively, were absorbed in the rumen and did not reach the portal blood (Bergman and Wolff, 1971). The organization of the components of the rumen epithelium in

sheep was determined by Dobson et al. (1956). The mucosal surface is dominated with papillae that vary in shape and size in relation to their location in the rumen. In most cases, the papillae are tongue-like and occasionally appear to be conical. The papillae core contains dense collagen fibers and have a rich blood and lymphatic supply that is connected to the basal layer of stratified squamous epithelial cells. The next layer of cells are highly keratinized and closely packed together. The most superficial layer of cells that come in contact with the rumen digesta are also keratinized but are sometimes absent due to sloughing into the ruminal cavity. The rumen epithelium is able to absorb VFA and transport them into blood via diffusion, but can be affected by pH and intracellular metabolism of VFA (Bergman, 1990).

Of the three main VFA produced in the rumen, butyrate is metabolized the most by rumen epithelium. Nearly 90% of butyrate is absorbed by the rumen epithelium and in turn is converted into ketone bodies or oxidized to produce CO<sub>2</sub> (Bergman, 1990). The process of ketone formation via rumen epithelium is referred to as alimentary ketogenesis to distinguish it from hepatic ketogenesis. In a study by Pennington (1951) butyrate, acetate, and propionate were incubated with rumen epithelial cultures and effects on ketone body production were investigated. The presence of butyrate produced significantly larger concentrations of ketones than the other two VFA. The amount of butyrate utilized to form ketone bodies ranged from 59 to 74%. When butyrate was supplied at 100 μmoles/culture (normal concentration) the percentage of butyrate converted to ketones was 70%. When the amount of butyrate was divided in half the conversion percentage was 65%. This suggests that the rumen epithelium has a high affinity for converting butyrate into usable ketones. Alimentary ketogenesis is important in regards to the fact that in other species, the production of ketones is limited to the liver. Ruminants produce high concentrations of VFA from the fermentation of feed, contributing to a



more acidic rumen environment which inhibits the survival of microbes. The ketogenic activity of the rumen epithelium is important in equalizing this acidic environment thus preventing ruminal acidosis and maintaining an adequate environment for microbial digestion of feeds.

Although butyrate is mainly absorbed and utilized by the rumen epithelium, it also has significant impacts on the liver, lower gastrointestinal tract and blood supply. Butyrate that is not metabolized in the rumen is transported to the liver via the hepatic portal vein. Here the liver converts butyrate into butyryl-CoA with the enzyme butyryl-CoA synthetase. The product butyryl-CoA is then readily transformed into acetyl-CoA, ketones, or longer chain fatty acids (Bergman, 1990). Butyrate significantly inhibits propionate utilization in the liver. When incubated in sheep hepatocyte cultures, 2 mM of butyrate decreased the hepatic conversion of propionate to glucose by 63% (Demigne et al., 1986). This inhibitory effect is not seen under normal conditions because the circulating amount of butyrate in blood is minimal due to rapid metabolism via the rumen epithelium. Glucose production in the liver is highly regulated by the secretion of insulin, which can be increased with the administration of butyrate or propionate.

An experiment was conducted to determine the physiological role of butyrate on the concentrations of plasma insulin and glucagon and their responses to butyrate infused at varying rates. Butyrate was infused intravascularly through the femoral vein of sheep at rates of 0, 1, 2, 4, 8, 16, 32, and 64  $\mu\text{mol}\cdot\text{kg BW}^{-1}\cdot\text{min}^{-1}$  and concentrations of plasma insulin and glucagon were determined. Plasma insulin was increased ( $P < 0.01$ ) when butyrate infusion rates were 2  $\mu\text{mol}\cdot\text{kg BW}^{-1}\cdot\text{min}^{-1}$  or higher. Plasma glucagon concentrations were also increased ( $P < 0.05$ ) at infusion rates of 32 and 64  $\mu\text{mol}\cdot\text{kg BW}^{-1}\cdot\text{min}^{-1}$ . From these results it can be inferred that insulin concentrations were not affected by glucagon because glucagon required greater infusion rates to elicit a response. Thus it appears that in sheep, butyrate is capable of stimulating the secretion of

insulin from the pancreas and can also have an effect on blood glucose levels due to its activity as a substrate for gluconeogenesis (Sano et al., 1995). Herrick et al. (2017) ruminally dosed lactating cows with a single dose of SB at either 1 g/kg of BW or 2 g/kg of BW and compared the metabolic effects to cows treated with 2 L of water (control) or 3.5 g/kg of BW of lactose. Plasma  $\beta$ -hydroxybutyrate (**BHB**) concentrations were greater ( $P < 0.01$ ) in SB vs. control or lactose treated cows. Sodium butyrate treated cows also tended to have greater ( $P = 0.06$ ) concentrations of plasma insulin than control or lactose treated cows. This supports the results of the sheep study, that butyrate elicits an increase in insulin secretion which may indirectly affect glucose metabolism. In addition to its absorption in the rumen and gastrointestinal tract, butyrate is also utilized by the mammary gland.

The inclusion of SB in the diet of lactating cows has shown to impact milk constituents and production. Huhtanen et al. (1993) used 4 mid-lactation cows in a 4 X 4 Latin square, with each experimental period lasting 2 weeks. They were intraruminally infused with increasing levels of butyrate. Milk yield was unchanged by butyrate infusions. The concentrations of milk fat ( $P < 0.01$ ) and protein ( $P < 0.05$ ) both increased as butyrate infusion rates increased. Due to the changes in milk component composition, the resulting milk fat yield increased ( $P < 0.05$ ). Lactose concentrations decreased ( $P < 0.01$ ) linearly with increasing rates of butyrate infusion as did lactose yield. Overall, butyrate infusion did not have major impacts on milk production but did have positive effects on milk composition. At a molecular level, Kleiber et al. (1954) determined the relative mechanism by which butyrate is incorporated into milk constituents. They injected 1-C<sup>14</sup> and 2-C<sup>14</sup>-labeled butyrate into the jugular veins of 4 lactating cows. They traced the transfer of the carbon from butyrate into lactose, casein, milk albumin, and butter fat and estimated the rates at which the transfer occurred. Around 6% of the 1-C<sup>14</sup> and 22% of the 2-

C<sup>14</sup>-labeled butyrate were found in the milk constituents. More of 1-C<sup>14</sup> and 2-C<sup>14</sup> were found in lactose and casein than in milk fat. In conclusion, butyrate took on a glyconeogenic role instead of a lipogenic role.

### ***Effects of Sodium Butyrate on Growth, Digestive Ability, and Health***

Butyrate plays a key role in stimulating the maturation of the ruminal epithelium in dairy cattle and has significant impacts on the digestive capabilities and health of other species of animals. Abdelqader and Al-Fataftah (2015) conducted a study testing the effects of supplementing butyric acid to the diets of heat-stressed broilers. They investigated the effects of butyric acid on the performance of broilers as well as its effects on their intestines. Butyric acid stimulated an increase in intestinal villi height, villi surface area, absorptive epithelial cell area and intestinal weight. These results indicate that butyric acid stimulates epithelial cell proliferation and supports the repair of intestinal damage caused by heat-stress. Butyric acid not only improves intestinal integrity but it also has positive effects on growth rates and feed efficiencies of heat-stressed birds. Sodium butyrate has positive effects on the proliferation of rumen epithelial cells when administered intraruminally. Sakata and Tamate (1978b) biopsied rumen papillae of sheep to determine the effects of sodium butyrate supplementation. Sheep were fed sodium butyrate (2g/kg body weight per day) at either a rapid or slow rate. Both doses were administered intraruminally once a day, the rapid dose was given within 10 s and the slow dose was given over the course of 20 to 24 hr. Sheep given a rapid dose of sodium butyrate had an increase in mitotic indices ( $P < 0.01$ ) the day following treatment when compared to indices before administration. Sheep fed slowly did not show any increases in mitotic index, mostly because the mode of administration stimulates only moderate butyrate production in the rumen.

Rapidly increasing the amount of intraruminal SB will stimulate the proliferation of rumen epithelial cells, thus enhancing the functioning ability of the rumen.

Another study of the effects of intraruminal butyrate was conducted by Moolchand et al. (2013). Fifteen ruminally-fistulated goats at 120 d of age and an average weight of 20 kg were allocated into 1 of 2 treatment groups for infusion with or without SB. Goats were fed 200 g of concentrate a day and hay was provided ad libitum. Animals on the SB treatment were infused with 0.3 g/kg of BW of sodium butyrate once a day for 28 d at a rate of 10-15 seconds. Rumen fluid samples were taken before infusion and 0.5, 1.0, 1.5, 2.5, and 3.5 h after the infusion and stored for VFA analysis. Goats were slaughtered at d 28, the stomach was removed and all compartments were weighed, contents were collected from the rumen, omasum, and abomasum and weighed. Rumen tissue samples were taken from 6 goats of each treatment, 4 papillae with 3 visual fields on each were examined for individual goats. Papillae were evaluated for height and thickness and epithelial layer thickness was measured. On d 26 the rate of liquid passage from the rumen was estimated. The concentration of butyrate significantly increased ( $P < 0.01$ ) and remained elevated for 3.5 hours after infusing SB. Papillae height increased ( $P < 0.005$ ), distance between papillae decreased ( $P < 0.05$ ), and thickness of epithelium increased ( $P < 0.05$ ) in goats infused with SB. Increasing papillae height, thickness, and epithelial density also increase the absorptive area of the papillae. When expressed as a percentage of total stomach weight, the rumen in goats infused with SB was significantly greater ( $P < 0.05$ ) than for the controls, with values of 89.09 and 86.71% respectively. Weight of digesta in the rumen, as a percentage of total stomach weight, tended to be greater ( $P < 0.06$ ) in SB infused goats (91.72%) when compared to control goats (89.81), which suggests that SB infusion caused a longer retention time of feeds in the rumen. This study illustrates that sodium butyrate infusion can improve the absorptive

capacity of papillae by enhancing their size and density in the rumen, while also prolonging the retention time of feeds in the rumen. The combination of these effects support improved rumen efficiency and digestive abilities which, in turn, can result in the enhancement of growth performance.

