Feeding and Supplementation Strategies During Weaning in Dairy Calves

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Abstract

The objectives of this research were to test the effects of limiting forage in pre-weaned calves, and supplementing butyrate during weaning on the incidence of sub-acute ruminal acidosis. For study 1, mean ruminal pH had a tendency to be lower, and duration and severity of sub-acute ruminal acidosis were greater in calves limit-fed forage. In study 2, there were no differences in average daily gain, blood plasma glucose or β-hydroxybutyrate concentrations, or ruminal pH between pre-weaning dietary treatments. For post-weaning calves, calves fed a butyrate supplemented starter tended to have greater starter intake and average daily gain, but lower mean ruminal pH, leading to longer duration of sub–acute ruminal acidosis. Based on these data, limiting forage intake prior to weaning increases sub-acute ruminal acidosis while supplementing butyrate during the weaning transition increases feed intake, weight gain, and sub-acute ruminal acidosis immediately after weaning.

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Dedication

I would like to dedicate this thesis to my parents, Brian and Kathryn McCurdy, to the calves that made this research possible, and to everyone who has helped me achieve this milestone in my life.

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Introduction

Sub-acute ruminal acidosis is an issue within the dairy industry affecting most cows during the first 60 days of lactation (Steele et al., 2011; Plazier et al., 2009; Dionissopoulos et al., 2013). While this issue has been studied extensively in older cows, it has not been studied as closely in young calves where low ruminal pH can cause severe developmental retardation of ruminal papillae if left untreated (Steele et al., 2009; Van Soest, 1982a). Once detected, producers can either feed slower fermenting feeds such as long-stem forages, or ruminal buffers such as bicarbonate to young calves, which are similar to feeding strategies used in older cows to manage sub-acute ruminal acidosis (Van Soest, 1982 a; Plazier et al., 2009). These are the two common feeding strategies used in adult cows that are known to increase ruminal pH greater than 5.8, which is the threshold for sub-acute ruminal acidosis (Aschenbach et al., 2011; Van Soest, 1982a,b). In calves, feeding forage is a major controversy because of issues such as rumen fill where bulky forages leave less room for intake of calf starter. Calf starter is the major driver behind ruminal papillae growth and proliferation because of its fermentation products, which are mainly propionate and butyrate (Bergman, 1990; Van Soest, 1982a). Propionate and butyrate are responsible for development of the absorptive qualities of the ruminal epithelium making these volatile fatty acids of interest in calves to promote rumen development (Gorka et al., 2009; Govil et al., 2017; Guilloteau et al., 2010). When supplemented, propionate and butyrate cause papillae height and density to increase compared to non-supplemented calves by increasing the mitotic index of rumen epithelial cells, which increases cell proliferation (Baldwin et al., 2004; Sakata and Tamate, 1978; Dirksen et al., 1985). For the purpose of this thesis, only butyrate was supplemented in young calves therefore we will mainly discuss the effects of butyrate on the ruminal epithelium from here forward. Forages help maintain a constant ruminal pH, increase salivary output, and increase rumen musculature during the pre-weaning period, which is important for rumination in the post-weaning period (Khan et al., 2011a; Yang and Beauchemin, 2006). Research has been completed on the physical form of forage, and maximum inclusion rate of forages in the diet where inclusion between 5 to 10% optimize body weight gain as well as starter intake, but no research has been done on the minimal inclusion rate to alleviate the symptoms of sub-acute ruminal acidosis (Coverdale et al., 2004; Montoro et al., 2013; Laarman and Oba, 2011). Likewise, research has been completed on

inclusion of supplemental butyrate in milk and calf starter during the pre-weaning period at 0.3% (as-fed basis), but supplementation of butyrate in starter during weaning has not been explored (Gorka et al., 2009; Gorka et al., 2011; Guilloteau et al., 2009). Older production animals have increased productivity when supplemented with butyrate between 1 to 4% (as-fed basis) where 1% is midway between the recommended pre- and post-weaning supplementation percentages (Guilloteau et al., 2010). With the addition of butyrate during the weaning transition, starter intake and rumen pH could be positively affected for greater transition success during the post-weaning period. With these changes to feeding strategies in dairy calves, sub-acute ruminal acidosis may become less of an issue in developing ruminants.

Chapter 1

Literature Review

1.1 Liquid Feed

During the pre-weaning phase, calves are not true ruminants because they rely largely on the abomasum and input of milk for their nutrient intake (Khan et al., 2016; Drackley, 2008; Heinrichs et al., 1995). Whole milk is one of the most nutritionally beneficial sources of nourishment in the young calf diet containing on average 3.4% fat, 3.3% protein, 4.9% carbohydrate in the form of lactose, and about 87% water, which is a crucial for hydration especially in species that will not voluntarily consume water until one to two weeks after birth (USDA Nutrient Database, 2018; Khan et al., 2016; McGrath et al., 2016). Liquid feeds such as whole milk, milk replacer, or colostrum, can be consumed either via suckling from a teat or bottle, or via bucket feeding (Godden et al., 2009; Appleby et al., 2001; Kung et al., 1997). Suckling shunts milk directly to the omasum from the esophagus via closure of the esophageal groove, which bypasses the reticulorumen altogether in calves less than 16 weeks of age (Drackley, 2008; Abe et al., 1979). A bucket fed calf can consume up to 1.6 L of milk in approximately 40 seconds, which would take a bottle fed calf 2 to 6 minutes (Abe et al., 1979; Drackley, 2008). Rapid consumption of milk decreases the amount of time for curd formation in the abomasum, decreasing digestibility and absorption in the small intestine (Abe et al., 1979). However, when feeding by bucket, grain intake increases, which increases dry matter intake at weaning due to the increase in the rate of passage of milk (Appleby et al., 2001; Jasper et al., 2002). There are benefits to feeding using both methods, but bottle feeding increases health parameters compared to bucket feeding.

1.1.1 Colostrum

Colostrum is the first milk produced post-partum and has a different concentration of nutrients in comparison to whole milk whereby on average it contains 8.0% fat, 14.0% protein, 1.2% lactose, and 77% water (McGrath et al., 2016). The low levels of lactose in colostrum cause viscosity due to lactose being the main osmoregulator in milk (McGrath et al., 2016). As lactation progresses, the lactose content in milk increases slowly, which leads

to an increase in the water content until it reaches 4.9% commonly found in Holstein milk (McGrath et al., 2016; USDA Database, 2018).

In the dairy industry, newborn calves must receive a minimum of one colostrum feeding directly after birth and it is recommended that they receive two feedings because cattle do not transfer immunoglobulins or vitamins well in utero, which leaves a calf's immune system underdeveloped early in life (Desjardins-Morissette et al., 2018; Godden et al., 2009; Hammon and Blum, 1998). Colostrum has elevated concentrations of fat soluble vitamins, A, D, E, and K, which decrease rapidly over the course of 5 to 6 milkings. However, these vitamins are crucial for calf survival because there is minimal transfer from the dam in utero where most transfer occurs during late gestation (Eaton et al., 1947; McDowell et al., 1996; Kume and Toharmat, 2001). When the dam is supplemented with fat soluble vitamins pre-partum, colostrum concentration of vitamins increases, which is directly correlated with improved immune function in calves fed colostrum from these dams (Eaton et al., 1947; McDowell et al., 1996; Kume and Toharmat, 2001). This increase is also observed in immunoglobulin concentrations when dams are vaccinated against pathogens such as rotavirus prior to calving to encourage antibody production for immunoglobulin G, A, and M (Staack, 1992; McGrath et al., 2016). Immunoglobulin G, A, and M comprise about 70% to 80% of the total protein in colostrum, but only 1.0% in whole milk so it is essential to feed colostrum from dams prior to their 5^{th} or 6^{th} milking (McGrath et al., 2016; Buhler et al., 1998; Godden et al., 2009).

The initial feeding is ideally given via teat or bottle feeding as opposed to esophageal tubing to increase the chance of absorption of immunoglobulins and fat soluble vitamins with the least amount of colostrum input (Buhler et al., 1998; Desjardins-Morissette et al., 2018; Godden et al., 2009). When suckled from a bottle, passive immunity increases due to greater absorption of the colostrum in the small intestine because absorption capacity decreases by up to 50% in the first 12 hours of life (Buhler et al., 1998; Godden et al., 2009; Hammon and Blum, 1998).

When feeding limited amounts of quality colostrum, bottle feeding becomes crucial for adequate immunoglobulin absorption. Feeding from a bottle also requires less colostrum, 1.5L, to achieve maximum immunoglobulin absorption where feeding from esophageal tubing

doubles the required amount (Godden et al., 2005, 2009; Desjardins-Morissette et al., 2018). A lack of colostrum feeding decreases villi development in the intestinal mucosa because colostrum contains insulin-like growth factor 1, which positively affects growth of intestinal villi (Buhler et al., 1998). When calves do not receive enough colostrum, insulin-like growth factor 1 quantity is also decreased, which can retard absorption efficiency in pre-weaned calves (Buhler et al., 1998). Absorption efficiency in the intestine is needed for optimal uptake of nutrients from milk.

1.1.2 Whole Milk and Milk Replacer

Pre-weaned calves are fed either whole milk or milk replacer. In the United States, Western producers tend to feed more milk replacer and Eastern producers feed more fluid milk (Heinrichs et al., 1995; Appleby et al., 2001). When calves are fed whole milk, average daily gain, body weight at weaning, and papillae length, width, and density increase with a decrease in morbidity and mortality (Godden et al., 2005; Hill et al., 2008a; Gorka et al., 2011). In contrast, when calves are fed milk replacer, starter intake and feed efficiency increases (Godden et al., 2005; Hill et al., 2009a; Donovan et al., 2002). Overall, feeding milk improves calf health when comparing calf mortalities, and incidence of disease such as pneumonia and scours, but is more variable in nutrient content than a commercial milk replacer (Godden et al., 2005; Hill et al., 2008a; Quigley et al., 2006).

When fed *ad libitum*, calves can consume milk or milk replacer up to 20% of their body weight (as-fed basis) (Khan et al., 2011a; Kehoe et al., 2007; Jasper et al., 2002). In production settings, milk is restricted to 10% of body weight (as-fed basis) in an effort to encourage consumption of calf starter (Drackley, 2008; Khan et al., 2007a,b). Decreasing the provision of milk ration from 20 to 10% of body weight results in a decrease of 30 day body weight from 64.2 to 54.2 kg, depressing average daily gain (Khan et al., 2007a,b). The cost associated with extra milk fed at 20% instead of 10% of body weight may be economically viable depending on the size of the farm. Smaller operations with 23 or less calves are better suited to feeding milk replacer at 10% of body weight whereas operations with more than 23 calves economically should feed whole milk (Godden et al., 2005). When growth is taken into consideration, it may be feasible for producers to feed greater than 10% of body weight

depending on the size of their operation (Drackley, 2008; Khan et al., 2007 a, b; Godden et al., 2005).

1.1.3 Nutrient Composition of Whole Milk and Milk Replacer

Whole milk contains 3.4% fat, 3.3% protein and 4.9% lactose (USDA Nutrient Database, 2018). Milk replacer nutrient content can vary depending on the ingredients used (Drackley, 2008). In milk replacers, a crude protein content of 23 to 28% is positively correlated to average daily gain and negatively correlated with starter intake (Hill et al., 2006; Hill et al., 2009a,b). When feeding milk replacer at 10% of body weight, the optimal crude protein content is 25% (dry matter basis), whereas optimal crude protein content at 20% of body weight is 27% (dry matter basis) (Hill et al., 2009b,c; Soberon et al., 2012).

While optimal crude protein content of milk replacer is elevated compared to the traditional 20 % crude protein, 20% fat milk replacer, optimal fat content is considerably lower. Fat content at 23% decreases digestibility of milk replacer due to limited pre-gastric lipase activity in the abomasum (Drackley, 2008; Hill et al., 2009a). When increasing fat content from 14 to 23%, there is a linear decrease in apparent digestibility, a linear increase in body fat deposition, an a quadratic decrease in starter intake (Hill et al., 2009a; Tikofsky et al., 2001; Diaz et al., 2001). Average daily gain, digestibility, and starter intake are slightly lower for a milk replacer with 20 to 21% fat compared to that of a milk replacer with 17% fat (Hill et al., 2009a,b; Tikofsky et al., 2001). As a result, milk replacer fed between 10 to 20% of body weight, containing 27% crude protein and 17% fat improves average daily gain, digestibility and starter intake in a young calf (Hill et al., 2009b; Tikofsky et al., 2001; Diaz et al., 2001).

1.2 Calf Starter

The addition of solid feed to the rumen increases rumen development and establishes a microbial population within the rumen, which is imperative to a successful transition to adult diets (Khan et al., 2008a,b; Drackley, 2008). Acetate, propionate, and butyrate are the main products from fermentation of calf starter, whereby the latter two are involved in increasing proliferation of stratified squamous epithelial tissue to form absorptive papillae and porous tissue within the rumen for nutrient transfer (Khan et al., 2016; Drackley, 2008). Volatile

fatty acids, products of carbohydrate fermentation specifically, are extremely important for rumen and papillae development (Figure 1.1, 1.2), and are the main energy source for the calf once they are weaned (Drackley, 2008; Khan et al., 2011a).