Young ruminants do not have a fully developed rumen and, because of this, rely on their abomasum and lower gut, specifically the small intestine, for digestion. The duodenum, the first section of the small intestine, utilizes pancreatic juices to further digest food received from the abomasum. Pancreatic secretions contain a variety of enzymes that help aid in the digestion process. Increasing levels of these juices can improve nutrient digestibility and feed efficiency. Guilloteau et al. (2010a) evaluated the effects of oral and duodenal SB supplementation to eight calves. The study began on 54 d of life and ended at 88 d of life. Calves were divided into 2 groups to be fed SB orally or duodenally and then further divided into 2 subgroups; a control group or a SB supplemented group. These divisions allowed researchers to more effectively evaluate the effects of each treatment. The first portion of the study utilized 4 duodenally cannulated calves that were fed equal amounts of milk replacer (**MR**) and infused with a saline solution (control) or sodium butyrate. Pancreatic juices were collected every 5 min for 2 consecutive hours and blood samples were obtained before infusions and every 10 min after infusions for 90 min. Sodium butyrate did not have an effect on pancreatic juice, protein, or chymotrypsin flow rate but did increase lipase flow rate ( $P < 0.05$ ) in comparison to saline infusion. Overall, duodenal infusion of SB showed little effect on pancreatic secretions.

Calves in the oral supplementation group were fed equal amounts of MR either with SB (3 g/kg of dry matter) or without. Pancreatic juice secretions were measured at 3 periods for a complete 24 hours. Blood samples were collected during times that pancreatic juice collection

was not occurring. Fecal samples were obtained for 4 complete days during each of the 3 periods. Calves fed SB tended to have higher DM and nitrogen (N) digestibilities than calves not fed SB. Sodium butyrate supplementation also significantly increased digestibility of fat, ash, and calcium ( $P < 0.05$ ). In comparison with calves on control diet, SB had a tendency to increase total pancreatic juice secretion relative to body weight ( $P < 0.10$ ) and total protein secretion was increased 1.4 fold ( $P < 0.05$ ). Total daily production in relation to body weight for chymotrypsin ( $P < 0.10$ ) and lipase ( $P < 0.05$ ) were increased by 52% and 40% respectively. Sodium butyrate also stimulated a reduction in the decrease of pancreatic juice flow rate after feeding while maintaining maximal duodenal flow of digesta. This reduction shows that SB is capable of altering circadian digestion kinetics. The increase in total pancreatic juices relative to body weight and increase in chymotrypsin and lipase explains the increase in nutrient digestibility. Increasing the amount of pancreatic juices will enhance the digestive abilities of the small intestine, allowing for improved breakdown of proteins. These results support the theory that SB administered orally will improve digestibility of nutrients in calves fed milk replacer (Guilloteau et al., 2010a).

The inclusion of sodium butyrate in milk replacer has been shown to have positive effects on the growth performance and digestive abilities of preweaned dairy calves. Stimulating the maturation of gut tissues, specifically through the proliferation of epithelial cells, plays an important role in establishing a functional rumen environment and healthy calf. Guilloteau et al. (2009) compared the effects of SB and flavomycin on the growth performance and intrastinal development of dairy calves. Eighty-eight calves were divided into 2 groups and fed MR with either flavomycin (16.5 mg/kg of dry matter) or SB (3 mg/kg of dry matter). Levels of milk replacer and starter grain were increased with respect to age. After slaughter at 151 d of age 8

calves from each treatment group were chosen as the most representative of the 2 groups. The small intestine was removed and each section was analyzed for length and physical characteristics of the villi. The duodenum of calves fed SB tended to be longer and have longer villi than the duodenum of calves fed flavomycin. In the other sections of the small intestines there were no significant differences between treatments. There were no differences in dry matter intakes. Calves fed SB had higher body weight gains, mainly during the first 2 mo, than calves fed flavomycin. Sodium butyrate-fed calves also had greater body weights than calves on flavomycin, which was most pronounced during the final 2 mo of the study. Overall, calves fed SB had increased feed efficiency in comparison to calves supplemented with flavomycin. These results support the hypothesis that sodium butyrate can be useful as a growth promoter in milk-fed calves. Increased duodenal length and villi length are indicative of enhanced maturation of the small intestine. A more mature small intestine will aid in more effective absorption of nutrients which explains the increased feed efficiency response. The supplementation of sodium butyrate not only increases the absorptive abilities of the small intestine but also has had major impacts on rumen papillae growth and rumen development.

In a study by Górká et al. (2011b), 21 bull calves were split into 3 groups and fed whole milk, milk replacer, or milk replacer supplemented with sodium butyrate. Sixty percent of the milk protein was replaced with soy protein to ensure slower small intestine development. Calves were fed the same amounts of liquid feed based on dry matter content and starter grain was available ad libitum. Calves fed whole milk had higher dry matter intakes during the final week of the study than calves fed milk replacer and milk replacer with supplemental SB. Calves fed whole milk gained more weight ( $P < 0.01$ ) and had higher average daily gains throughout the study than calves fed milk replacer and milk replacer with SB. Calves fed whole milk tended to

have more days (2.2 vs. 1.6) exhibiting signs of scours ( $P = 0.07$ ) and more days (1.3 vs. 0.7) treated with electrolyte therapy ( $P = 0.07$ ) than calves on milk replacer. When comparing performances of calves fed milk replacer, the calves supplemented with SB tended to have higher average daily gains ( $P < 0.09$ ) during the first week and greater body weights ( $P < 0.10$ ) during the second week of trial than calves not supplemented with SB. Addition of SB to milk replacer had no effect on starter grain intake. Sodium butyrate addition in milk replacer resulted in a higher mitotic index in the jejunum ( $P = 0.01$ ) and lower apoptotic index ( $P < 0.01$ ). When compared to calves fed only milk replacer, SB addition led to greater reticulorumen weight, reticulorumen weight as a percent of whole stomach weight, rumen papillae width and length ( $P < 0.05$ ). Mitotic indices are used to determine the rate of epithelial cell proliferation and apoptotic indices are indicative of the rate at which cells die (Sakata and Tamate, 1978a). Therefore the increase in mitotic index and decrease in apoptotic index in this study indicate that SB addition results in increasing proliferation of epithelial cells in the jejunum. Increasing epithelial cell growth will improve the absorptive ability of the small intestine and support higher growth rates. Rumen papillae are a major contributor to the digestive and absorptive functions of a ruminant. Establishing a large concentration of long, wide rumen papillae will ensure a highly functioning rumen. Calves that were fed SB in milk replacer had more pronounced papillae growth than calves that were not supplemented SB, which means they will be more efficient in breaking down feed and absorbing nutrients.

Supplementing sodium butyrate into milk replacer has not only been shown to improve rumen development but also the small intestine. Because calves are typically fed MR in conjunction with starter mixture (**SM**), Górká et al. (2014) conducted a study testing the synergistic effects of the inclusion of sodium butyrate in both milk replacer and starter mixture.



Twenty-eight Holstein bull calves began the study at 5 d of age and were allocated into 1 of 4 treatment groups and fed: (1) MR and SM without SB ( $\text{MR}^-/\text{SM}^-$ ); (2)  $\text{MR}^-$  and SM supplemented with SB encapsulated with a triglyceride matrix ( $\text{SM}^+$ , 0.6% as fed;  $\text{MR}^-/\text{SM}^+$ ); (3) MR supplemented with crystalline SB ( $\text{MR}^+$ , 0.3% as fed) and  $\text{SM}^-$  ( $\text{MR}^+/\text{SM}^-$ ); or (4)  $\text{MR}^+$  and  $\text{SM}^+$  ( $\text{MR}^+/\text{SM}^+$ ). Calves were fed milk replacer (22% CP and 18% fat in DM) twice daily at amounts equal to 10% initial body weight. Starter mixture (38% CP in DM) mixed with whole corn grain (50/50; wt/wt) was offered as the starter diet (24% CP and 15% NDF in DM) daily for ad libitum intake. Calves remained on treatments for 3 wk until they were slaughtered and the gastrointestinal tract was removed. The duodenum, proximal and distal jejunum, and ileum were weighed and measured. Tissue samples were taken from each of the sections and analyzed for villus length, crypt depth, and tunica mucosa and tunica muscularis thickness. Dry matter intake with MR was not different among treatments, however starter diet dry matter intake was increased during the final week on study when SB was supplemented into SM. Sodium butyrate addition into SM tended to increase small intestine and jejunum weights ( $P \leq 0.07$ ). Absolute ileum weight and length were highest for calves on  $\text{MR}^+/\text{SM}^+$  treatment. Crypt depth and tunica mucosa thickness in the duodenum was higher for  $\text{MR}^-/\text{SM}^+$  than calves on other treatments. Calves supplemented with SB in MR had lower villus height in the proximal jejunum and villus height, crypt depth, and tunica mucosa thickness in the middle jejunum ( $P \leq 0.04$ ) when compared with calves not supplemented SB in milk replacer. Supplementation of SB in MR and SM both increased the mitotic index ( $P \leq 0.04$ ) and decreased apoptotic index (MR and SM interaction,  $P < 0.01$ ) in the middle jejunum. These changes lead to a higher mitotic:apoptotic ratio which indicates accelerated enterocyte maturation, differentiation, and turnover. Even though there was a significant effect on mitotic and apoptotic indices, SB supplementation in

MR did not have a pronounced effect on intestinal mucosa growth. However, decreased mucosa thickness requires less energy expenditure for small intestine functions. Therefore, SB addition to MR can be deemed to have positive effects on small intestine growth.

In summary, sodium butyrate was added to starter mixture, there were significant effects on the structural and functional developments of the small intestine. Total weight and villus height in the distal jejunum were higher in calves fed SM with SB than calves fed SM without. Calves supplemented with SB had increased starter dry matter intake which would result in greater amounts of digesta passing through the gastrointestinal tract to the small intestine. This may have led to the improved mucosa structure in the duodenum. Even though SB stimulated small intestine development when fed in both MR and SM, there were more pronounced effects found in calves fed SB in MR. This could be due to insufficient amounts of SB reaching the duodenum because it was absorbed and utilized by ruminal epithelium. Although sodium butyrate had positive effects on improving development of the small intestine, there were no synergistic effects of SB addition to both MR and SM. Sodium butyrate supplementation enhanced the digestive and absorptive abilities of the gastrointestinal tract of young calves. Solid feed is an important stimulator of epithelial cell proliferation and the maturation of the rumen. Increasing the absorptive abilities of calves is not only associated with improved growth but can also positively affect the health of the animal.

Coccidiosis in calves is associated with significant economic losses, health impacts due to intestinal damage, and in severe cases mortality. This disease is caused by the protozoan species *Eimeria*, specifically *E. bovis* and *E. zurnii* (Quigley et al., 1997). Adding a coccidiostat or ionophore into the diet of calves has shown to decrease the prevalence of the parasites and improve growth performance. The antibiotic monensin is a common additive in the United States

that improves feed efficiency and reduces the incidence of scouring due to coccidian infection. Recently, the European Union banned the use of any antibiotics in the dairy industry, including monensin. The search for a new additive that acts similar to monensin and butyrate has gained prominence. Butyrate is able to act directly on the gastrointestinal tract and indirectly on the lower gut. Sodium butyrate supplementation, as previously stated, improved epithelial cell proliferation and tissue repair in the small intestine. These effects prove beneficial in controlling lower gut health disorders, such as scouring (Guilloteau et al., 2010). Studies conducted with humans, showed that butyrate delivery via enema produced a therapeutic effect in relation to inflammatory bowel disease (Okamoto et al., 2000). With this in mind, sodium butyrate could be useful in decreasing coccidiosis and improving the health of dairy cattle.

## CONCLUSION

The agricultural industry is depended upon to support the thriving world population, which is set to increase by nearly 1 billion people in years to come. A larger population means that demands for resources and products will be much greater than in the past. To cope with increasing demands, dairy producers are faced with the obstacle of increasing milk production without significant economic loss. Reducing the present-day costs on dairy farms is the main goal for nearly all producers. Raising replacement heifers is one of the largest expenses on a farm. Proper nutrition and management of young stock will ensure that they reach maturity at an early age and enter the milking herd where they can begin to produce profits. Encouraging solid feed intake early on will stimulate rumen development, which is essential for optimal digestion of feedstuffs. The fermentation of carbohydrates produces VFA that are important for the growth and health of the animal. One of the VFA produced, butyrate, is utilized by tissues in the rumen to increase proliferation of rumen papillae and the epithelium. Increasing the length and

concentration of rumen papillae directly increases the absorptive capacity of the rumen. The animal can utilize the nutrients absorbed for energy and partition that energy for their growth and development. Improving growth performance of dairy calves and cattle can increase their productivity.

Butyrate supplementation in the diets of young heifers has shown to improve growth rates and rumen development. Calves fed milk replacer with supplemental sodium butyrate had increased papilla heights and thickness and epithelial density when compared to calves that were not supplemented with sodium butyrate. Sodium butyrate in milk replacer also tended to decrease the number of days that calves exhibited scours and were treated with electrolyte therapy. Calves fed sodium butyrate had increased average daily gains and final body weights without affecting dry matter intake. Sodium butyrate inclusion has been shown to improve not only rumen function but also has significant impacts on the small intestine.

Inclusion of sodium butyrate in starter grain increased the mitotic index of small intestine enterocytes and decreased the apoptic index. This suggests that sodium butyrate was able to maintain the growth of small intestine epithelial cells, which improves the absorptive functions of the lower gastrointestinal tract. Sodium butyrate was also shown to stimulate increased secretion of pancreatic juices which are utilized for digesting feeds. Improving the digestive abilities of dairy cattle allows animals to grow at optimal rates without increasing dry matter intakes. Increasing feed efficiency will decrease the feed cost on the farm, while still raising healthy and productive animals. Sodium butyrate is an extremely beneficial feed additive in the diets of dairy cattle. It is associated with improved rumen development in calves, which stimulates increased body weight gains and health. It has shown to positively affect lower gut health and decrease the incidence of scours. No research has been conducted investigating its use

on the growth performances of postweaned heifers. Increasing growth performances in postweaned heifers can ensure that they reach maturity at a younger age and enter the milking herd sooner, shortening the payback period and improving economic benefits for the producer.

**CHAPTER 2: SUPPLEMENTATION OF SODIUM BUTYRATE TO POSTWEANED  
HEIFER DIETS: EFFECTS ON GROWTH PERFORMANCE, NUTRIENT  
DIGESTIBILITY, AND HEALTH**

**INTRODUCTION**

The cost of raising replacement heifers constitutes nearly 15-20% of all the expenses on a dairy farm (Heinrichs, 1993). Establishing a well-developed, functional rumen in dairy calves is essential for improving growth performance and feed efficiency, which can indirectly decrease costs of raising replacement heifers. Increased solid feed intake in calves is capable of stimulating the proliferation of microbial populations in the rumen, which in turn ferment carbohydrates (Bergman, 1990). The end-products of this breakdown are rapidly converted into volatile fatty acids (**VFA**) in the rumen. The three main VFA produced are acetate, propionate, and butyrate. Of these three, butyrate is the most readily absorbed by the rumen epithelium (Beever, 1993).

Mature rumen epithelia are characterized by the prominence of papillae, which are embedded in the mucosal surface. They are surrounded by blood and lymphatic vessels which support their ability to efficiently absorb VFA, particularly butyrate (Dobson et al., 1956). Butyrate is metabolized by the epithelial cells for energy, mainly through conversion into ketone bodies or carbon dioxide (**CO<sub>2</sub>**) (Bergman, 1990). The energy is utilized to stimulate individual papilla growth and also increase papillae concentration in the rumen (Tamate et al., 1962). A greater surface area for absorption of nutrients, improves the functioning ability of the rumen and other components of the gastrointestinal tract.

Research has shown that the inclusion of SB in the diets of ruminants positively impacts growth rates, feed efficiency, and gut health. Sander et al. (1959) tested the effects of intraruminally infused SB on the development of the rumen in young calves. Twelve dairy calves at 2 wk of age were fitted with rumen fistulae and allocated to 1 of 6 treatments: control, glucose, sodium acetate, sodium propionate, sodium chloride, or SB. The solutions were given twice daily before feeding and calves were slaughtered at the end of the 3 wk study period. Sodium butyrate increased weight gain of calves compared to control and other treatments. Calves on SB also had more extensive rumen papillary growth than calves not supplemented SB. The supplementation of SB in milk replacer has shown to have positive effects on the growth performances of dairy calves.

Guilloteau et al. (2009) investigated the differences between growth performance and gastrointestinal morphology of calves fed the growth promoter flavomycin or SB. Eighty-eight milk-fed calves were split into 1 of 2 treatment groups: supplementation of SB (3 g/kg of dry matter (**DM**)) or flavomycin (16.5 mg/kg DM) into milk replacer (**MR**). The duodenum in calves fed SB was longer and villi length was increased when compared to calves fed flavomycin. Sodium butyrate improved body weight gains for calves during the first 2 mo on study. There were no significant differences in dry matter intake (**DMI**) between treatments. These results point to an increased feed efficiency in calves fed SB, they maintained similar DMI but improved growth rates.