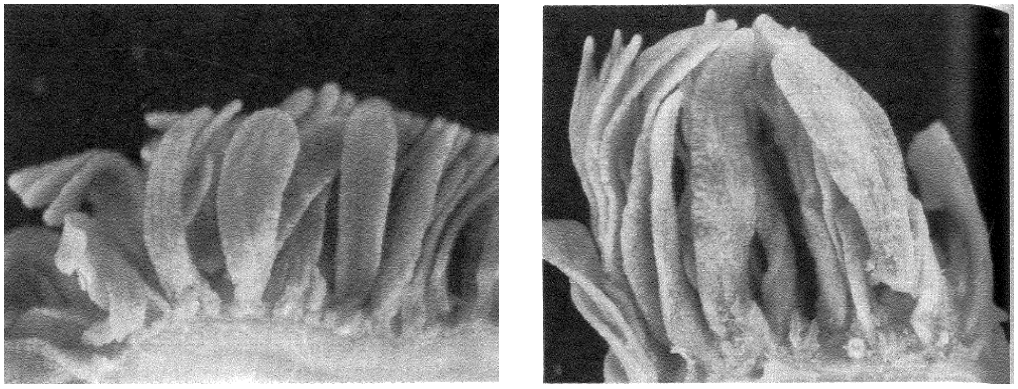

Figure 1.1. Modified from Dirksen et al., 1985, Rumen papillae after feeding forage only (left) for 7-8 weeks then feeding concentrates (right) for the next 7-8 weeks. Papillae are taller, wider, and more dense when cows are fed concentrates.

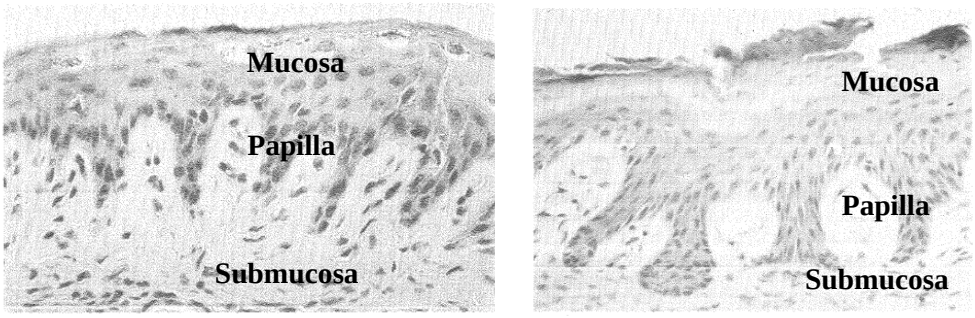

Figure 1.2. Modified from Dirksen et al., 1985, Rumen papillae histological section after feeding forage only (left) for 7-8 weeks then feeding concentrates (right) for the next 7-8 weeks. The muscosa is thicker and papillae are wider and longer when cows are fed concentrates.

Initiating intake of calf starter early in life and ensuring increasing intake during the pre-weaning period is crucial to maximizing the health and success of a ruminant animal (Khan et al., 2008a; Franklin et al., 2003; Quigley et al., 1994). To encourage intake of solid feed, calf starter is offered from birth and is composed of grains, molasses, and other carbohydrate sources that provide simple and complex sugars that are palatable and digestible

(Drackley, 2008; Hill et al., 2008c). Palatability and digestibility increase intake, which is a function of the physical form of the calf starter (Bach et al., 2007).

1.2.1 Physical Form of Calf Starter

The common forms of starter are texturized, multi-particle starter, pellets, or ground. Particle size is a factor to consider when feeding calf starter to optimize processes such as the rates of passage and ruminal fermentation (Terre et al., 2015; Laarman et al., 2012a; Froetschel et al., 1987). Ground starter has a particle size of 0.01 to 0.04 mm, pellets are 20 to 27 mm, and coarse, texturized starter has over 75% of the particles greater than 1.19 mm (Porter et al., 2007; Hill et al., 2008c; Khan et al., 2011b). As particle size decreased, chewing time decreased, and the rate of passage increased from 51 to 93 % in dairy cows fed corn based diets (Yang and Beauchemin, 2006a; Coverdale et al., 2004). A decrease in chewing decreases saliva production and rumen buffering (Drackley, 2008; Franklin et al., 2003; Coverdale et al., 2004).

Intake can be heavily influenced by the physical form of starter and is optimized when calves are fed a texturized calf starter. When feeding texturized calf starter, calves consume starter at a rate above 80% compared to calves fed pellets (Franklin et al., 2003; Bach et al., 2007; Hill et al., 2008b). Compared to pelleted starter, calves fed texturized starter increase their daily gain from 0.51 to 0.64 kg/d (Franklin et al., 2003; Coverdale et al., 2004; Porter et al., 2007). Further, calves fed texturized starter and ground starter are weaned earlier compared to calves fed pellets (Franklin et al., 2003; Coverdale et al., 2004; Porter et al., 2007).

1.2.2 Nutrient Composition of Calf Starter

When evaluating a calf starter, crude protein, fat, and starch are the three principal nutritional components (Drackley, 2008; Akayezu et al., 1994). Increasing crude protein from 15 to 26% (dry matter basis) results in a linear increase in body weight and starter intake during the pre-weaning phase (Akayezu et al., 1994; Hill et al., 2007). Crude protein above 20% increases body weight at weaning but had the opposite effect post-weaning, although starter intake increased linearly throughout (Akayezu et al., 1994; Hill et al., 2007). Average daily gain, intakes, feed efficiency and growth parameters are optimal at a crude protein level

between 18.0 to 19.6% in calf starter because there is a quadratic effect on these parameters above or below this threshold (Akayezu et al., 1994; Drackley, 2008; Hill et al., 2007).

During the pre-weaning phase, calf starter is not the main source of fat in the diet because milk is still heavily relied upon for nutrients (Drackley, 2008; Khan et al., 2016). The main purpose for fat in any type of starter is to decrease the amount of dust in the feed, or to be used as a binding agent for the pelleted portion (Khan et al., 2016; Bach et al., 2007). For these purposes, fat must not be included in excess of 3 to 4%, otherwise palatability, digestibility, and, in turn starter intake will drastically decrease (Khan et al., 2016). The optimal goal is to keep fat around 1% (dry matter basis) unless dust and energy content are a concern because most of the energy from starter intake will come from starch fermentation, not fat inclusion (Khan et al., 2016; Hill et al., 2008b).

Carbohydrates are the most important energy source in starter with the most common being corn although other cereals such as wheat, barley, oats, sorghum and rice are also used (Khan et al., 2008a,b). In terms of starch content, corn is similar to rice and sorghum (70 to 73%), but requires processing to increase starch digestion. Alternatively, wheat contains more starch at 77%, and barley and oats contain less starch at 57%, but these grains require minimal processing (Khan et al., 2016; Khan et al., 2008a,b). Dry matter intake increases linearly for ground, steam-flaked, steam rolled, whole, or dry-rolled grains respectively (Govil et al., 2017). Processing increases surface area and starch digestibility, and changes particle size of the products when compared to whole grains (Govil et al., 2017). Steam processing increases digestibility from 75 to 80% for corn due to an increase in surface area and gelatinization of starch to pectin, which is more digestible (Govil et al., 2017; Huntington, 1997; Ferraretto et al., 2013). Steam processing also disrupts the protein matrix of corn, which also increases digestibility (Govil et al., 2017; Huntington 1997; Ferraretto et al., 2013). Barley is 17%, and wheat is 25% more digestible than corn however, corn based diets increased dry matter intake 2.6 kg/d more than if diets are barley or wheat based (Ferraretto et al., 2013; Huntington, 1997).Therefore, multiple cereal grains can be used as the principal starch source, but their differences in starch content and availability affects their efficacy in calf performance.

A principal part of corn's popularity over wheat, oats, and barley as a starch source in calf starters stems from increased feed intake, average daily gains, ruminal pH and feed efficiency (Khan et al., 2008a,b). Compared to other grains, corn improves rumen development parameters such as rumen weight, volatile fatty acid concentration, and papillae density at weaning (Khan et al., 2008a,b). Post-weaning, both oats and wheat have an average daily gain similar to corn (Coverdale et al., 2004; Khan et al., 2008a,b; Hill et al., 2008b). Barley has a greater average daily gain (0.49 and 0.41 kg/d) due to its greater overall intake (0.71 and 0.50 kg/d) than corn when no forage is provided because its particle size is smaller and fermentation rate is more rapid. When forage is provided, the average daily gain and intakes are similar (Mirzaei et al., 2017; Khan et al., 2008a,b; Suarez-Mena et al., 2016b).

Due to the increasing demand for cereal grains in industrial processes such as biofuels, grains are expensive to feed so alternative feeds and byproducts become appealing in growing calf diets (Suarez et al., 2006; Laarman et al., 2012b). Feeding beet pulp, triticale dried distiller's grains and solids, cottonseed hulls, soybean hulls, or ground corn compared to whole corn had no effect on intake, average daily gain, or structural growth parameters in pre-weaned calves (Laarman et al., 2012b; Suarez et al., 2007; Terre et al., 2015). However, the addition of triticale dried distiller's grains increased starter intake whereas fibrous substitutes like cottonseed and soybean hulls decreased average daily gain when added to calf starter (Hill et al., 2008 a,b; Laarman et al., 2012b). This is likely due to the elevated fiber content of cottonseed and soybean hulls whereas triticale dried distiller's grains and beet pulp have greater starch contents (Hill et al., 2008a,b; Laarman et al., 2012b; Terre et al., 2015). Overall, use of alternative feeds to replace cereal grains has mixed results, and the impact on calf performance varies widely from feed to feed.

1.3 Forage

There is some debate as to whether or not calves should be fed forage during the pre-weaning period. Some studies have reported a decrease in starter intake when fibrous forage is provided along with a decrease in average daily gain in pre-weaning calves, which is attributed to gut fill (Hill et al., 2008a,b; Porter et al., 2007). However, other studies conclude that forage provision pre-weaning increases starter intake, feed efficiency, rumen muscle development, weight, and pH, and decreases negative plaque accumulation leading to

parakeratosis with no impact on performance in comparison to calves fed no forage (Suarez et al., 2006; Khan et al., 2011a; Coverdale et al., 2004). While calf starter is important for papillary development and volatile fatty acid production, forage is important for maintenance of rumen pH, chewing, and rumen musculature development in calves (Khan et al., 2011b; Allen, 1997; Khan et al., 2016). By feeding forages during the pre-weaning phase, rumen health increases and the transition from liquid to solid feed has greater success with calves maintaining dry matter intake post-weaning (Suarez-Mena et al., 2016; Khan et al., 2016; Gorka et al., 2011).

1.3.1 Forage Types and Inclusion Rates

Inclusion of forages in calf starter has varying effects depending on the type of forage and the inclusion rate. Alfalfa and mixed grass are the most commonly added forages in calf starter, but other sources include bromegrass, barley or wheat straw, oat hay, and barley or corn silages. Inclusion of alfalfa hay at 5 to 10% (dry matter basis) in comparison to no added forage increased dry matter intake, average daily gain, body weight, ruminal pH, and volatile fatty acid concentration (Castells et al., 2013; Beiranvand et al., 2014). Grass hay tends result in similar benefits as alfalfa hay such as similar dry matter intake and feed efficiency when added at 5% (dry matter basis) to calf starter (Castells et al., 2013; Montoro et al., 2013). Oat hay fed prior to weaning also has similarities including body weight gain at weaning and average daily gains of 0.66 kg/d and 0.62 kg/d for those fed alfalfa and grass hay respectively (Castells et al., 2013; Montoro et al., 2013). Bromegrass hay addition is another alternative to alfalfa hay inclusion; dry matter intake, body weight, average daily gain, and feed efficiency increased with the addition of 7.5% (dry matter basis) (Castells et al., 2013; Coverdale et al., 2004). Addition of bromegrass resulted in similar growth performance to that of oat hay (Castells et al., 2013; Coverdale et al., 2004). Lastly, silage is another option for including forage in calf starter with the common silages being barley and corn silage (Yang et al., 2006a,b; Suarez et al., 2007). Therefore, calf performance can be maintained with a variety of forage sources.