Górka et al. (2011b) conducted a study to investigate the effects of feeding whole milk versus milk replacer with or without supplemental SB. Milk replacer protein was replaced with soy protein to ensure slower development of the small intestine. Calves fed whole milk had higher DMI during the final week of the study and gained more weight overall when compared

to calves fed milk replacer. Sodium butyrate tended to increase average daily gains during the first week ( $P < 0.09$ ) and body weights during the second week of trial ( $P < 0.10$ ). Calves fed SB had higher mitotic indices in the jejunum ( $P = 0.01$ ) and lower apoptotic indices ( $P < 0.05$ ) than calves fed milk replacer without SB. Mitotic indices are used to determine the rate of epithelial cell proliferation, which when increased reflects enhanced absorptive function of the small intestine.

To date, no research has been conducted testing the effects of SB supplementation into the diets of postweaned heifers. Enhancing the proliferation of epithelial cells in both the rumen and the small intestine will elicit positive growth responses in heifers (Baldwin et al., 2004; Guilloteau et al., 2010). Improving growth performance in prepubertal heifers could help to decrease the age at which they are first bred, which will ultimately save producers money. The objective of this study was to determine the effects of varying levels of SB on the growth performance, nutrient digestibility, and health of postweaned dairy heifers. We hypothesize that as SB levels are increased calf growth performances and nutrient digestibility will also increase. Subsequently, the information collected was used to determine the most beneficial feeding amount of SB and what effects it has on postweaned 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. 151002).

Forty Holstein heifers with a mean age of 84 d and average BW of 100.88 kg were blocked by date of birth and randomly assigned to 1 of 4 treatments: (1) carrier (control; **CON**);



(2) 0.25 g SB/kg of BW + carrier (**0.25SB**); (3) 0.50 g SB/kg of BW + carrier (**0.50SB**); (4) (0.75 g SB/kg of BW + carrier (**0.75SB**)). All heifers were given 100 g of carrier (soybean meal) per day and 0.25SB, 0.50SB, and 0.75SB treatments were adjusted weekly according to individual body weights. Heifers entered the study on the first Tuesday of the 12 wk of life and remained on the study for approximately 15 wk. Heifers were individually fed a total mixed ration (**TMR**) with treatments top-dressed at 1030 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 ( $6.17 \times 4.88$  m,  $6.32 \times 4.8$  m) were utilized, each having the capacity to hold 8 heifers. Heifers had unlimited access to water through automatically refilling water troughs and no competition for stall space. A 1 wk training period was allotted in order to train heifers to use Calan feeding doors (American Calan Inc., Northwood, NH). Each heifer was assigned a specific door and assisted with opening the door until they were able to enter and exit on their own.

Heifers were fed the formulated TMR (Table 1 or Table 2) at 1030 h daily in individual feed tubs to allow for daily feed intake measurements. Feed was mixed and distributed using a motorized feeding vehicle (Data Ranger; American Calan Inc.). The ration was fed to obtain 5 to 10% feed refusals and the amount fed was adjusted daily according to individual intakes. Treatments were hand mixed into each heifer's feed.

### ***Feed Analysis***

The amount of feed offered and refused was measured daily at 1100 h to determine dry matter intake (**DMI**). Samples of TMR and feed refusals from each heifer were obtained daily

and frozen at -20°C for future analysis. Frozen samples were thawed and placed in a forced hot-air convection oven to dry at 55°C for 48 h to determine DMI (Binder, Bohemia, NY). Ort samples were composited weekly by heifer, and TMR samples were composited monthly. Samples were ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Samples were sent to a commercial laboratory for nutrient analysis (Dairy One Forage Laboratory, Ithaca, NY). 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  $\alpha$ -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***

Heifers were weighed and skeletal measurements were taken before starting treatments, and every Tuesday at 830 h throughout the 14wk on study. Heifers were measured for hip height, withers height, heart girth, and body length. Hip and withers heights were measured using a sliding-scale height stick with a bubble level. Heart girth and body lengths were measured using a weight tape. Heifers were weighed on a platform scale (Cardinal, Northeast Scale Co. Inc., Hooksett, NH).

Blood samples were obtained from the jugular vein using a 20-gauge needle prior to the administration of treatments. Once heifers were assigned to treatments blood samples were

collected every Tuesday at 830 h for the duration of the study. Samples were collected in 2 10-mL vacutainer tubes, 1 containing anticoagulant EDTA and 1 without anticoagulant (Monoject, Covidien Inc., Mansfield, MA). Concentrations of BHB were obtained utilizing a hand-held electronic blood glucose and ketone monitoring system (Nova Max Plus, Nova Biomedical, Waltham, MA; Deelen et al., 2016). Whole blood 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  $1,278 \times g$  at  $4^{\circ}\text{C}$  for 20 min (5430R, Eppendorf, Hamburg, Germany). Serum was stored in 2 aliquots at  $-20^{\circ}\text{C}$  until further analysis of plasma urea nitrogen (PUN). 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.

### ***Digestibility Measurements***

Chromium oxide (Sigma-Aldrich Corp., St. Louis, MO) was used as a marker to estimate apparent total-tract nutrient digestibility. The equation used to estimate digestibility was  $100 - [100 \times (\% \text{ chromium in feed} / \% \text{ chromium in feces}) \times (\% \text{ nutrient in feces} / \% \text{ nutrient in feeds})]$ . Each of the 40 heifers underwent the digestibility phase at 47 d on study until 54 d on study. Chromium oxide was dosed at 4 g/d and split into 2 gelatin capsules (Torpac Inc., Fairfield, NJ). Doses were administered each day at 0800 h and 2000 h as an oral bolus for 7 d. Total mixed ration samples were taken d 2 through d 5 and individualorts samples were collected d 3 through d 6. Orts and TMR samples were then composited over the sampling days. Fecal grab samples were collected for the last 4 d every 12 h to represent a 24-h period (d 4: 1100 and 2300 h; d 5: 0200 and 1400 h; d 6: 0500 and 1700 h; d 7: 0800 and 2000 h) by stimulating defecation or collecting directly from rectum. Samples over the 4 d period were combined to obtain a single

composite and frozen at  $-20^{\circ}\text{C}$ . Fecal samples were thawed at room temperature and emptied into aluminum trays to be dried in a forced-air oven at  $55^{\circ}\text{C}$  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 Analab (Fulton, IL) for analysis. Feed samples were analyzed for CP, ADF, NDF, oil, organic matter, and starch, and chromium concentrations (AOAC method 990.08; AOAC International, 1999) were determined from the fecal samples.

### ***Coccidia Count***

Fecal samples were obtained from each heifer prior to the start of treatment and biweekly thereafter. 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

Weekly DMI, ADG, feed efficiency (ADG/DMI), BW, skeletal measurements and blood metabolites were analyzed as a randomized complete block design with repeated measures using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) according to the following model:  $Y_{ijkl} = \mu + B_i + S_{Bj} + W_k + \beta X_{ij} + SBW_{jk} + E_{ijkl}$ , where  $Y_{ijkl}$  = the dependent variable;  $\mu$  = the overall mean;  $B_i$  = the random effect of block  $i$  ( $i = 1, \dots, 10$ );  $S_{Bj}$  = the fixed effect of the  $j$ th SB amount ( $j = 0, 0.25, 0.50, 0.75$  mg/kg BW);  $W_k$  = the fixed effect of the  $k$ th week on study ( $k = 1-14$ );  $\beta$  = the regression (covariate coefficient);  $X_{ij}$  = the covariate measurement;  $SBW_{jk}$  = the fixed interaction between the  $j$ th SB amount and the  $k$ th week; and  $E_{ijkl}$  = the residual error  $\sim N(0, \sigma^2_e)$ . 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 from repeated measurements in the experimental units (heifers). All variables, except average hip height, BHB gain, body length gain, and average coccidia count were modeled using a first-order autoregressive covariance spatial structure. First-order autoregressive resulted in the smallest Bayesian information criteria of the 4 covariate structures tested: compound symmetry, unstructured, Toeplitz, and first-order autoregressive. Average hip height and BHB gain were modeled using Toeplitz 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. Body length gain was modeled using compound symmetry covariance spatial structure as it resulted in the smallest Bayesian information criterion. Covariate *P*-values for BHB and body length were > 0.25; therefore they were removed from the model. Average coccidia count was model using unstructured covariance spatial structure as it resulted in the smallest Bayesian information criterion. Single degree of freedom contrasts for linear, quadratic, and cubic effects were determined for all variables.