More important than forage source is the inclusion rate. When included above 5%, grass hay causes a decline in average daily gain, body weight, feed efficiency and intake in calves (Hill et al., 2008b; Montoro et al., 2013). Similarly, inclusion of bromegrass hay above

7.5% causes a reduction in starter intake and body weight post-weaning, just as inclusion of alfalfa above 10% causes a similar reduction in performance (Coverdale et al., 2004; Castells et al., 2013). In all, hay forage sources mixed in with calf starter should be kept at a maximum of 5 to 10% depending on the forage sources. Where straw is included at more than 15%, calves show decreased dry matter intake, average daily gain, and body weight (Suarez et al., 2007; Terre et al., 2015). Unlike dry forages, silage results in optimal feed efficiency and average daily gain when inclusion is less than 30% inclusion (Suarez et al., 2007). The differences in inclusion rate are likely due to nutrient density and palatability of these feeds where silage is the most palatable with the greatest carbohydrate content. Overall, dry forages can be mixed with starter in limited amounts, typically 10% or less, while silages show potential to be included at greater rates.

1.3.2 Physical Form of Forages

Like calf starters, forages are commonly included in rations as chopped, ground or pelleted. Chopped forage increases dry matter intake, feed efficiency, digestibility, chewing, and ruminal pH because the fermentation and rumination rates are slower compared to pelleted and ground forage (Jahani-Moghadam et al., 2015; Montoro et al., 2013; Yang and Beauchemin, 2006a). Chopped and pelleted forages have similar average daily gain, body weight, and dry matter intake in calves, but these parameters decrease when forage is ground (Jahani-Moghadam et al., 2015; Montoro et al., 2013). Ground forage also promotes non-nutritive behaviors such as inter-sucking, tongue rolling, and head-butting, whereas chopped and pelleted forages encourage chewing as an alternative (Yang and Beauchemin, 2006a; Montoro et al., 2013; Mirzaei et al., 2017). Chopped forages provide bulk in the rumen, which strengthens the rumen musculature, increasing rumen mixing while providing a scratch factor for the ruminal epithelium (Van Soest, 1982a). Ground or pelleted forages do not have the same effect on rumen health although growth parameters are similar between chopped and pelleted forages in calves (Van Soest, 1982a; Montoro et al., 2013; Mirzaei et al., 2017). Chopped forage and pelleted forage can be used interchangeably without compromising performance in young calves but rumen development is better with addition of chopped forage (Hill et al., 2008c; Jahani-Moghadam et al., 2015; Montoro et al., 2013).

The physical form of forage matters because of changes in particle size. Particle size resulting from forage chopping is categorized as long (greater than 9.5 mm), medium (7.15 mm), a combination of long and short chop, and short (less than 4.8 mm) (Yang and Beauchemin, 2006a,b; Jahani-Moghadam et al., 2015). When determining the optimal chop length for inclusion in a calf ration, there is an increase in dry matter intake for medium particle size but a linear decrease in digestibility as particle size increases beyond medium chop (Yang and Beauchemin, 2006a,b). Both long and medium chop forages increased ruminal pH but short chop decreased ruminal pH (Suarez-Mena et al., 2016a; Nemati et al., 2015). Medium compared to long and small chop lengths increased average daily gain (0.58, 0.53, and 0.46 kg/d), and dry matter intake (2.0, 1.9, and 1.5 kg/d), respectively and increased rumen health compared to calves fed no forage (Nemati et al., 2015; Khan et al., 2016). Feeding medium chop forage is optimal in calf rations where short chop forage is the least beneficial forage source.

1.4 Rumen Development

Rumen development is the transition of a calf from metabolic reliance on glucose to volatile fatty acids (Baldwin et al., 2004; Baldwin and Jesse, 1992; Van Soest, 1982a). This process begins with ingestion of solid feed that deposit microbes such as bacteria, fungi, and protozoa into the rumen fluid, establishing a population that ferments feed (van der Giezen et al., 2002; Van Soest, 1982a,b; Akin and Borneman, 1990). Calves will not become true ruminants until 4 to 6 weeks of age when the rumen has developed both physically, and metabolically including having an established microbial population and rumen fluid, growth in rumen size and substrate flow, papillae establishment, and development of transport proteins (Baldwin et al., 2004; Quigley, 2001). The products of microbial fermentation will stimulate the rumen wall, promoting rumen development prior to weaning (Van Soest, 1982a,b).

In the pre-weaned calf, the rumen is only 25 to 35% of the forestomach until weaning, whereby it will contribute 62 to 80% of the total forestomach volume post-weaning (Baldwin et al., 2004; Van Soest, 1982a). For this transition to occur, changes must begin in the luminal cells and surrounding muscularis tissue to aid in feed breakdown and absorption (Baldwin et al., 2004; Quigley, 2001; Van Soest, 1982a). As rumen size changes, calves on a

solid feed regimen will begin to exhibit luminal changes in the size, length, width, and density of papillae made of non-secretory, non-glandular, keratinized stratified squamous cells that are first to come into contact with digesta (Graham and Simmons, 2005; Connor et al., 2013; Groenewald et al., 1993).

1.4.1 Rumen Anatomy

The layers of the rumen include a serous membrane, muscular tunic, and an epithelium, which is made of tunica mucosa (Van Soest, 1982a; Graham and Simmons, 2005). The tunica mucosa consists of four layers of cells, which are the strata basale, spinosum, granulosum, and corneum, where the stratum basale lines the serosa and the stratum corneum lines the lumen (Graham and Simmons, 2005; Baldwin, 1998; Van Soest, 1982a). The epithelial layers are responsible for protection, absorption, and transport of nutrients from lumen to serosa and can be as thick as 15 or as thin as 4 cell layers depending on the type of diet fed (Van Soest, 1982a; Baldwin, 1998). These cells can live for up to 6 days before becoming part of the dead exterior layer of the ruminal epithelium (Baldwin, 1998). Passive transport across the epithelium occurs for volatile fatty acids, water, ammonia and urea, which are the main energy sources for ruminants (Van Soest, 1982a; Graham and Simmons, 2005). Active transport occurs with charged molecules such as sodium, chloride, and other minerals in non-keratinized cells (Van Soest, 1982a; Graham and Simmons, 2005).

The strata basale and spinosum are the only true living layers of the ruminal epithelium containing almost all of the epithelial mitochondria (Baldwin, 1998). Cellular organization, mitochondrial density, and metabolic activity decrease rapidly from the stratum basale to corneum (Baldwin, 1998; Graham and Simmons, 2005). The stratum granulosum has tight junctions (Figure 1.3) formed by desmosomes, which create a barrier for absorption across the epithelium but contains dead cells as does the stratum corneum (Baldwin, 1998, Van Soest, 1982a). The stratum corneum is the most luminal layer of cells that are completely dead and keratinized (Baldwin, 1998; Van Soest, 1982a). This layer contains large gaps between cells making the stratum corneum most subject to sloughing (Baldwin, 1998).

Epithelial barrier integrity is maintained at the stratum granulosum where permeability decreases due to the density of desmosomes and tight junction proteins such as claudin-1, plasma membrane connexin-2, zona occludens-1, and membrane associated kinase proteins (Graham and Simmons, 2005; Graham et al., 2007; Groenewald et al., 1993). Together, these cellular proteins regulate cell-to-cell interactions, creating structural connections that prevent para-cellular transport of nutrients and bacteria. This layer is subject to rapid proliferation, abrasion, and absorption stress as a result (Van Soest, 1982a; Baldwin, 1998). Moving away from the stratum granulosum, abundance of tight junction proteins gradually decreases and proteins involved in nutrient transport and metabolism increase in abundance (Graham and Simmons, 2005; Graham et al., 2007).

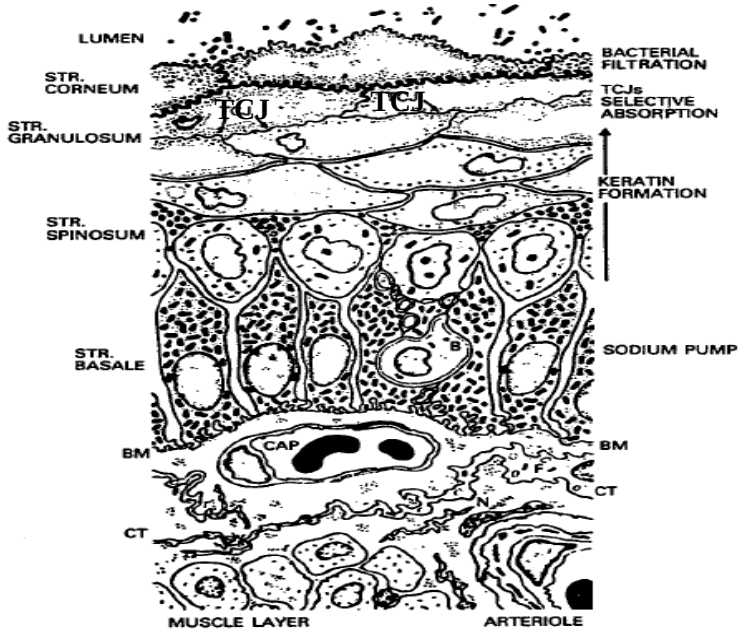

Figure 1.3: Modified from Van Soest, 1982a. Fully developed, cross sectional layers of the ruminal epithelium exhibiting different cellular layers from lumen to serosa where tight junctions (TCJ) can be seen in the stratum granulosum.

The stratum basale has the greatest level of metabolic activity due to the presence of transport proteins such as Na^+/K^+ ATPase, and monocarboxylate transporter 1 and 2, which are the main mediators of volatile fatty acid absorption (Graham et al., 2007; Baldwin, 1998; Aschenbach et al., 2011). The NHE (Na^+/H^+) and NBC (Na^+/HCO_3) families are responsible for maintaining a proton gradient in relation to the concentration of free protons within the

rumen digesta (Graham et al., 2007; Aschenbach et al., 2011). NHE1, 2, 3, and NBC1 are found within the strata basale, spinosum, and granulosum (Graham et al., 2007; Aschenbach et al., 2011). The nutrient transporters have a direct effect on ruminal development and growth in young calves.

1.4.2 Papillae and Musculature Development

When calves are fed only milk, ruminal tissue does not develop papillae (Baldwin et al., 2004; Connor et al., 2013). The lumen has a smooth appearance with minimal pigmentation even if milk enters the rumen, which is comparable to the rumen of a newborn calf in both physical and metabolic capacity (Van Soest, 1982a; Baldwin et al., 2004; Connor et al., 2013). Longest projections reach no more than 0.5 mm, which is similar to calves fed only milk and forage pre-weaning because ruminal papillae show minimal development or pigmentation and will reach a range of 0.5 to 1.5 mm in height (Connor et al., 2013). When calves are fed milk and starter compared to only milk, the fermentation rate increases, which increases volatile fatty acid concentrations and lowers ruminal pH (Laarman and Oba, 2011; Connor et al., 2013). This also results in the length of papillae to increase up to 2.5 mm pre-weaning, with the papillae also having darker pigmentation (Laarman et al., 2012a; Laarman and Oba, 2011; Connor et al., 2013). Longer papillae and darker pigmentation indicates better rumen development (Connor et al., 2013).

While starter is important, bulky forages do increase the integrity, strength, and size of the tunica muscularis, which aids in rumination and digesta mixing within the rumen (Baldwin et al., 2004; Van Soest, 1982a,b). Bulky feeds are the main driver behind rumen musculature and size development, but other indigestible material will have this effect on both layers of tunica muscularis (Baldwin et al., 2004; Van Soest, 1982a,b). Although forages stimulate rumen musculature, they have minimal effect on the development of papillae within the rumen. Consequently, a combination of both rapidly fermentable carbohydrates and bulky forages are necessary for optimal development of ruminal tissue.

1.4.3 The Role of Butyrate in Rumen Development

Optimal development of papillae is stimulated by an elevated concentration of volatile fatty acids in the rumen, specifically propionate and butyrate. When fed concentrates, acetate,

propionate and butyrate are the main volatile fatty acids, where acetate is the most abundant with forage fermentation, and lactic acid comes from addition or leakage of milk to the rumen (Bergman, 1990; Guilloteau et al., 2010; Balch and Rowland, 1957). When cows were fed a diet composed of 70% forage and 30% concentrate, the ratio of acetate, propionate, and butyrate was 70:19:11 (Bergman, 1990; Balch and Rowland, 1957). Diets composed of 60% concentrate and 40% forage had ratios of 46:42:12 (Bergman, 1990; Balch and Rowland, 1957). While these are concentrations found in adult cows, calves should have similar ratios due to the nature of carbohydrate fermentation (Figure 1.4)and similarity between feeds provided to calves and adult cows (Ferraretto et al., 2013; Balch and Rowland, 1957; Baldwin, 1998). When fed a high concentrate diet, and butyrate comprises 10 to 20% of the total volatile fatty acid concentration in the rumen in calves, ruminal papillae begin to grow and develop reaching up to 15 mm in length (Bergman, 1990; Baldwin, 1998).

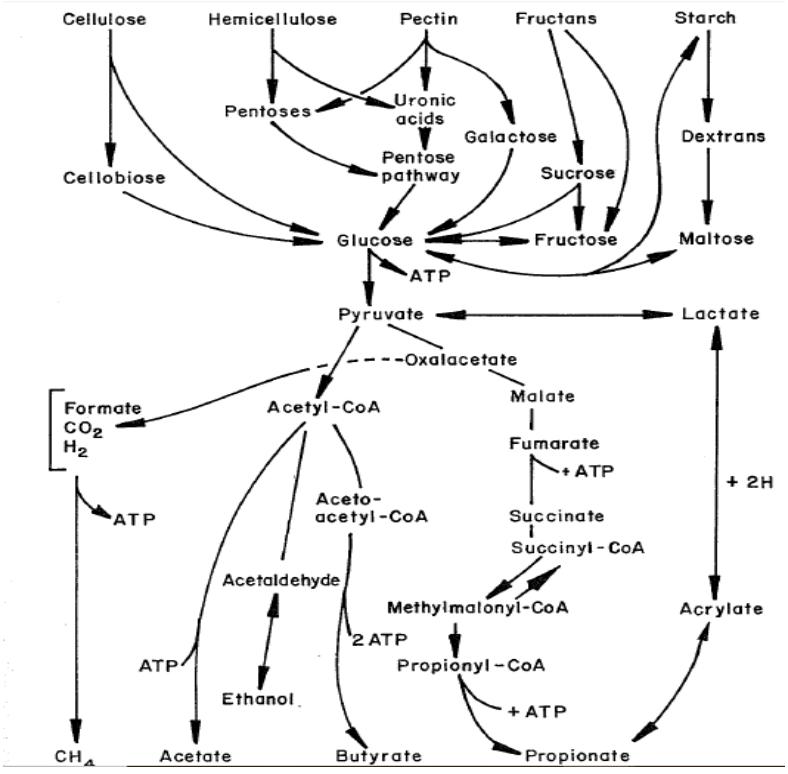

Figure 1.4. Modified from Van Soest, 1982b. Pathways of carbohydrate metabolism in the rumen. Final products include acetate, propionate, butyrate and methane from carbohydrate fermentation.

Butyrate can be measured directly from rumen fluid however; this process is invasive but a metabolic derivative, β-hydroxybutyrate, can be measured in blood and is a product of butyrate metabolism in the rumen epithelium (Veech et al., 2003; Gorka et al., 2018; Graham et al., 2007). Volatile fatty acids in the rumen are absorbed through the ruminal epithelium, leaving less than 10% for passage into the omasum (Baldwin, 1998). In the ruminal epithelium, acetate, propionate, and butyrate are respectively metabolized to CO_2, lactate and pyruvate, and acetoacetate and β-hydroxybutyrate (Baldwin, 1998; Baldwin and McLeod, 2000). In 75% forage, and 75% concentrate diets, acetate, propionate, and butyrate were fully oxidized at a rate of 58.3 to 53.9, 46.4 to 47.0, and 15.5 to 21.8 nmol/($1*10^6$ cells 120 min), compared to glucose values of 28.4 to36.8 nmol/($1*10^6$ cells 120 min) respectively (Baldwin and McLeod, 2000). When in the presence of butyrate, glucose oxidized much more rapidly in neonatal calves from day 0 to 14, 12.5 to 70, and 2.5 to 6.2 nmol/($1*10^6$ cells 120 min) for glucose only and glucose with butyrate respectively (Baldwin and Jesse, 1992). In the presence of glucose, butyrate oxidation decreased from day 0 to 14 where butyrate alone was 18.7 to 70 and butyrate with glucose was 12.5 to 12.7 nmol/($1*10^6$ cells 120 min) (Baldwin and Jesse, 1992). Normal blood glucose concentrations for a calf are 80 to 126 mg/dL pre-weaning and 73 to 97 mg/dL post-weaning (Suarez-Mena et al., 2017; Mohri et al., 2007; Daniels et al., 1974).

Ketogenesis only occurs within the ruminal epithelium and the liver (Baldwin, 1998; Baldwin and McLeod, 2000). This enables β-hydroxybutyrate to be measured from the blood or urine non-invasively whereas acetate and propionate are metabolized to a variety of intermediates (Baldwin, 1998; Baldwin and McLeod, 2000). Once butyrate and other volatile fatty acids have been absorbed across the tunica mucosa, they will enter the serosa where they are transported to the liver via the hepatic portal vein (Veech et al., 2003; Baldwin and Jesse, 1992; Baldwin, 1999). As calves grew, β-hydroxybutyrate concentrations increased from 3.2 to 36.7 mmol/L in the blood at day 4 and day 42 of life, showing a ten-fold increase in β-hydroxybutyrate that is negatively correlated with glucose oxidation (Baldwin and Jesse, 1992; Graham et al., 2006; Baldwin and McLeod, 2000). Normal β-hydroxybutyrate blood concentrations in calves are 0.0 to 0.7 mg/dL pre-weaning and 0.7 to 1.6 mg/dL post-weaning (Suarez-Mena et al., 2017). As a result, β-hydroxybutyrate can be effectively used as an

indirect indicator of rumen development because of its direct relationship to butyrate production in the rumen (Baldwin and Jesse, 1992; Van Soest, 1982a,b; Connor et al., 2013).

1.5 Weaning

Weaning is one of the most stressful times in the life cycle of the calf, whereby removal of milk can cause a decrease in solid feed intake and body weight during the transition from pre-ruminant to ruminant, and this increases the risk of disease (Hamada et al., 1976; Weary et al., 2008; Baldwin et al., 2004). The typical calf is weaned between 6 and 8 weeks of age, but weaning can occur as early as 4 weeks (Drackley, 2008; Quigley, 2001; Baldwin et al., 2004). There are several approaches to weaning, including basing the decision to withdraw milk on age or solid feed intake, or whether or not calves should be abruptly weaned, or if a more gradual weaning process is optimal for growth and health parameters (Roth et al., 2009; Sweeney et al., 2010; Kehoe et al., 2007).

1.5.1 Time of Weaning

Weaning according to age is a common method because it has the least amount of discrepancy as to when the calf should wean, but can be detrimental because there is no way to fully know how well developed the rumen or intestinal system actually is (Quigley, 2001; Roth et al., 2008; Hamada et al., 1976). Common ages to wean include 4, 5, 6, 7 and 8 weeks of age for dairy calves (Quigley, 2001; Sweeney et al., 2010). Concentrate-dependent weaning is becoming more popular as more information is generated on rumen development, whereby calves can be weaned early and have a greater success rate during the weaning transition than those weaned by age (Quigley, 2001; Khan et al., 2011b; Kehoe et al., 2007). When calves are weaned based on concentrate intake, they must be consuming enough nutrients from solid feed to be able to be completely sustained nutritionally from that intake, which falls around 0.68 to 0.90 kg of starter consumed per day for a minimum of two consecutive days (Quigley, 2001; Roth et al., 2009; Roth et al., 2008). This intake rate can be achieved early in the life of the calf and most calves will reach this threshold at 4 to 5 weeks of age (Quigley, 2001; Roth et al., 2009). However, calves that are sick at any point during the first few weeks of life will not be able to wean this early and must be kept on milk longer to maintain adequate nutrient intake (Quigley, 2001; Kehoe et al., 2007; Roth et al., 2009).

Calves weaned on an intake basis tend to have accelerated physiological development due to increased grain intake; however, grain and morbidity are inversely related (Roth et al., 2009). As long as intake is elevated, there is no difference in productivity or morbidity post-weaning between calves weaned at 3, 4, 5, and 6 weeks of age (Kehoe et al., 2007; Connor et al., 2013; Quigley et al., 1991). Calves weaned at 4 weeks of age had optimal grain intake and volatile fatty acid concentrations in weeks 5 to 8 at 1.5 kg/d compared to calves weaned later at 6 weeks of age with an intake of 1.0 kg/d (Quigley et al., 1991; Roth et al., 2009). When calves are weaned later than 6 weeks, grain intake is less as is forage intake post-weaning compared to calves weaned at 4 weeks (Quigley et al., 1991; Khan et al., 2011b). Weaning at the age of 4 weeks is an optimal goal when calves are consuming enough starter because they have the greatest productivity with the least input cost and has the best performance benefits post-weaning (Quigley et al., 1991; Kehoe et al., 2007; Khan et al., 2011b). This weaning strategy at 4 weeks of age is most successful when calves are weaned in a step-down manner, whereby nutrients from milk are reduced over the period of 2 weeks and must be well managed if calves are not consuming more than 0.68 kg/d of starter for 2 or more consecutive days (Sweeney et al., 2010; Roth et al., 2009; Khan et al., 2007a,b).

1.5.2 Weaning Method

Once weaning begins, the process can be either abrupt or gradual. Abrupt weaning occurs when calves are fed milk until a certain point in time and are then weaned immediately from that point onward, whereas step-down weaning varies between one to two weeks of gradual reduction of milk volume with the same nutrient concentrations, or diluting the milk, causing gradual reduction of nutrients with the same milk volume (Khan et al., 2007a,b; Kehoe et al., 2007).

Abrupt weaning requires the least amount of labor during the weaning transition due to the immediate removal of milk from the calf diet (Roth et al., 2008; Weary et al., 2008). During abrupt weaning, weight gain and dry matter intake decline and a large depression in growth ensues in the first 2 to 3 weeks following removal of milk (Sweeney et al., 2010; Roth et al., 2008; Khan et al., 2011b). Abruptly weaned calves had a weight gain of 0.89 kg/d prior to weaning and 0.87 kg/d post-weaning, whereas conventionally-weaned calves had body weight gains of 0.95 and 1.10 kg/d and dry matter intake of 1.5 to 2.0 and 1.0 to 1.7 kg/d,

respectively (Sweeney et al., 2010; Roth et al., 2009; Khan et al. 2007a,b). Abrupt weaning decreases weight gain and dry matter intake post-weaning compared to conventional weaning.

When weaned conventionally in a step-down method over 4, 10, or 22 days, calves weaned over the course of 22 days and 10 days had the greatest gains and dry matter intake but weaning over the duration of 10 days had the greatest gains with the least input of milk (Sweeney et al., 2010; Khan et al., 2007a,b). Step-down weaning with addition of water to reduce milk concentration during days 45 to 50 at 10% of body weight (as-fed basis) decreased solid feed intake, body weight gain, and feed efficiency (Khan et al., 2007a,b; Jasper and Weary, 2002). Calves fed at 20% of body weight (as-fed basis) for 30 days, 10% of body weight for 31 to 45 days, and with water diluted milk for the final 5 days had greater intakes, body weight and feed efficiency than abruptly weaned calves (Khan et al., 2007a,b; Jasper and Weary, 2002). Weaning over the duration of 10 to 20 days optimizes weight gain, solid feed intake, rumen pH, total volatile fatty acid concentrations, rumen development, and psychological behaviors as opposed to abrupt weaning (Weary et al., 2008; Sweeney et al., 2010; Khan et al., 2007a,b).

1.6 Ruminal Fermentation and Buffering

To ensure a successful weaning transition, the rumen microbial populations must be able to effectively ferment solid feed. The main microbes found in the rumen include bacteria, protozoa, and anaerobic fungi (Van Soest, 1982b; van der Giezen et al., 2002; Orpin et al., 1977). Bacteria are anywhere from 60 to 90% of the ruminal microbial biomass, protozoa are 10 to 50%, and fungi comprise 8 to 20% of the biomass (Van Soest, 1982b; Akin and Borneman, 1990). Bacteria are predominantly anaerobic and are subdivided into four main groups, amylolytes, cellulolytes, proteolytics, and methanogens (Van Soest, 1982b; Allen, 1997). Amylolytes and cellulolytes ferment non-structural and structural carbohydrates, respectively, which affect concentrations of volatile fatty acids (Van Soest, 1982b, Allen, 1997). Consequently, amylolytic bacteria play an important role in the fermentation of calf starter and have a relatively rapid reproductive rate compared to cellulolytic bacteria (Van Soest, 1982b; Allen, 1997). Cellulolytes ferment forages and increase their population size slowly (Van Soest, 1982b; Allen, 1997). Cellulolytic bacteria thrive at a pH of 6.7 and amylolytic bacteria have an optimal pH of 5.8 to 6.5, whereas

facultative lactic acid producers begin to proliferate below 6.2 (Aschenbach et al., 2011; Van Soest, 1982b). When the volatile fatty acid concentration of lactic acid reaches 50 to 90% of total rumen acid and the pH drops below 6.0, bacteria such as the cellulolytes and protozoan families that maintain rumen pH around 6.7 to 7.0 begin to decrease in population (Van Soest, 1982a,b; Aschenbach et al., 2011).

1.6.1 Clearance of Volatile Fatty Acids from the Rumen

With the microbes fermenting feed, an excessive amount of volatile fatty acids could accumulate, which must be cleared to avoid sub-acute ruminal acidosis (Van Soest, 1982a,b; Aschenbach et al., 2009; Chiba, 2007). Volatile fatty acids are mainly removed from the rumen by an ion gradient and need to be in great quantity for proper absorption into the blood, which is more alkaline than the rumen (Van Soest, 1982a; Aschenbach et al., 2009). This difference in ion concentration drives volatile fatty acid movement from the rumen towards serosal absorption either by passive diffusion or active transport (Van Soest, 1982a,b; Aschenbach et al., 2011). Acetate, propionate and butyrate are 30, 50, and 90% metabolized by the ruminal epithelium respectively (Bergman, 1990; Penner et al., 2010). Volatile fatty acids are removed predominantly via absorption across the rumen wall, neutralization by saliva, and passage from the rumen to the omasum (Allen, 1997; Aschenbach et al., 20009).