Final hip and withers heights, heart girth, body length, and blood metabolites were analyzed as a randomized complete block design using the MIXED procedure of SAS 9.4 (SAS Institute Inc.) according to the following model:  $Y_{ij} = \mu + B_i + SB_j + \beta X_{ij} + e_{ij}$ , where  $Y_{ij}$  = the dependent variable;  $\mu$  = the overall mean;  $B_i$  = the random effect of block  $i$  ( $i = 1, \dots, 10$ );  $SB_j$  = the fixed effect of the  $j$ th SB amount ( $j = 0, 0.25, 0.50, 0.75$  mg/kg BW);  $\beta$  = the regression (covariate coefficient);  $X_{ij}$  = the covariate measurement; and  $e_{ij}$  = the residual error  $\sim N(0, \sigma^2 e)$ . In this model, the random effect of heifer within block subclass was used as the error term for the effect of treatment. Degrees of freedom were calculated using the Kenward-Roger approximation

option of the MIXED procedure. Single degree of freedom contrasts for linear, quadratic, and cubic 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., Cary, NC) according to the following model:  $Y_{ij} = \mu + B_i + SB_j + e_{ij}$ , where  $Y_{ij}$  = the dependent variable;  $\mu$  = the overall mean;  $B_i$  = the random effect of block  $i$  ( $i = 1, \dots, 10$ );  $SB_j$  = the fixed effect of the  $j$ th SB amount ( $j = 0, 0.25, 0.50, 0.75$  mg/kg BW);  $X_{ij}$  = the covariate measurement; and  $e_{ij}$  = the residual error  $\sim N(0, \sigma^2_e)$ . Degrees of freedom were calculated using the Kenward-Roger approximation option of the MIXED procedure. Single degree of freedom contrasts for linear, quadratic, and cubic effects were determined.

Initial BW, skeletal measurements, serum glucose, PUN, BHB, and coccidia oocyst counts served as covariates. Week was used as a repeated measure for all variables except for digestibility data. For all variables, significant treatment and interaction effects were noted at  $P \leq 0.05$  and trends were noted at  $0.05 < P \leq 0.15$ . Any data points with values greater or lesser than 2.5 standard deviations away from the mean were removed from the data set.

## RESULTS

The nutrient analysis of the TMR is presented in Table 3. Nutrient analysis of refusals by treatment are shown in Table 4. Dry matter intake, feed efficiency, ADG, BW, and skeletal measurements are presented in Table 6.

Two heifers from treatment 0.50SB were given 4 cc/d of Penicillin from 12/30 to 1/2 to treat fevers (body temperature  $> 39.17^\circ\text{C}$ ). One heifer in the control group was treated with 4

cc/d of Penicillin from 1/2 to 1/5 and 3 cc/d of Penicillin from 2/6 to 2/9 for treatment of fever. A heifer from treatment 0.50SB was treated for coccidiosis with 45.36 g/d of Corid® (Hubbard Feeds) from 11/30 to 12/5. Another control heifer was treated with 45.36 g/d of Corid® to treat coccidiosis on 2 occasions: 11/16 to 11/22 and 11/30 to 12/5. Because the nutrient digestibility period was set on the dates of coccidiosis treatment, the digestibility period was moved to the following week for treated heifers.

Heifers average initial body weights were  $100.88 \pm 11.17$  kg (mean  $\pm$  SD) upon entering the study. Dry matter intake was as expected for heifers of this age. Average daily gain tended to increase linearly as heifers were fed higher levels of SB ( $P = 0.12$ ). Average BW over the duration of the study increased as SB levels increased ( $P = 0.04$ ) with average weights of 164.37, 160.38, 159.0, and 157.50 kg (LSM  $\pm$  SEM), for treatments 0.75SB, 0.50SB, 0.25SB, and CON respectively. Average daily gains were 1.16, 1.15, 1.17, and 1.24 kg/d for CON, 0.25SB, 0.50SB, and 0.75SB, respectively and tended to increase linearly ( $P = 0.12$ ) as SB levels increased. Final body weights were 222.53, 215.51, 213.84, and 214.15 kg for treatments 0.75SB, 0.50SB, 0.25SB, and CON respectively and tended to increase linearly ( $P = 0.07$ ) as SB levels increased.

Overall gains are presented in Table 7. Overall body weight gains increased linearly ( $P = 0.02$ ) as SB treatment levels increased. Body weight gains were 111.47, 111.43, 118.10, and 121.50 kg for CON, 0.25SB, 0.50SB, and 0.75SB, respectively. Overall gains for hip height, withers height, heart girth, and body length gains were all similar. Average and final hip height, withers height, heart girth, and body length had no differences between treatments.

Fecal samples indicated the presence of coccidia, but the counts were low and varied greatly among calves in each treatment (Table 8). One heifer in treatment group 0.25SB received

a slightly different concentration of chromium oxide, but all samples were treated the same way during analysis. Average counts for CON, 0.25SB, 0.50SB, and 0.75SB were 573.48, 359, 411.96, and 536.98 coccidian oocysts per 150 g of feces. There was a significant quadratic effect ( $P = 0.03$ ) which indicates that SB fed at the level of 0.25 g/kg of BW was most effective in reducing the prevalence of *Coccidia*. Heifers in the final blocks of the study had numerically higher coccidian counts with a significant block effect ( $P = 0.0001$ ).

Plasma concentrations of PUN and BHB are presented in Table 8. Concentrations of average and final PUN were similar among treatments. Initial concentrations of BHB did not differ for treatments. Average BHB concentrations increased linearly ( $P = 0.009$ ) as SB levels increased with values of 0.54, 0.57, 0.60, and 0.61 mmol/L. Final BHB concentrations also increased linearly ( $P = 0.014$ ) as SB levels increased, averaging 0.49, 0.56, 0.51, 0.68 mmol/L.

Data collected during the digestibility measurement period are shown in Table 9. Chemical analysis of TMR for digestibility period is presented in Table 5. Starch, NDF, CP, and fat digestibility percentages were similar for all treatments. Dry matter digestibility tended to increase linearly with addition of higher levels of SB ( $P = 0.14$ ) with values of 62.74, 58.24, 65.65, and 67.22 for CON, 0.25SB, 0.50SB, and 0.75SB, respectively. Digestibility percentages of ADF for CON, 0.25SB, 0.50SB, and 0.75SB tended to increase linearly ( $P = 0.08$ ) were 35.94, 32.97, 44.89, and 46.31, respectively. Organic matter digestibility percentages tended to decrease linearly ( $P = 0.07$ ) as SB levels increased with values of 68.20, 69.45, 60.73, and 61.71 for CON, 0.25SB, 0.50SB, and 0.75SB, respectively.

## DISCUSSION



Our results are consistent with findings from previous studies that calves supplemented with SB in MR have improved feed efficiency and weight gain when compared to calves that are not fed SB. Guilloteau et al. (2009) observed that starter grain intake was not affected by SB supplementation. They also found that SB increased the length of the duodenum and villi, which improves absorptive capacity of the small intestine. Similarly, we found that DMI did not differ among treatments. Average daily body weight gain tended to increase as SB levels increased, from CON to the highest level of SB (0.75 g/kg BW) average daily body weight gain increased 6.9% ( $P = 0.12$ ). These responses may be direct effects of increased intestinal villi length, allowing the heifers to absorb more nutrients in the lower gut that might otherwise be expelled. The increased average daily body weight gain may also be a result of increased rumen papillae growth and concentration. Using ADG and DMI, we calculated feed efficiency for the heifers in all treatments. There was a linear trend ( $P = 0.08$ ) for increased feed efficiency as SB levels increased. Feed efficiency for heifers fed the highest level of SB increased 16.67% in comparison to heifers fed no SB. We also observed that as SB levels increased final body weights also increased, which relates to improved growth performance.

Overall, there was a significant linear response of increased treatment level on improved average BW and overall BW gains. We observed that when compared with heifers fed no SB, calves fed the highest levels of SB had 4.4% greater average BW and 3.9% greater final BW. Górká et al. (2011a) observed that calves that were supplemented with SB had higher average daily gains ( $P < 0.09$ ) and greater body weights ( $P < 0.10$ ) during the first weeks of treatment than calves without SB supplementation. They also observed that reticulorumen weight and papillae length and width were higher in calves fed SB. This correlates with an enhanced absorptive ability in the rumen due to improved papillae morphology and surface area. In our

results we witnessed a possible linear trend in improved digestibility percentages of ADF and ash as treatment levels of SB were increased, which may be direct results of increased papillae growth and absorptive capacity.

Heifers fed the highest level of SB (0.75 g/kg BW) had a 37.47% increase of ash digestibility when compared to heifers not fed SB. Dry matter digestibility for heifers fed the highest level of SB increased 7.14% from that of heifers fed no SB. Digestibility of ADF for heifers fed the highest level of SB (0.75 g/kg BW) had a 28.85% increase in digestibility in comparison to control heifers. The increased digestibility of DM, ADF, and organic matter support the work of Guilloteau et al. (2010a) which found that orally administered SB improved digestibility of fat, ash, and calcium in comparison to calves without SB supplementation. The study also detected an increase in pancreatic juice secretion ( $P < 0.10$ ) and a 52 and 40% increase in production of chymotrypsin and lipase, respectively. Pancreatic juices are important in aiding the small intestine with the breakdown of food, increasing the amount available will stimulate more efficient breakdown.

Besides BW, supplementing SB did not have an effect on improving skeletal measurements. Hip height, withers height, heart girth, and body length measurements were not different across treatments. Sodium monensin, sodium butyrate, and sodium propionate were supplemented to calves during pre and postweaning periods by Ferreira and Bittar (2010). Monensin is a commonly used ionophore, effective in improving feed efficiency and eventually growth rates of animals. Animals on all treatments had similar growth performance, there were no differences in withers height, heart girth, or hip width. Sodium butyrate calves had decreased starter intake during one period of the study but skeletal and BW gains remained similar among all treatments. This suggests that although the calves had lower intakes they still continued to

grow at the same rates as calves fed monensin or sodium propionate. Although no significant growth was detected, these results indicate that SB may be used as a replacement for monensin.