Volatile fatty acids are passively diffused across the rumen wall through the apical membrane in their lipophilic protonated form, which can be as little as 1% diffusion when the pH is greater than 6.8 (Aschenbach et al., 2009; Gabel et al., 2002). Optimal pH for absorption is below 5.6 because volatile fatty acids will become more protonated (Nagaraja and Titgemeyer, 2007; Gabel et al., 2002). The more lipophilic an acid is, the easier it will pass through the ruminal epithelium, which is why 14% of total butyrate, and only 7% of total acetate are able to diffuse through the ruminal epithelium (Aschenbach et al., 2009; Walter and Gutknecht, 1986; Gabel et al., 2002). Diffusion creates a continual gradient within the rumen for butyrate because it is rapidly metabolized once it enters ruminal epithelial cells (Aschenbach et al., 2009; Baldwin, 1998).

Acetate is removed from the rumen via active transport from sodium and hydrogen exchange in the ruminal epithelium through the NHE transporters (Aschenbach et al., 2009;

Laarman et al., 2013). Apical NHE transporters can be inhibited when potassium concentrations in the rumen are low, impairing acetate transport by up to 70% with minimal effect on propionate and butyrate absorption (Aschenbach et al., 2009; Laarman et al., 2013). When transport is reduced, volatile fatty acids accumulate in the rumen, decreasing the pH (Allen, 1997; Aschenbach et al., 2009). There are mechanisms in place to increase the ruminal pH such as the addition of bicarbonate, which not only acts as a buffer but is also involved in acetate absorption via non-diffusive, bicarbonate mediated transporters (Aschenbach et al., 2009; Laarman et al., 2013). Buffers are molecules that resist the change in pH by either donation or acceptance of protons.

Bicarbonate is most active at a pH of 6.1 where concentrations can range from 0.5 to 22.5 m*M* in the rumen, and are 22.5 m*M* in the blood, which creates a gradient for absorption of acetate into the blood (Van Soest, 1982a; Aschenbach et al., 2009). Acetate (10 m*M* and 0.5 m*M*) had greater uptake when bicarbonate was at a concentration of 1.1 m*M* compared to 22.5 m*M*, at a ruminal pH of 6.1 compared to 7.4 (Aschenbach et al., 2009). Optimal uptake of acetate for 10 and 0.5 m*M* at a pH of 6.1 was 13 and 0.7 nmol/mg*min, respectively (Aschenbach et al., 2009). In transition, intracellular pH decreases with increased uptake of acetate, which causes upregulation of the NHE transporters to increase intracellular pH via ion exchange (Penner et al., 2010). Transporters that are bicarbonate-dependent have a three-fold increase in volatile fatty acid uptake when bicarbonate is present, which enables rapid removal of volatile fatty acids from the rumen to stabilize ruminal pH overall (Aschenbach et al., 2009; Aschenbach et al., 2011). Bicarbonate can be synthesized within the rumen from external CO_2, or can be transported from the serosal side of the ruminal epithelium where a sodium-bicarbonate co-transporter is active during periods of decreased ruminal pH (Aschenbach et al., 2009; Kramer et al., 1996).

Bicarbonate from these sources becomes a proton donor, making acetate more diffusible where normal concentrations within the rumen are greater than 60 m*M* (Aschenbach et al., 2009; Aschenbach et al., 2011). However, in the presence of 28 m*M* chloride as a co-transporter, uptake of acetate, propionate, and butyrate decreased from 25 mM each to 15, 10, and 20 m*M* respectively, as chloride concentration increased from 28 to 40 m*M* (Aschenbach et al., 2009; Kramer et al., 1996). When the concentration of volatile

fatty acids was greater than 30 m*M* in the rumen, chloride uptake increased from 5 to 15 m*M* per hour when either acetate or propionate was removed from the mixture (Aschenbach et al., 2009; Kramer et al., 1996).

In the bicarbonate independent transport, acetate uptake decreased from 12 to 6 nmol/mg*min when compared to bicarbonate dependent transport (Aschenbach et al., 2009). When bicarbonate is not present, acetate uptake still occurs but is inhibited by the presence of nitrates where 50 m*M* concentration of nitrates decreased acetate uptake from 6 to 3 nmol/mg*min (Aschenbach et al., 2009; Aschenbach et al., 2011). With the presence of chloride and nitrates, acetate uptake decreases and is higher in bicarbonate dependent rather than independent transporters at a pH of 6.1 (Aschenbach et al., 2009).

Saliva contains multiple buffering ions with the two major buffers being bicarbonate, and phosphate (Van Soest, 1982a; Yang and Beauchemin, 2006a; Silanikove and Tadmor, 1989). Saliva also contains sodium, potassium, mucins, lipase, urea, sulfate, calcium, and magnesium (Van Soest, 1982a; Yang and Beauchemin, 2006a; Silanikove and Tadmor, 1989). Salivary excretions in adult cattle can be between 100-190 l/d when on a high forage diet and can be as low as 40 l/d when on high concentrate diets (Silanikove and Tadmor, 1989; Van Soest, 1982a). Production of saliva is most effective from elevated chewing levels of high fiber diets (Yang and Beauchemin, 2006a,b; Silanikove and Tadmor, 1989). In high concentrate diets, minimal or no forage diets, or pelleted forage diets, chewing is greatly reduced in adult cows (Yang and Beauchemin, 2006a,b; Van Soest, 1982a; Silanikove and Tadmor, 1989). Since saliva is the main buffering source for the rumen, pH is depressed especially in diets that only consist of rapidly fermentable concentrates, which cause sub-acute ruminal acidosis (Plazier et al., 2009; Steele et al., 2011; Steele et al., 2009).

1.6.2 Subacute and Acute Ruminal Acidosis

While it has been established that growing calves must consume as much starter as possible to be successful during the weaning transition and post-weaning period, rapid increases in starter consumption can have detrimental effects on rumen health due to the increased accumulation of volatile fatty acids that can result in disorders, including acidosis (Laarman and Oba, 2011; Steele et al., 2011; Steele et al., 2009). Ruminal acidosis occurs

when non-structural carbohydrates are fermented too rapidly producing elevated amounts of volatile fatty acids that are not readily absorbed across the ruminal epithelium causing a decrease in mean ruminal pH less than 5.0 for greater than 180 min/d (Krause et al., 2006; Aschenbach et al., 2011; Gozho et al., 2005).

Accumulation of volatile fatty acids such as acetate, propionate, and butyrate contribute to a decrease in ruminal pH where the pKa's of these acids are 4.80, 4.88, and 4.82, respectively (Krause and Oetzel, 2006; Allen, 1997). The pKa determines how strong an acid is, whereby the strongest acids have the lowest pKa's. Lactic acid has a pKa of 3.86 and has the greatest impact on lowering ruminal pH. When transitioning cows from a high forage diet to a high concentrate diet, lactate using microbes that convert lactic acid to acetate, propionate, and butyrate, are unable to rapidly increase populations, leading to a decrease in ruminal pH as concentrate intake increases (Allen, 1997; Van Soest, 1982a; Krause and Oetzel, 2006). Signs and symptoms of acute ruminal acidosis include reluctance to eat, a painful abdomen, lethargy, and laminitis which are all easily noticeable in an animal (Van Soest, 1982a,b; Cook et al., 2004).

Unfortunately, there is another type of acidosis that occurs much more frequently and is difficult to detect, sub-acute ruminal acidosis (Allen, 1997; Kuenen et al., 2002; Plazier et al., 2009). Sub-acute ruminal acidosis is the condition in which mean ruminal pH is less than 5.8 for greater than 180 min/d (Gozho et al., 2005; Aschenbach et al., 2011; Garrett et al., 1999). It is different from acute ruminal acidosis because acute ruminal acidosis is caused by accumulation of lactic acid at a pH less than 5.0 whereas sub-acute ruminal acidosis is caused by accumulation of volatile fatty acids, acetate, propionate, and butyrate at a pH of less than 5.8 (Nagaraja and Titgemeyer, 2007; Allen, 1997; Krause and Oetzel, 2006). If left untreated, the ruminal epithelium can accrue permanent damage and abcesses will form in the liver (Stone et al., 2004; Dirksen et al., 1985; Garrett et al., 1999). Minimizing the incidence of sub-acute ruminal acidosis requires inclusion of slower fermenting, structural carbohydrates typically found in forages, and ensuring adequate buffering capacity of the rumen via routes such as saliva production (Osborne et al., 2004; Plazier et al., 2009; Aschenbach et al., 2009).

Rumen pH fluctuates considerably during a 24-hour period in cattle on high grain diets and is typically between 5.5 to 6.5 due to short ruminal papillae adapted to a high forage diet

(Nagaraja and Titgemeyer, 2007; Nagaraja et al., 1981; Krause and Oetzel, 2006). When fed high grain diets, papillary length begins to increase, increasing uptake of volatile fatty acids (Nagaraja and Titgemeyer, 2007; Keunen et al., 2002). Salivary secretion is also reduced, which can result in extended time at pH of less than 5.8 and typically inflammation of the ruminal epithelium, resulting in decreased absorptive capacity (Krause and Oetzel, 2006; Enemark, 2009; Silanikove and Tadmor, 1989). A pH in this range promotes the growth of lactic acid producing bacteria, which once established, maintain a low pH in the rumen specifically around feeding time (Nagaraja and Titgemeyer, 2007; Krause and Oetzel, 2006). The non-physiological accumulation of volatile fatty acids causes a continual decrease in pH in addition to a reduction in the metabolism of lactate as a result of changes in microbial distribution of the rumen and death of microbes such as protozoa and cellulolytic bacteria (Nagaraja and Titgemeyer, 2007; Krause and Oetzel, 2006; Bergman, 1990).

Once lactic acid fermenting bacteria are the primary fermenters, total fermentation capacity decreases and feed consumption begins to vary as a result of decreased osmolarity and water accumulation in the rumen (Nocek, 1997; Nagaraja et al., 1981; Nagaraja and Titgemeyer, 2007). Variable feed consumption greater than 10% of dry matter intake, and inconsistent meal times only increase the growth of lactic acid producing bacteria after meals (Nagaraja and Titgemeyer, 2007; Enemark, 2007). This cycle will continue where cows will then reduce then increase intake to 90 then 110% of their normal dry matter intake for a duration of 3 to 4 days each, beginning an episodic feeding pattern that will continually promote abnormal papillae growth and production of toxic substances within the rumen (Nagaraja and Titgemeyer, 2007; Nagaraja et al., 1981; Nocek, 1997). Parakeratosis can result from damaged ruminal epithelial cells, and microtears in the epithelium can translocate bacteria into the serosa, transferring bacteria to other organs such as the liver (Scanlan and Hathcock, 1983; Nagaraja and Titgemeyer, 2007). The free bacteria cause liver abscesses and laminitis, and can further cause abscesses in the lungs and kidneys if the condition persists (Scanlan and Hathcock, 1983; Nagaraja and Titgemeyer, 2007).

Effective fiber in the diet is key to increasing mastication and rumination, which begins between 2 to 4 weeks of age, causing an increase in overall salivary output (Baldwin et al., 2004; Van Soest, 1982a; Mertens, 1997). When fed high forage diets, the incidence of

irregular feeding habits and ruminal pH fluctuation decreases (Nagaraja and Titgemeyer, 2007; Allen, 1997). Effective fiber is defined as the portion of fiber in the diet that stimulates chewing and contributes to the formation of the fiber mat in the rumen (Yang and Beauchemin, 2006a,b; Mertens, 1997; Allen, 1997). This fiber aids in abrasion of the ruminal epithelial cells, stimulating ruminal contractions and providing resistance for musculature development and enhancement which, is commonly referred to as the scratch factor (Mertens, 1997; Aschenbach et al., 2011; Baldwin et al., 2004). Effective fiber aids papillary growth by removing tough, keratinized cells on the external papillae, allowing for further hyperplasia from exposure to volatile fatty acids (Aschenbach et al., 2011; Baldwin et al., 2004; Van Soest, 1982a). Without a fiber source in the diet of the young calf, the rumen will develop issues such as sub-acute ruminal acidosis leading to parakeratosis, rumenitis, and other metabolic diseases that have been extensively studied in adult cows (Dirksen et al., 1985; Allen, 1997; Stone et al., 2004).

1.6.3 Rumen Pathologies

Left untreated, sub-acute ruminal acidosis can lead to parakeratosis. Parakeratosis is the accumulation and keratinization of cell layers forming a mucous membrane that leads to excess sloughing of hardened tissue found in organs such as the rumen (Steele et al., 2009; 2011; Khafipour et al., 2011). It is caused by inadequate removal of accumulated keratinized cells due to a loss in scratch factor from diets containing minimal roughage (Krause et al., 2006). During parakeratosis, papillae will start to keratinize and branch in a polydactyl fashion, creating weak, thin, non-absorptive extensions with excess sloughing of the stratum corneum (Aschenbach et al., 2011; Van Soest, 1982a; Steele et al., 2009). Deep crevices between papillae also disappear under this condition, reducing total surface area and absorption capabilities (Steele et al., 2009; 2011; Plazier et al., 2009). Parakeratosis can occur from reduced roughage intake in the rumen and results from sub-acute ruminal acidosis because ruminal epithelial cells do not have protective mucus, leaving them highly susceptible to changes in ruminal pH (Plazier et al., 2009; Steele et al., 2009; Kleen et al., 2003).

Rumenitis provides a direct pathway for the passage of microbes into the epithelium and blood, which can cause ruminal ulcers and liver abscesses (Plazier et al., 2009; Emmanuel et al., 2007). With the increase in permeability and translocation of ruminal bacteria,

abscesses decrease liver function and are difficult to detect, which can lead to the spread of bacteria to other organs including the heart, lungs, and kidneys (Plazier et al., 2009; Scanlan and Hathcock, 1983; Krause and Oetzel., 2006). In calves, these conditions do not tend to arise during the pre-weaning period but become more prevalent during the post-weaning period if the forage source is inadequate (Plazier et al., 2009; Allen, 1997; Kuenen et al., 2002). Rumenitis can lead to laminitis from over accumulation of histamine that causes destruction of micro-vessels in the hoof, and even death can occur if left untreated, but it is still unclear if the cause of rumenitis is from sub-acute ruminal acidosis alone or if lipopolysaccharides are the main cause of rumentitis (Cook et al., 2004; Gozho et al., 2005; Khafipour et al., 2009).

Under the conditions of sub-acute ruminal acidosis, there is an increase in lipopoylsaccharides within the rumen (Cook et al., 2004; Gozho et al., 2005; Khafipour et al., 2009). Lipopolysaccharides are also known as endotoxins that are found within the rumen and comprise the cellular structure of gram negative bacteria (Nocek, 1977; Emmanuel et al., 2007; Nagaraja and Titgemeyer, 2007). When in contact with the ruminal epithelium, lipopolysaccharides elicit an immune response which causes inflammation of the epithelium and increased permeability (Nocek, 1997; Emmanuel et al., 2007; Nagaraja and Titgemeyer, 2007). Lipopolysaccharides are released from ruminal bacteria when the ruminal pH decreases below 5.5 through bacteriolysis (Emmanuel et al., 2007; Nocek, 1997; Leive, 1965). When sub-acute ruminal acidosis was induced by feeding alfalfa pellets, lipopolysaccharide concentrations increased but there was no immune response as was reported when concentrate content of the diet was increased (Khafipour et al., 2009; Khafipour et al., 2011; Emmanuel et al., 2007). When sub-acute ruminal acidosis was induced with grain, there was instead an increase in *Escherichia coli* bacteria and virulence factors, causing an inflammatory response from the ruminal epithelium (Khafipour et al., 2009; Khafipour et al., 2011). *Escherichia coli* is a gram negative bacteria that contains lipopolysaccharides in its cellular structure and is common in the rumen (Emmanuel et al., 2007; Leive, 1965). When the pH is less than 5.5, lipopolysaccharides from *Escherichia coli* are released, causing an inflammatory response from the ruminal epithelium (Emmanuel et al., 2007; Leive, 1965). Supplementing butyrate under these conditions decreases virulence

and increases tissue repair, development and renewal but will not increase ruminal pH (Guilloteau et al., 2010; Sakata and Tamate, 1978).

Lipopolysaccharides also contribute to presence of laminitis in cows experiencing sub-acute and acute ruminal acidosis (Stone et al., 2004; Nocek, 1997). Since lipopolysaccharides can cause vasoconstriction and dilation once in the blood, it is possible for them to destroy small blood vessels such as hoof capillaries, ultimately leading to a loss of normal blood supply to the hoof (Cook et al., 2004; Nocek, 1997; Stone et al., 2004). Once this occurs, the hoof begins to deteriorate and lose function, causing laminitis and severe structural damage especially when cows are routinely housed on hard surfaces such as concrete (Nocek, 1997; Stone et al., 2004; Cook et al., 2004). Prevention of sub-acute, and acute ruminal acidosis is key to eliminating future ruminal, metabolic, and structural problems.

1.7 Summary

Dairy calves are not true ruminants until solid feed consumption exceeds 680 g/d of starter (Quigley, 2001). Once this threshold is met, weaning from milk to solid feed only can occur, which has the best results for optimal starter intake and weight gain if it occurs over the course of ten days (Sweeney et al., 2010; Khan et al., 2007a,b). Feeding forage during the pre-weaning period, regardless of chop length or form, increases productivity in comparison to feeding no forage. However, a medium chop forage source increases weight gains, starter intake, and digestibility than long or short chop, or pelleted forage (Jahani-Moghadam et al., 2015; Montoro et al., 2013). Inclusion of forage between 5 to 10% is optimal for these parameters but exceeding 10% forage inclusion, and 30% inclusion for silage decreases weight gain and starter intake (Coverdale et al., 2004; Castells et al., 2013). Forage also stabilizes ruminal pH, reducing the incidence of sub-acute ruminal acidosis in young calves (Laarman and Oba, 2011; Kleen et al., 2003). Supplementing butyrate during the pre-weaning period in milk and starter increases weight gain, reticulorumen weight, and papillae growth, length, width, and density, which helps weaning calves transition more successfully (Gorka et al., 2009; Gorka et al., 2011). When adding forage and supplemental butyrate to the pre-weaning diet, calves are more successful in the post-weaning period due to an increase in rumen development (Gorka et al., 2011; Khan et al., 2011a). The hypothesis for the first study, the effect of limiting forage intake on ruminal pH in pre-weaned Jersey calves, is if

forage is limited to 90 g/d to pre-weaned calves, then there will be no impact on ruminal pH. For the second study, the effects of supplemental butyrate on sub-acute ruminal acidosis and weaning in Holstein calves, the hypothesis was if butyrate is supplemented at 1% inclusion in calf starter during the two week weaning transition then ruminal pH will be affected.

Chapter 2

The Effect of Limiting Forage Intake on Ruminal pH in Pre-Weaned Jersey Calves

2.1 Abstract

The objective of this study was to determine the effect of limiting forage intake in pre-weaned calves on ruminal pH during the pre-weaning period. Twelve Jersey bull calves (1.92 ± 0.75; mean ± SD) days of age were randomly assigned by birth weight to one of two treatments, *ad libitum* forage (AL) or limit-fed forage (LF) at 90 g/d. All animals were fed the same milk replacer (28% crude protein and 18% fat; dry matter basis) at a rate of 1,200 g/d and the same texturized starter (21.1 % crude protein and 36.5% starch; dry matter basis) *ad libitum* from birth until weaning. Once calves reached an intake of 680 g/d of starter for three consecutive days, ruminal pH was measured for seven days. Individual feed intake was recorded daily, weights were recorded, and jugular blood samples were collected weekly. Rumen fluid was sampled at slaughter along with records of weaning age and body weights. During the pre-weaning period, starter intake, feed efficiency, plasma glucose and β-hydroxybutyrate concentration, volatile fatty acid concentration, minimum and maximum ruminal pH, average daily gain, body weight and age at weaning were not different between treatments although blood metabolites exhibited characteristics of handling stress. Butyrate concentration in the rumen fluid had a tendency to be greater in ad libitum calves compared to limit-fed calves (12.3 ± 2.7 vs. 5.8 ± 3.6 mean ± SE respectively $P = 0.06$). Forage intake for AL calves increased beginning at week 9 (254.89 ± 33.60 vs. 70.94 ± 39.76 mean ± SE; respectively $P < 0.01$). There were tendencies for body weight (121.65 kg ± 6.12 vs. 112.43 kg ± 4.59 mean ± SE; respectively $P = 0.10$) and age at weaning to be lower in AL than LF calves (96.26 d ± 8.11 vs. 82.86 d ± 6.12 mean ± SE; respectively $P = 0.08$). Mean ruminal pH, (6.38 ± 0.16 vs. 5.98 ± 0.23 mean ± SE; respectively $P = 0.09$), duration pH <5.8 (min/d) (796 ± 145 vs. 261 ± 133 mean ± SE; respectively $P = 0.03$), and area under curve pH <5.8 (pH*min/d) (249 ± 47 vs. 60 ± 43 mean ± SE; respectively $P = 0.02$) measured with a ruminal pH logger were greater in AL compared to LF calves. In conclusion, limiting forage intake to 90 g/d has similar performance parameters and starter intake when compared to *ad*

libitum fed calves but does not control the incidence of sub-acute ruminal acidosis at a pH less than 5.8 during the pre-weaning period.

2.2 Introduction

Calf starter fermentation drives rumen papillae growth and development, which makes increasing starter intake an important goal in the pre-weaning period. Excess fermentation may lead to sub-acute ruminal acidosis, which is difficult to detect in young calves because of challenges in its diagnosis (Dirksen et al., 1985). In adult cows, excess fermentation and acid build-up in the rumen is reduced by inclusion of forage in the diet, given the well-established positive correlation between forage intake and rumen pH (Allen, 1997; Zebeli et al., 2008).

Forage feeding has traditionally been discouraged in young dairy calves due to concerns of a decrease in intake and production of propionate and butyrate compared to feeding concentrate (Zitnan et al., 1998; Castells et al., 2013). Propionate and butyrate drive development of ruminal papillae, but high forage diets decrease butyrate and propionate production, which reduces weight gain and performance during the pre-weaning period (Hill et al., 2008a,b). However, forage inclusion can increase development of rumen musculature, increase rumen pH, and growth performance, which can increase rumen motility (Castells et al., 2013; Terre et al., 2015). Several studies have reported that feeding between 5 to 10% forage inclusion in calf rations is optimal for growth, body weight gain, dry matter intake, and limits non-nutritive behaviors (Coverdale et al., 2004; Jahani-Moghadam et al., 2015; Montoro et al., 2013). Even with varied particle size of forage, dry matter intake, rumen pH, and average daily gain all increased with medium chop forage (Nemati et al., 2015). In pre-weaned Holstein calves fed forage *ad libitum*, forage intake above 80 g/d minimized the occurrence of sub-acute ruminal acidosis (Laarman and Oba 2011).

Currently, little is known about the effect of limiting forage intake on calf starter consumption and rumen pH in pre-weaned calves. The objective of the study was to determine the effect of limiting forage intake to 90 g/d to pre-weaned calves on starter intake, ruminal pH, and incidence of sub-acute ruminal acidosis during the pre-weaning period.

2.3 Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee (AUP # 2016-16). All calves were treated with ampicillin (0.90 mg/kg of body weight) upon arrival for 3 days at the recommendation of the attending veterinarian.

2.3.1 Animals, Management, and Treatments

Jersey bull calves (n = 12) at 0 to 5 days of age were fed 4 L of colostrum at birth, were individually housed on sand, and split into two treatments upon arrival in a completely randomized design. Treatments were either *ad libitum* forage or limited forage at 90 g/d of long-stem alfalfa hay beginning at week 2 of life. Calves were fed 8 L/d containing 1,200 g/L of milk replacer (28% crude protein and 18% fat; dry matter basis) (Calva Advantage, Calva Products LLC. Acampo, CA) (Table 2.1) twice daily at 0700 and 1800 hours in equal allotments. An energy supplement (K-Cal Energy Supplement, Calf Solutions Products, Chilton, WI) was added at 20 g/d (7% crude protein and 60% fat; dry matter basis) when the average ambient temperature for the week was less than 0°C. Texturized calf starter (AMPLI-CALF Starter 20, Land O'Lakes Purina Animal Nutrition LLC., Tulare, CA) (21.1% crude protein and 36.5% starch; dry matter basis) and water were available always. Forage was offered starting at 14 days of age. Intakes of grain, forage, and milk replacer were recorded daily. Any refusal of milk greater than 2 L over the course of the day was fed through esophageal tubing upon recommendation of the attending veterinarian due to an outbreak of Salmonellosis. Calves showing any reduced intake or signs of dehydrations or scours were offered electrolytes (RE-SORB, Zoetis Services LLC., Parsippany, NJ) twice daily in addition to their milk replacer allotment.

Body weights were recorded weekly and a jugular blood sample was collected by restraining calves on the ground in a sodium heparin tube for glucose and β-hydroxybutyrate analysis. Blood was spun at 3,000 × g for 20 minutes at 4°C, and plasma was aliquoted and frozen at -20°C for metabolite analysis. Calves were given a ruminal pH logger (Dascor Inc., Escondido, CA) calibrated to a pH of 4.00 and 7.00 prior to administration and before data was downloaded in a supine position, which recorded ruminal pH every two minutes for seven days once the calves reached a threshold of 680 g/d of starter intake for three

consecutive days. At the conclusion of day seven, age and weight were recorded and calves were euthanized by captive bolt and exsanguination. A final blood sample was then collected from the jugular/arterial flow. Rumen fluid samples were collected from the rumen through four layers of cheese cloth and snap-frozen in liquid nitrogen for volatile fatty acid analysis.