In this study, there was a significant quadratic effect ( $P = 0.03$ ) of SB on reducing the prevalence of coccidian oocysts in the feces. This response suggests that SB supplemented at the level of 0.25 g/kg of BW was the most effective treatment in reducing coccidian count. Górká et al., (2011a) observed that calves supplemented with SB in SM had less days experiencing scours than calves without SB in SM ( $P = 0.03$ ). Calves with SB in SM also tended to have less days being treated with electrolytes ( $P = 0.09$ ) in comparison to calves without SB in SM. A second study by Górká et al., (2011b) determined that calves fed SB in MR had increased mitotic ( $P = 0.01$ ) when compared to MR without SB at 4.8 vs. 3.0% respectively. Apoptotic index was decreased with SB addition to MR ( $P < 0.01$ ) when compared to MR with no supplementation, 4.0 and 7.4% respectively. These results support the findings that calves fed sodium butyrate had less incidence of scours. Elevated mitotic indices of the intestinal epithelial cells indicate an increased rate of cell division. This elevated cell growth improves the capability of the intestines to repair the damage inflicted by scours. Heifers in the final blocks of the study had numerically higher coccidian counts with a significant block effect ( $P = 0.0001$ ). As the study progressed, the number of heifers that had been housed in the pens increased which may have led to higher concentrations of oocysts in the environment. Therefore, heifers in the final blocks that were exposed to this may have been at a greater risk of coccidiosis, which explains the higher coccidian counts.

In this study, there was no effect of sodium butyrate on PUN concentrations in heifers as also observed by Krehbiel et al. (1992). Increasing levels of ruminal butyrate in Holstein steers

did not affect concentrations of PUN. When ruminal butyrate is found in high concentrations, it inhibits the uptake of propionate by the liver which decreases glucose production.

The results from this study indicate that SB can stimulate improved body weight gain and increase feed efficiency. Dry matter intakes were not different for treatments but there was a calculated trend for increased feed efficiency as SB levels were fed in higher amounts. As SB levels increased, average daily gains, average body weights, and final body weights of heifers also increased. Increasing the levels of SB fed tended to increase digestibility of DM, ADF, and organic matter which are relative to increased absorptive capacity of the gastrointestinal tract. Butyrate is the main VFA, utilized by the rumen to increase growth and concentration of rumen papillae. Increasing the available surface area for nutrient absorption will allow the ruminant to more effectively absorb and utilize nutrients obtained from fermentation. This energy can be used to optimize body weight and skeletal growth gains. Improving growth performance can ensure heifers reach breeding age younger and therefore can enter the milking herd sooner. Therefore, more research should be conducted to investigate the usefulness of sodium butyrate on improving feed efficiency and growth performance and decreasing the prevalence of scours and coccidiosis outbreaks.

## LIST OF REFERENCES

- Abdelqader, A. and A. Al—Fataftah. 2015. Effect of dietary butyric acid on performance, intestinal morphology, microflora composition and intestinal recovery of heat-stressed broilers. *Livestock Sci.* 183:78-83.
- Anderson, K.L., T.G. Nagaraja, and J.L. Morrill. 1987. Ruminant metabolic development in calves weaning conventionally or early. *J. Dairy Sci.* 70:1000-1005.
- AOAC International. 1998. *Official Methods of Analysis*. 16th ed. AOAC International, Arlington, VA.
- AOAC International. 1999. *Official Methods of Analysis*. 16th ed. AOAC International, Gaithersburg, MD
- AOAC International. 2006. *Official Methods of Analyses*, 18th ed. AOAC International, Gaithersburg, MD.
- Arthington, J.D., S.D. Eicher, W.E. Kunkle, and F.G. Martin. 1987. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. *J. Anim. Sci.* 81:1120-1125.
- Ash, R. and G.D. Baird. 1973. Activation of volatile fatty acids in bovine liver and rumen epithelium. *Biochem. J.* 136:311-319.
- Bach, A., A. Gimenez, J.L. Juaristi, and J. Ahedo. 2007. Effects of physical form of a starter for dairy replacement calves on feed intake and performance. *J. Dairy Sci.* 90:3028-3033.
- Baile, C.A., C.L. McLaughlin, and W.V. Chalupa. 1981. Effects of monensin fed to replacement dairy heifers during the growing and gestation period upon growth, reproduction, and subsequent lactation. *J. Dairy Sci.* 65:1941-1944.
- Baldwin, R.L. and M.J. Allison. 1983. Rumen metabolism. *J. Anim. Sci.* 57:461-477.
- Beever, D.E.. 1993. Rumen Function. Ch 9: pg 186-215 in *Quantitative Aspects of Ruminant Digestion and Metabolism*. J.M. Forbes and J. France, ed. CAB International, Wallingford, United Kingdom.
- Beharka, A.A., T.G. Nagaraja, J.L. Morrill, G.A. Kennedy, and R.D. Klemm. 1998. Effects of form of the diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. *J. Dairy Sci.* 81:1946-1955.
- Bergen, W.G. and D.B. Bates. 1984. Ionophores: their effect on production efficiency and mode of action. *J. Anim. Sci.* 58:1465-1483.
- Bergman, E.N. and J.E. Wolff. 1971. Metabolism of volatile fatty acids by liver and portal-drained viscera in sheep. *Am. J. Phys.* 221: 586-592.
- Bergman, E.N., W.E. Roe, and K. Kon. 1965. Quantitative aspects of propionate metabolism and gluconeogenesis in sheep. *Am. J. Phys.* 211:793-799.

- Bergman, E.N.. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev.* 70:567–590.
- Bornstein, B.T. and H.A. Barker. 1947. The nutrition of clostridium kilyuyveri. *J. Bacteriol.* 55:223-230.
- Brownlee, A., 1956. The development of rumen papillae in cattle fed on different diets. *British Vet. J.* 112:373-375.
- Bryant, M.P. and N. Small. 1955. The anaerobic monotrichous butyric acid-producing curved rod-shaped bacteria of the rumen. *J. Bacteriol.* 72:16-21.
- Bull, L.S., L.J. Bush, J.D. Friend, B. Harris, Jr., and E.W. Jones. 1965. Incidence of ruminal parakeratosis in calves fed different rations and its relation to volatile fatty acid absorption. *J. Dairy Sci.* 48:1459-1466.
- Callaway, T.R., T.S. Edrington, J.L. Rychlik, K.J. Genovese, T.L. Poole, Y.S. Jung, K.M. Bischoff, R.C. Anderson, and D.J. Nisbet. 2003. Ionophores: their use as ruminant growth promotants and impact on food safety. *Curr. Issues Intest. Microbiol.* 4:43-51.
- Chapman, D.E., T.M. Hill, D.R. Elder, and P.S. Erickson. 2017. Nitrogen utilization, preweaning nutrient digestibility, and growth effects of Holstein dairy calves fed 2 amounts of a moderately high protein or conventional milk replacer. *J. Dairy Sci.* 100:279-292.
- Counotte, G.H.M., R. A. Prins, R.H.A.M. Janssen, and M.J.A. DeBie. 1981. Role of *Megasphaera elsdenii* in the fermentation of DL [2-13C] lactate in the rumen of dairy cattle. *Appl. Environ. Microbiol.* 42:649–655.
- Cowles, K.E., R.A. White, N.L. Whitehouse, and P.S. Erickson. 2006. Growth characteristics of calves fed on intensified milk replacer regimen with additional lactoferrin. *J. Dairy Sci.* 89:4835-4845.
- Davis Rincker, L.E., M.J. VandeHaar, C.A. Wolf, J.S. Liesman, L.T. Chapin, and M.S. Weber Neilsen. 2011. Effect of intensified feeding of heifer calves on growth, pubertal age, calving age, milk yield, and economics. *J. Dairy Sci.* 94:3554-3567.
- Davis, C. L., and J. K. Drackley. 1998. *The Development, Nutrition, and Management of the Young Calf.* Iowa State University Press, Ames
- Demigne, C., C. Yacoub, C. Remesy, and P. Fafournoux. 1986. Propionate and butyrate metabolism in rat or sheep hepatocytes. *J. Biochem and Biophys.* 875:535-542.
- Dobson, J., W.C.B. Brown, A. Dobson, and A.T. Phillipson. 1956. A histological study of the organization of the rumen epithelium of sheep. *J. Exp. Phys.* 41:247-253.
- Eckert, E., H.E. Brown, K.E. Leslie, T.J. DeVries, and M.A. Steele. 2015. Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage. *J. Dairy Sci.* 98:6315-6326.
- Ellis, J.L., J. Dijkstra, A. Bannink, E. Kebreab, S.E. Hook, S Archibeque, and J. France. 2015. Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high grain-fed beef cattle. *J. Anim. Sci.* 90:2717-2726.