2.3.2 Sample Analysis

Commercial enzymatic kits were used for glucose (Glucose assay 997-03001, Wako Diagnostics Inc., Mountain View, California), and β-hydroxybutyrate (3HB assay 417-73501/413-73601, Wako Diagnostics Inc., Mountain View, CA) analysis. 1.5 µL of sample plasma and 175 µL of buffer solution (60 mmol/L phosphate, 5.3 mmol/L phenol, pH 7.1), and 135 µL of color reagent (0.13 U/mL mutarotase, 9.0 U/mL glucose oxidase, 0.65 U/mL peroxidase, 0.50 mmol/L 4-aminoantipyrine, 2.7 U/mL ascorbate oxidase) were analyzed at 37°C at a wavelength of 505 nm in a SpectraMax i3x plate reader (Molecular Devices LLC., San Jose, CA) in triplicate with a CV of less than 5.0 for each sample. For β-hydroxybutyrate analysis, 1.5 µL of sample plasma and 135 µL of R1 (4.27 mmol/L β-Thionicotinamide adenine dinucleotide mixed with 20 mmol phosphate buffer, 5 IU/mL acetoacetate carboxylase, 0.018% sodium azide, pH 7.0) were gently shaken and incubated at 37 °C for 5 minutes. 45 µL of R2 (3200 IU/mL 3-hydroxybutyrate dehydrogenase, 2.65 mmol/L β-nicotinamide adenine dinucleotide disodium mixed with 0.20 mL Good's buffer, 0.053% sodium azide, pH 9.0) was added after incubation and was gently shaken for 2 minutes at 37 °C where initial absorbance readings were taken at a wavelength of 405 nm and subsequent readings were taken every 30 seconds for the next two minutes on a SpectraMax i3x plate reader (Molecular Devices, LLC., San Jose, CA) in triplicate with a CV of less than 5.0 for each sample. Rumen pH was analyzed for minimum, mean, maximum, duration of ruminal pH less than 5.8 and area under the curve of ruminal pH less than 5.8. Volatile fatty acids were analyzed for acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate, and isocaproate acids. For volatile fatty acid analysis, 1 mL of rumen fluid was aliquoted into a 2 mL microcentrifuge tube combined with 250 µL of 25% meta-phosphoric acid and vortexed to mix. The solution was left to precipitate at 22°C for 30 minutes and was then centrifuged at 24,750 x g for 20 minutes at 22°C in an Avanti centrifuge; rotor i.d. JA 18.1 (Beckam Coulter, Indianapolis, IN). The supernatant was decanted into a new 2 mL

microcentrifuge tube and frozen at -20°C for 12 hours, thawed, and centrifuged at 13,000 x g for 10 minutes at 22°C. The supernatant was then transferred to gas chromatography vials and analyzed on an Agilent 6890 series gas chromatographer with a 7673 series injector (Agilent, Wilmington, DE), with a DB-FFAP column using hexane and acetone solvents and 2-ethylbutyric acid 99% (ACROS Organics, Geel, Belgium; Smiricky-Tjardes et al., 2003; Bal et al., 2000).

2.3.3 Statistical Analysis

Recorded body weights were used to determine average daily gain using PROC REG in SAS (SAS 9.4; SAS Inst. Inc., Cary, NC). All data on nutrient, rumen pH, and blood metabolites were analyzed using PROC MIXED in SAS (SAS 9.4; SAS Inst. Inc., Cary, NC) for a completely randomized design according to the following model.

$$Y = \mu + t_i + w_j + t \times w_{ij} + \varepsilon_{ijk}$$

Where Y is the response, μ is the overall mean, t_i is the treatment effect, w_j is the time effect, $t \times w_{ij}$ is the interaction between time and treatment, and ε_{ijk} is the residual term. Temporal blood metabolite data was analyzed accounting for repeated measures through the inclusion of additional terms for time (week) and metabolite × time interaction in the model described previously. The variance-covariance structure of the repeated measures was modeled separately with an appropriate structure fitted using the lowest values of the fit statistics based on the Akaike information criteria. Data on body weight at weaning, age at weaning, feed efficiency and volatile fatty acid profile were analyzed using the MIXED procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC) according to the following model.

$$Y = \mu + t_i + \varepsilon_{ij}$$

Where Y is the response, μ is the overall mean, t_i is the treatment effect, and ε_{ij} is the residual term. Significance was declared at $P \leq 0.05$ for the mean ± standard error of measurement, and tendencies were declared at $0.05 < P \leq 0.10$.

2.4 Results and Discussion

2.4.1 Production Performance

During weeks 2 to 8 of age, there were no differences observed in forage intake across treatments. After week 9, *ad libitum* fed calves began to consume a greater amount of forage beyond that of limit-fed calves ($P < 0.01$; Figure 2.1). Similar to previous observations (Nemati et al., 2015), total forage intake increased from week 9 forward for pre-weaned calves ($P < 0.01$) and there was an effect on treatment by week interaction ($P < 0.01$). Although forage intake was greater in *ad libitum* than limit-fed calves, concentrate intake was similar between treatments for the duration of the study ($P = 0.67$) and was independent of forage intake (Figure 2.2). Concentrate intake increased as calves aged ($P < 0.01$) and there was an effect on treatment by week interaction ($P < 0.01$). Slaughter body weights were not different between treatments ($P = 0.10$; Figure 2.3). However, there was a tendency for calves on the *ad libitum* treatment to be slaughtered several days earlier than those on the limit-fed treatment (Figure 2.4). This tendency may be due to greater numerical feed efficiency (0.51 ± 0.05 vs. 0.47 ± 0.03; $P = 0.24$; Figure 2.6) in calves fed *ad libitum* forage, however, feed efficiency and average daily gain were not different across treatments ($P = 0.61$; Figure 2.5).

Increasing forage content in calf rations is controversial due to the concern of rumen fill and reduced concentrate intake. However, forage is important to decrease the growth of abnormal, polydactyl papillae and to enhance rumen development (Drackley, 2008). Compared to pelleted or ground forage, chopped forage increased dry matter intake, gain to feed ratio, digestibility, and decreased non-nutritive behaviors, while maintaining a similar body weight, hip height and heart girth (Jahani-Moghadam et al., 2015; Montoro et al., 2013). Terre et al. (2015) did not observe differences in intake and growth when feeding a texturized starter with straw, texturized starter without straw, or pelleted starter with straw to pre-weaned calves. Porter et al. (2007) observed an increase in dry matter digestibility when feeding starter with a high than starter with a low amount of roughage in weaned calves. Digestibility was not measured in the current study, but it may explain the tendencies for limit-fed compared to *ad libitum* calves to have a greater body weight and age at weaning with similar average daily gain. However, because the age at weaning was based on concentrate intake,

the tendency for calves on the limit-fed diet to wean at a later age than most *ad libitum* calves likely allowed for more time for body weight to increase although feed efficiency was not different across treatments. This could be attributed to the greater intake of forage by the *ad libitum* treatment, which increased ruminal pH possibly because rumen musculature was more developed in *ad libitum* calves, which could have caused an increase in rumen motility although neither rumen musculature or rumen motility were measured in this study. When ruminal pH is consistently low, less than 5.0, lactic acid producing bacteria can accumulate, which increases lactic acid in the rumen (Nagaraja and Titgemeyer, 2007; Nocek, 1997). This accumulation decreases the ruminal pH and osmolarity, increasing volume of water in the rumen, which decreases feed intake as a result (Nagaraja and Titgemeyer, 2007; Nocek, 1997). When feeding larger quantities of milk with or without forage, Khan et al. (2011a) did not observe difference in total dry matter intake or starter intake at weaning, but provision of forage increased ruminal pH, rumen weight, and dry matter intake after week 6 of life, which is consistent with our results. Placement of the ruminal pH logger could have had an impact on rumen motility and pH measurements in this study, however, all loggers were retrieved from the ventral sac of the rumen but depending on rumen motility, the mixing of the rumen contents can cause differences in the regional pH. Castells et al. (2013) reported that when forage intake was increased beyond 10%, gut fill increased, but this did not reduce concentrate consumption and total dry matter intake, which is likely the case in our study. The benefits of feeding forage *ad libitum* were manifested in week 8 to 9 where intake surpassed that of the limit-fed calves but because the average daily gains were similar as were the body weights, it suggests that feeding 90 g/d of forage to calves is sufficient to maintain normal growth parameters.

2.4.2 Blood Metabolites

Plasma glucose ($P = 0.43$) and β-hydroxybutyrate ($P = 0.69$) concentrations were not different across treatments (Figure 2.7, 2.8). This is in contrast to Quigley et al. (1991) who recorded a decrease in plasma β-hydroxybutyrate in correspondence to increased forage intake. However, forage intake was restricted in this study to 90 g/d for the limit-fed treatment and β-hydroxybutyrate concentration increased with age of the calf ($P < 0.01$) regardless of treatment, and had no interaction effect between treatment and week ($P = 0.38$),

which is consistent with Quigley et al. (1991). Khan et al. (2008a,b) reported that as calf age increased, glucose concentration decreased, which is not consistent with our observations. The glucose concentrations in this study increased with age ($P < 0.01$), which may be a result of stable milk replacer intake until slaughter, but there was no interaction effect between treatment and week ($P = 0.41$). However, there was large variability in our blood sampling technique where the duration of sampling time for each calf caused variation in the actual time of sampling for each calf on a weekly basis. Calves were due to be sampled one hour post-feeding but some calves had to wait as long as 2 hours to be sampled depending on the week. These animals were under acute stress during blood sampling due to our method of restraint. Some calves took as long as 10 minutes to sample whereas other calves may have taken less than one minute. This varied from calf to calf each week which is why our results align more closely to blood glucose and β-hydroxybutyrate values reported by Suarez-Mena et al. (2017). Sampling time of day did not have an effect on blood glucose or β-hydroxybutyrate concentrations but age increased β-hydroxybutyrate (0.07 to 0.27 mmol/L) and decreased glucose (98.4 to 80.3 mg/dL) from week 0 to 7 of life (Suarez-Mena et al., 2017). Suarez-Mena et al. (2017) also reported that when acute stress was induced, β-hydroxybutyrate concentrations decreased but glucose was unaffected, which is consistent with this study.

2.4.3 Rumen Parameters

The volatile fatty acid profiles did not differ across treatments ($P > 0.05$; Table 2.2), however, there was a tendency for total concentration of butyrate to be greater in *ad libitum* calves compared to limit-fed calves (12.3 ± 2.7 vs. 5.8 ± 3.6; respectively, mean ± SE; $P = 0.06$). The concentrations of acetate, propionate, and butyrate are consistent with the ratios described by Bergman, (1990) where in adult cows, the ratio can range from 40:40:20 to 75:15:10 for acetate, propionate, and butyrate respectively. The ratio observed in this study where samples of rumen fluid were taken at slaughter is 70:20:10 respectively, which is consistent with volatile fatty acid concentrations in adult cows, suggesting that these calves were consuming enough solid feed at weaning to rely solely on volatile fatty acids from starter and hay fermentation as their main energy source.

Mean rumen pH tended to be greater in *ad libitum* calves ($P = 0.09$), which can be related to the increase in forage intake from week 9 to the end of the study (Table 2.3; Figure 2.1). Laarman and Oba, (2011) observed an increase in rumen pH with the addition of forage, and the relationship between dry matter intake of forage and area under the curve <5.8 pH min*d hit a breakpoint of 0 at 80 g/d of forage intake indicating that 80 g/d is sufficient to alleviate sub-acute ruminal acidosis in calves. This was not consistent with results in the current study as limit-fed calves fed at 90 g/d experienced sub-acute ruminal acidosis 3 times as long ($P = 0.03$) and 4 times as severe ($P = 0.02$) when compared with the *ad libitum* treatment. It is possible that rumen pH could have been distributed differently had calves been fed forage twice daily as opposed to once daily for the limit-fed treatment. Ruminal pH fluctuates and decreases at feeding time in calves (Khan et al., 2008a,b; Suarez et al., 2007). By measuring consistently over the course of seven days for each calf, we gain a more accurate representation of ruminal pH over a 24 hour period ultimately showing that 90 g/d of forage is not sufficient to control sub-acute ruminal acidosis in pre-weaned calves.

2.5 Conclusion

Providing a forage source of 90 g/d is sufficient to maintain body weight at weaning, average daily gain, and age at weaning similar to calves fed *ad libitum* forage. Blood plasma glucose and β-hydroxybutyrate were similar between treatments. Starter intake and feed efficiency did not differ by treatment and no effect of rumen fill was witnessed in this trial although it was not measured. These data indicate that pre-weaned calves can be fed 90 g/d of forage without compromising performance or starter intake. However, 90 g/d of forage intake was not sufficient to alleviate sub-acute ruminal acidosis indicating that forage provision must be increased to minimize the negative effects of sub-acute ruminal acidosis during the pre-weaning period.