- Feed Additive Compendium. 2017. Miller Publishing Company, Minnetonka, MN.
- Ferreira, L.S. and C.M.M. Bittar. 2010. Performance and plasma metabolites of dairy calves fed starter containing sodium butyrate, calcium propionate, or sodium monensin. *Animal*. 5:239-245.
- Gilchrist, F.M.C. and H.M. Schwartz. 1972. Microbiology of the rumen in relation to the nutrition and physiology of the animal. *S. Afr. J. Anim. Sci.* 2:161-167.
- Górka, P., Z.M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, J.J. Holst, P. Guilloteau, and R. Zabielski. 2011a. Effect of Method of Delivery of Sodium Butyrate on Rumen Development in Newborn Calves. *J. Dairy Sci.* 94:5578-5588.
- Górka, P., Z.M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, and R. Zabielski. 2011b. Is rumen development in newborn calves affected by different liquid feeds and small intestine development? *J. Dairy Sci.* 94:3002-3013.
- Górka, P., P. Pietrzak, A. Kotunia, R. Zabielski, and Z.M. Kowalski. 2014. Effect of method of delivery of sodium butyrate on maturation of the small intestine in newborn calves. *J. Dairy Sci.* 97:1026-1035.
- Greenwood, R.H., J.L. Morrill, and E.C. Titgemeyer. 1997. Using dry feed intake as a percentage of initial body weight as a weaning criterion. *J. Dairy Sci.* 80:2542-2546.
- Guilloteau P., R. Zabielski, J.C. David. 2009. Sodium butyrate as a growth promoter in milk replacer formula for young calves. *J. Dairy Sci.* 92:1038–1049.
- Guilloteau, P., G. Savary, Y. Jaguelin-Peyrault, V. Romw, L. Le Normand., and R. Zabielski. 2010a. Dietary sodium butyrate supplementation increases digestibility and pancreatic secretion in young milk-fed calves. *J. Dairy Sci.* 93:5842-5850.
- Guilloteau, P., L. Martin, V. Eeckhaut, R. Ducatelle, R. Zabielski, and F. Van Immerseel. 2010b. From the gut to peripheral tissues: the multiple effects of butyrate. *Nutr. Res. Rev.* 23:366-384.
- Hegland, R.B., M.R. Lambert, N.L. Jacobson, and L.C Payne. 1957. Effect of dietary and managemental factors on reflex closure of the esophageal groove in the dairy calf. *J. Dairy Sci.* 40:1107-1113.
- Heinrichs, J. (2001). "Photos of Rumen Development." Retrieved 8/16/2017, 2017, from [http://extension.psu.edu/animals/dairy/nutrition/calves/rumen/photos-of-rumen-development/rumen-and-reticulum-of-calves-at-6-weeks/image\\_galleryzoom](http://extension.psu.edu/animals/dairy/nutrition/calves/rumen/photos-of-rumen-development/rumen-and-reticulum-of-calves-at-6-weeks/image_galleryzoom).
- Heinrichs, A.J., C.M. Jones, S.M. Gray, P.A. Heinrichs, S.A. Corenelisse, and R.C Goodling. 2013. Identifying efficient dairy heifer producers using production costs and data envelopment analysis. *J. Dairy Sci.* 96:7355-7362.
- Heinrichs, A.J.. 1993. Raising dairy replacements to meet the needs of the 21<sup>st</sup> century. *J. Dairy Sci.* 76:3179-3187.
- Herrick, K.J., A.R. Hippen, K.F. Kalsheur, D.J. Schingoethe, D.P. Casper, S.C. Moreland, and J.E. van Eys. 2017. Single-dose infusion of sodium butyrate, but not lactose, increases plasma  $\beta$ -hydroxybutyrate and insulin in lactating dairy cows. *J. Dairy Sci.* 100:757-768.

- Hino, T., K. Miyazaki, and S. Kuroda. 1991. Role of extracellular acetate in the fermentation of glucose by ruminal bacterium *Megasphaera elsdenii*. *J. Gen. Appl. Microbiol.* 37:121-129.
- Hopkins, B.A.. Effects of the method of calf starter delivery and effects of weaning age on starter intake and growth of Holstein calves fed milk once daily. *J. Dairy Sci.* 80:2200-2203.
- Huber, J. T., A. G. Silva, O. F. Campos, and C. M. Mathieu. 1984. Influence of feeding different amounts of milk on performance, health, and absorption capability of baby calves. *J. Dairy Sci.* 67:2957–2963
- Huhtanen, P., H. Miettinen, and M. Ylinen. 1993. Effect of increasing ruminal butyrate on milk yield and blood constituents in dairy cows fed a grass silage-based diet. *J. Dairy Sci.* 76:1114-1124.
- Jasper, J., and D. M. Weary. 2002. Effects of ad libitum milk intake on dairy calves. *J. Dairy Sci.* 85:3054–3058.
- Jaster, E.H., G.C. McCoy, and R.L. Fernando. 1989. Dietary fat in milk or milk replacers for dairy calves raised in hutches during the winter. *J. Dairy Sci.* 73:1843-1850.
- Kleiber, M., A.L. Black, M.A. Brown, J. Luick, C.F. Baxter, and B.M. Tolbert. 1954. Butyrate as a precursor of milk constituents in the intact dairy cow. *J. Biol. Chem.* 210:239-247.
- Krehbiel, C.R., D.L. Harmon, and J.E. Schnieder. 1992. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. *J. Animal Sci.* 70:904-914.
- McGuffey, R.K., L.F. Richardson, and J.I.D Wilkinson. 2001. Ionophores for dairy cattle: current status and future outlook. *J. Dairy Sci.* 84:E194-E203.
- Miller, T.L. and S.E. Jenesel. 1978. Enzymology of butyrate formation by *Butyrivibrio fibrisolvens*. *J. Bact.* 138:99-104.
- Moolchand, M., J. Wang, H. Gui., and Z. Shen. 2013. Ruminal butyrate infusion increased papillae size and digesta weight but did not change liquid flow rate in the rumen of goats. *J. Animal and Plant Sci.* 23:1516-1521.
- NAHMS (National Animal Health Monitoring System). 2014. Dairy Heifer Raiser, 2011. Accessed June 20 2017. [https://www.aphis.usda.gov/animal\\_health/nahms/dairy/downloads/dairyheifer11/HeiferRaiser.pdf](https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairyheifer11/HeiferRaiser.pdf)
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, D.C.
- Okamoto, T., M. Sasaki., T. Tsujikawa., Y. Fujiyama., T. Bamba, and M. Kusunoki. 2000. Preventative efficacy of butyrate enemas and oral administration of *Clostridium butyricum* M588 in dextran sodium sulfate-induced colitis in rats. *J. of Gastro.* 35:341-346.
- Orskov, E.R.. 1972. Reflex closure of the oesophageal groove and its potential application in ruminant nutrition. *S. Afr. J. Anim. Sci.* 2:169-172.



- Otterby, D.E. and J.W. Rust. 1965. Effects of age and diet on rumen and blood components of the young calf. *J. Dairy Sci.* 48:1716-1718.
- Pennington, R.J. 1951. Metabolism of short-chain fatty acids in the sheep. *Biochem. J.* 51:251-258.
- Quigley, J.D. III, J.J. Drewry, L.M. Murray, and S.J. Ivey. 1997. Effects of lasalocid in milk replacer or calf starter on health and performance of calves challenged with *Eimeria* species. *J. Dairy Sci.* 80:2972-2976.
- Russell, J.B. and H.J. Strobel. 1987. Effects of additives on in vitro ruminal fermentation: a comparison of monensin and bacitracin, another Gram-positive antibiotic. *J. Anim. Sci.* 66:552-558.
- Russell, J.B. and H.J. Strobel. 1989. Minireview: effect of ionophores on ruminal fermentation. *J. Appl. and Enviro. Microb.* 55:1-6.
- Sakata, T. and H. Tamate. 1978. Rumen epithelium cell proliferation accelerated by propionate and acetate. *J. Dairy Sci.* 62:49-52.
- Sander, E.G., R.G. Warner, II. N. Harrison, and J.K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of the rumen mucosa in the young calf. *J. Dairy Sci.* 42:1600-1605.
- Sano, H., S. Tano, H. Takahashi, and Y. Terashima. 1995. Dose response of plasma insulin and glucagon to intravenous n-butyrate infusion in sheep. *J. Dairy Sci.* 73:3038-3043.
- Stadtman, E.R. and H.A. Barker. 1949. Fatty acid synthesis by enzyme preparations of *Clostridium kluyveri* I. preparation of cell-free extracts that catalyze the conversion of ethanol and acetate to butyrate and caproate. *J. Biol. Chem.* 180:1085-1093.
- Stamey, J.A., N.A. Janovick, A.F. Kertz, and J.K. Drackley. 2012. Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program. *J. Dairy Sci.* 95:3327-3336.
- Sutton, J.D., A.D. McGilliard, M. Richard, and N.L. Jacobson. 1963. Functional development of rumen mucosa. II. Metabolic activity. *J. Dairy Sci.* 46:530-537.
- Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of Various Dietaries on the Anatomical Development of the Stomach in the Calf. *J. Dairy Sci.* 45:408-420.
- U.S. Census Bureau. 2017. Census Bureau Home Page. 2017. [www.census.gov/ipc/www/idb/country.php](http://www.census.gov/ipc/www/idb/country.php).
- USDA. 2007. Dairy 2007. Part I: Reference of Dairy Cattle and Management Practices in the United States, 2007. [https://www.aphis.usda.gov/animal\\_health/nahms/dairy/downloads/dairy07/Dairy07\\_dr\\_PartI.pdf](https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_dr_PartI.pdf).
- USDA. 2017. Long-term Projections. August 2017. <https://www.usda.gov/oce/commodity/projections/>.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.

Weiss, W.P. and B.A. Amiet. 1989. Effect of lasalocid on performance of lactating dairy cows. *J. Dairy Sci.* 73:153-162.

Yarlett, N., D. Lloyd, and A.G. Williams. 1985. Butyrate formation from glucose by the rumen protozoon *Dasytricha ruminantium*. *Biochem. J.* 228:187-192.

Table 1. *Ingredient composition of experimental diet (range of use Feb-Aug)*

Item	DM (%)
Corn Silage	39.98 ± 0.51
Hay Crop Silage	27.50 ± 4.50
Soy/Urea Mix <sup>1</sup>	10.93 ± 0.71
Energy Mix <sup>2</sup>	17.29 ± 4.57
Provail <sup>3</sup>	2.30 ± 0.03
PGI Heifer Balancer	2.0 ± 0.03

<sup>1</sup>Soy/Urea Mix contains 7.28% distillers grain, 69.14% soy bean meal, 21.83% canola meal, and 1.75% urea

<sup>2</sup>Energy Mix contains 5% molasses, 45.80% corn meal, 15.20% steam flaked corn, and 34% whole beet pulp

<sup>3</sup>Provail is a rumen undegradable protein (**RUP**) mix that contains blood meal and methionine at 3.9% CP

Table 2. Ingredient composition of experimental diet (range of use Aug-May)

Item	DM (%)
Corn Silage	40.89 ± 0.26
Grass Silage	32.34 ± 2.04
Soy/Urea Mix	13.15 ± 2.09
Energy Mix	9.44 ± 0.02
Blood Meal <sup>1</sup>	2.34 ± 0.005
PGI Heifer Balancer	1.93 ± 0.11

<sup>1</sup>Blood Meal was supplemented with 2.43 g of Smartamine<sup>®</sup>M that contained 2.38% methionine

Table 3. Nutrient analysis of experimental diet

Item	DM (%)
CP	15.83 ± 0.83
ADF	25.84 ± 2.63
NDF	40.82 ± 3.61
Starch	21.6 ± 3.65
NFC <sup>1</sup>	31.95 ± 4.20
Fat	3.92 ± 0.68
Ash	7.49 ± 0.60

<sup>1</sup>NFC = 100 - [CP% + (NDF% - NDICP%) + fat% + ash%].

Table 4. Nutrient analysis of refusals by treatment

Item (DM%)	Treatment <sup>1</sup>			
	CON	0.25SB	0.50SB	0.75SB
CP	14.98 ± 1.68	14.93 ± 1.54	15.02 ± 1.46	15.19 ± 1.20
ADF	26.12 ± 3.07	26.77 ± 2.47	26.10 ± 2.83	25.93 ± 2.70
NDF	41.58 ± 5.00	42.54 ± 3.60	40.9 ± 5.03	40.65 ± 3.63
Starch	20.43 ± 4.21	19.58 ± 2.90	20.9 ± 4.26	20.61 ± 3.32
NFC	32.26 ± 5.3	31.37 ± 3.05	32.49 ± 5.65	32.33 ± 3.47
Fat	3.98 ± 0.47	3.80 ± 0.36	3.79 ± 0.85	3.60 ± 0.414
Ash	7.22 ± 0.47	7.38 ± 0.46	7.82 ± 0.87	8.23 ± 0.98

<sup>1</sup>Treatment CON = 0 sodium butyrate; 0.25SB = 0.25 g/kg BW of sodium butyrate; 0.50SB = 0.50 g/kg BW of sodium butyrate; 0.75SB = 0.75 g/kg BW of sodium butyrate

Table 5. Nutrient analysis of diet fed during digestibility period

Item	DM%
CP	14.71 ± 1.30
ADF	21.04 ± 2.63
NDF	34.60 ± 4.18
Starch	2.54 ± 3.25
Ash	6.76 ± 0.62
Fat	20.55 ± 0.40

Table 6. Intake and performance of heifers during the 14 wk trial

Item	Treatment				SEM	<i>P</i> -value <sup>1</sup>			
	CON	0.25 SB	0.50 SB	0.75 SB		L	Q	C	Trt × Week
Initial BW, kg	96.87	97.46	108.66	100.54	3.56				
Average BW, kg	157.50	159	160.38	164.37	2.37	0.04	0.58	0.23	0.68
ADG, kg/d	1.16	1.15	1.17	1.24	0.04	0.12	0.32	0.36	0.37
Final BW, kg	214.15	213.84	215.51	222.53	3.36	0.07	0.26	0.30	
DMI, kg/d	5.09	4.84	5.19	4.91	0.24	0.85	0.93	0.25	0.99
Feed efficiency, ADG/DMI	0.24	0.26	0.23	0.28	0.01	0.08	0.12	0.01	0.07
Hip height initial, cm	97.98	97.85	100.65	99.31	0.67				
Hip height, cm	109.12	109.73	109.14	109.50	0.53	0.81	0.80	0.33	0.71
Hip height gain, cm/d	0.19	0.21	0.19	0.20	0.01	1.00	0.64	0.22	0.46
Hip height final, cm	117.63	119.18	117.82	118.36	0.62	0.76	0.38	0.08	
Withers height initial, cm	93.92	94.23	95.25	95.89	0.76				
Withers height, cm	105.55	105.39	106.02	104.69	0.39	0.29	0.13	0.06	0.60
Withers height gain, cm/d	0.20	0.20	0.20	0.19	0.01	0.71	0.59	0.45	0.28
Withers height final, cm	114.17	114.01	114.74	113.65	0.55	0.74	0.39	0.25	
Heart girth initial, cm	107.32	108.20	112.14	110.49	1.14				
Heart girth, cm	124.46	124.39	124.67	125.61	0.74	0.28	0.47	0.53	0.01
Heart girth gain, cm/d	0.28	0.28	0.29	0.30	0.01	0.39	0.68	0.73	0.0031
Heart girth final, cm	137.35	137.28	138.14	139.03	0.98	0.20	0.61	0.65	
Body length initial, cm	91.57	91.95	95.63	93.47	0.99				
Body length, cm	107.88	107.64	107.92	107.25	0.67	0.58	0.73	0.46	0.68
Body length gain, cm/d	0.27	0.28	0.27	0.27	0.01	0.66	1.00	0.78	0.57
Body length final, cm	120.22	120.29	119.59	119.77	1.02	0.75	0.96	0.81	

<sup>1</sup>*P*-value significant if < 0.05; trend if < 0.15



Table 7. Overall body weight and skeletal measurement gains

Item	Treatment				SEM	<i>P</i> -value		
	CON	0.25 SB	0.50 SB	0.75 SB		Linear	Quadratic	Cubic
BW, kg	111.47	111.43	118.10	121.50	3.43	0.02	0.62	0.63
Hip, cm	18.80	20.36	18.67	19.37	0.55	0.99	0.44	0.05
Withers, cm	19.43	19.24	19.88	18.73	0.52	0.54	0.37	0.21
Heart girth, cm	28.32	28.05	28.00	29.27	0.92	0.50	0.41	0.59
Body length, cm	26.92	27.18	26.35	26.61	0.91	0.66	1.00	0.78

Table 8. Coccidia count and blood parameters

Item	Treatment				SEM	<i>P</i> -value		
	CON	0.25SB	0.50SB	0.75SB		L	Q	C
Coccidia/ 150 g of feces	573.48	359.00	411.96	536.98	76.46	0.87	0.03	0.56
Initial PUN <sup>1</sup> , mg/dL	28.18	27.75	27.02	27.75	2.56	0.86	0.82	0.95
PUN average, mg/dL	31.84	32.82	30.36	30.75	1.30	0.33	0.83	0.60
Final PUN, mg/dL	31.18	32.57	32.26	33.42	2.19	0.51	0.96	0.53
Initial BHB <sup>2</sup> , mmol/L	0.46	0.59	0.47	0.58	0.05	0.25	0.79	0.02
Average BHB, mmol/L	0.54	0.57	0.60	0.61	0.02	0.009	0.59	0.51
Final BHB, mmol/L	0.49	0.56	0.51	0.68	0.04	0.014	0.25	0.009

Table 9. Apparent total-tract nutrient digestibility

Item	Treatment				SEM	P-value		
	Control	0.25 SB	0.50 SB	0.75 SB		L	Q	C
DMI, kg/d	5.05	4.52	5.55	5.12				
Digestibility %								
DM	62.74	58.24	65.65	67.22	3.19	0.14	0.34	0.64
CP	55.24	50.97	57.61	60.03	4.07	0.21	0.38	0.81
ADF	35.94	32.97	44.89	46.31	5.37	0.08	0.69	0.88
NDF	43.19	46.64	51.54	50.77	5.13	0.22	0.68	0.79
Starch	98.83	98.14	98.52	98.63	0.30	0.86	0.19	0.33
Organic Matter	68.20	69.45	60.73	61.71	3.62	0.07	0.97	0.75
Fat	74.01	71.32	75.73	76.96	2.85	0.28	0.48	0.79