Chapter 3

The Effects of Supplemental Butyrate on Sub-acute Ruminal Acidosis and Weaning in Holstein Calves

3.1 Abstract

The objectives of these studies were to determine the effects of supplemental butyrate on sub-acute ruminal acidosis, intake, and growth parameters during the weaning transition and post-weaning periods. In experiment 1, Holstein bull calves (n = 17) were split into two groups based on age and were then assigned to one of two treatments: calves fed milk replacer only (PRE-M), or fed milk replacer, starter, and forage (PRE-S). In experiment 2 (n = 16), all calves were fed starter and forage until a two-week weaning transition during weeks 7 and 8. Weaning was over the course of 14 days whereby 2 L of milk replacer was removed each week. During the weaning transition, calves received either no supplement (POST-S) or supplemental butyrate at 1% of calf starter (dry matter basis) (POST-B). Rumen pH was measured continuously for 7 days during week 6 prior to weaning (PRE-S and PRE-M) and during week 9 after completion of weaning (POST-S and POST-B). Thereafter, calves were euthanized and rumen fluid was sampled. In both studies, individual feed intake was recorded daily while weights were recorded and blood samples were collected weekly. In experiment 1, PRE-S calves had greater total volatile fatty acid mM (35.60 ± 5.60 vs. 11.80 ± 5.80 mean ± SE respectively; $P < 0.01$), propionate (23.10 ± 2.60 vs. 15.30 ± 2.70 mean ± SE respectively; $P < 0.01$), feed efficiency (1.55 ± 0.42 vs. 1.30 ± 0.19 mean ± SE respectively; $P = 0.02$), and plasma βHBA ($P = 0.04$). There was a tendency for PRE-S calves to have a greater A:P ratio (2.30 ± 0.30 vs. 1.60 ± 0.30 mean ± SE respectively; $P = 0.07$), and PRE-M calves had greater isobutyrate (5.60 ± 0.06 vs. 2.20 ± 0.50 mean ± SE respectively; $P < 0.01$), isovalerate (2.40 ± 0.20 vs. 1.70 ± 0.20 mean ± SE respectively; $P = 0.02$), and caproate (1.10 ± 0.10 vs. 0.30 ± 0.10 mean ± SE respectively; $P < 0.01$). There were no differences across treatments for ruminal pH parameters ($P > 0.10$), average daily gain ($P = 0.33$), or plasma glucose concentrations ($P = 0.81$). In experiment 2, POST-B calves had greater, average daily gain (0.91 ± 0.04 vs. 0.77 kg/d ± 0.04 mean ± SE; respectively; $P = 0.03$), propionate concentration (34.90 ± 2.70 vs. 27.90 ± 2.70 mean ± SE; respectively $P = 0.05$), and had a

tendency for starter intake (450.77 ± 218.61 vs. 249.53 g ± 218.61 mean ± SE; respectively; *P* = 0.09) to be greater than POST-S calves. POST-S calves had greater mean ruminal pH (6.39 ± 0.19 vs. 5.83 ± 0.18 mean ± SE; respectively *P* = 0.05), and had a tendency to have longer duration pH less than 5.8 (min/d) (730 ± 199 vs. 209 ± 213 mean ± SE respectively; *P* = 0.10) and greater A:P ratio (2.30 ± 0.30 vs. 1.60 ± 0.30 mean ± SE respectively; *P* = 0.07). Other ruminal pH parameters (P > 0.10), volatile fatty acid concentrations (*P* >0.10), feed efficiency (*P* = 0.73), and plasma glucose (*P* = 0.88) and βHBA (*P* = 0.65) concentrations were not different across treatments although both experiments did exhibit characteristics of handling stress for blood metabolite values. In conclusion, supplementing butyrate at 1% inclusion (dry matter basis) in calf starter during the weaning transition increased starter intake, propionate concentration, and average daily gain during the weaning and post-weaning periods but induces sub-acute ruminal acidosis.

3.2 Introduction

In newborn calves, the rumen is underdeveloped and physiologically nonfunctional until establishment of a microbial population occurs (Khan et al., 2011b). Consumption of solid feed, specifically calf starter, initiates fermentation process, which elevates the concentration of volatile fatty acids such as butyrate, which is known to initiate and encourage the development of rumen epithelial papillae (Drackley, 2008; Penner et al., 2011; Bergman, 1990). A smooth transition from a milk-based diet to solid feed is essential for maintaining adequate intake, minimizing weight loss and stress at weaning, and increasing butyrate production to drive the growth and differentiation of the rumen epithelium (Weary et al., 2009; Quigley et al., 1991; Sweeney et al., 2010). With a properly functioning absorptive rumen at weaning, uptake of volatile fatty acids increases, making more energy available to the calf, but also decreases the incidence of ruminal acidosis through removal of excess protons in the rumen (Baldwin and Jesse, 1992; Dirksen et al., 1985). Sub-acute ruminal acidosis often occurs in pre-weaned calves but goes unnoticed, which causes issues such as parakeratosis and non-absorptive, polydactyl papillae (Allen, 1997). During the weaning transition, the change in nutrient intake from milk replacer to rapidly fermentable, non-structural carbohydrates found in calf starter can cause rumen epithelial inflammation from rapid increases in volatile fatty acids in the rumen (Plazier et al., 2009; Baldwin et al., 2004).

Stable ruminal pH above 5.8 is crucial for normal rumen development. Therefore, improved development of the rumen from an early age has been explored by supplementing butyrate at different points in time (Khan et al., 2016; Connor et al., 2013). When supplemented in milk replacer and calf starter during the pre-weaning period, butyrate increased body weight, reticulorumen weight, ruminal papillae length, and width (Gorka et al., 2009, 2011). These results are consistent with supplementation of different butyrate salts that increase the integrity of the ruminal epithelium as well as the growth and density of ruminal papillae (Govil et al., 2017). Butyrate inclusion at 2.5% (dry matter basis) in lactating animals upregulated transcript abundance of transporter proteins including; monocarboxylate transporter 1 (MCT1), (Na^+/H^+) NHE3, and (Na^+/HCO_3) NBC1, indicating greater uptake of volatile fatty acids within the rumen while pH remained similar across treatments (Laarman et al., 2013). To our knowledge, butyrate supplementation of calf starter has not yet been investigated during the weaning transition. The objective of experiment 1 was to determine the effect of calf starter on ruminal acidosis, intake, and growth parameters in the pre-weaning period. The objective of experiment 2 was to determine the effect of supplemental butyrate on sub-acute ruminal acidosis, intake, and growth parameters during the weaning and post-weaning period.

3.3 Materials and Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee (AUP # 2016-32). All calves were treated with 0.90 mg/kg of body weight ampicillin upon arrival for 3 days at the recommendation of the attending veterinarian.

3.3.1 Animals and Treatments

Holstein bull calves (n = 33) from a single farm were fed 4 L of colostrum at birth, and were received in two groups, group 1 (n = 17), and group 2 (n = 16) two weeks apart with equal representation of the following age ranges; 5 days (n = 9), 10 days (n = 12), and 16 days (n = 12). All calves were individually housed on sand and fed 6 L/d with 1,200 g/L of (28% crude protein and 18% fat; dry matter basis; Calva Advantage, Calva Products LLC. Acampo, CA) (Table 3.1) milk replacer twice daily at 0630 and 1700 hours in equal allotments. Texturized calf starter (AMPLI-CALF Starter 20, Land O'Lakes Purina Animal Nutrition

LLC., Tulare, CA; 22.1% crude protein and 36.5% starch; dry matter basis), medium chopped alfalfa forage (19.8% crude protein, 35% ADF, and 42.2% NDF; dry matter basis), and water were available at all times for all treatments except pre-weaned, milk replacer only (PRE-M) calves in experiment 1. Starter was offered beginning at 16 days of age and forage was offered beginning at 21 days of age. Starter was supplemented with 1% (dry matter basis) of powdered butyrate (UltramixC, Nutriad, Hampshire, IL) during the weaning period for post-weaning calves on the butyrate treatment (POST-B). Intakes of grain, forage, and milk replacer were recorded daily. Any refusal of milk replacer greater than 2 L over the course of the day was fed through esophageal tubing upon recommendation of the attending veterinarian due to an outbreak of rotavirus and heat stress. Calves showing any reduced intake, dehydration, or scours were offered electrolytes (RE-SORB, Zoetis Services LLC., Parsippany, NJ) twice daily in addition to their milk replacer allotment.

Body weights were recorded weekly and a jugular blood sample was collected by restraining calves on the ground in a sodium heparin tube for glucose and β-hydroxybutyrate analysis. Blood was centrifuged at 3000 × g for 20 minutes at 4°C, and plasma was aliquoted and frozen at -20°C for metabolite analysis. Eight days prior to the scheduled slaughter date for each calf in both experiments, calves were given a ruminal pH logger (Dascor Inc., Escondido, CA) calibrated to a pH of 4.00 and 7.00 prior to administration and before data was downloaded in a supine position, which recorded the ruminal pH every two minutes for seven days. At the conclusion of day seven, age and weight at slaughter were recorded; then, calves were euthanized by captive bolt and exsanguination. A final blood sample was then collected from the jugular/arterial flow. Rumen fluid samples were collected from the rumen through four layers of cheesecloth and snap-frozen in liquid nitrogen for later volatile fatty acid analysis.

3.3.2 Experiment 1

The first group of Holstein bull calves (n = 17) were blocked by age and randomly assigned to one of two treatments in a randomized complete block design. Their dietary treatments were either; milk replacer only (PRE-M), or milk replacer, texturized starter, and alfalfa forage (PRE-S). These calves were slaughtered at six weeks of age and were not weaned during this trial.

3.3.3 Experiment 2

The second group of Holstein bull calves (n = 16) were blocked by age and randomly assigned to one of two treatments in a complete randomized block design. The dietary treatments were; milk replacer, texturized starter, and alfalfa forage (POST-S), or milk replacer, texturized starter, and alfalfa forage with 1% powdered, supplemental butyrate (UltramixC, Nutriad, Hampshire, IL) added to the starter during the 2 week weaning transition beginning at week 7 of age (POST-B). Supplementing at 1% (dry matter basis) was chosen because adult animals have a threshold of 4% (dry matter basis) supplemental butyrate in the ration and neonatal calves have been supplemented as low as 0.01% (dry matter basis; Gorka et al., 2009; Gorka et al., 2011). During weaning, calves have greater body weight than neonatal calves but do not weigh enough to consume 4% butyrate in the ration so a supplemental quantity of 1% (dry matter basis) was chosen for this study. Calves were weaned over the course of 14 days, reducing intake by 2 L every seven days. Once weaned, butyrate starter was replaced with normal starter until calves were slaughtered at 10 weeks of age.

3.3.4 Sample Analysis

Commercial enzymatic kits were used for glucose (Glucose assay 997-03001, Wako Diagnostics Inc., Mountain View, California) and β-hydroxybutyrate (3HB assay 417-73501/413-73601, Wako Diagnostics Inc., Mountain View, CA) analysis. For glucose analysis, 1.5 μL of sample plasma and 175 μL of buffer solution (60 mmol/L phosphate, 5.3 mmol/L phenol, pH 7.1), and 135 μL of color reagent (0.13 U/mL mutarotase, 9.0 U/mL glucose oxidase, 0.65 U/mL peroxidase, 0.50 mmol/L 4-aminoantipyrine, 2.7 U/mL ascorbate oxidase) were analyzed at 37 °C at a wavelength of 505 nm in a SpectraMax i3x plate reader (Molecular Devices LLC., San Jose, CA) in triplicate with a CV of less than 5.0 for each sample. For β-hydroxybutyrate analysis, 1.5 μL of sample plasma and 135 μL of R1 (4.27 mmol/L β-Thionicotinamide adenine dinucleotide mixed with 20 mmol phosphate buffer, 5 IU/mL acetoacetate carboxylase, 0.018% sodium azide, pH 7.0) were gently shaken and incubated at 37 °C for 5 minutes. 45 μL of R2 (3200 IU/mL 3-hydroxybutyrate dehydrogenase, 2.65 mmol/L β-nicotinamide adenine dinucleotide disodium mixed with

0.20 mL Good's buffer, 0.053% sodium azide, pH 9.0) was added after incubation and was gently shaken for 2 minutes at 37 °C where initial absorbance readings were taken at a wavelength of 405 nm and subsequent readings were taken every 30 seconds for the next two minutes on a SpectraMax i3x plate reader (Molecular Devices, LLC., San Jose, CA) in triplicate with a CV of less than 5.0 for each sample. Rumen pH was analyzed for minimum, mean, and maximum pH, duration of ruminal pH less than 5.8 and area under the curve of ruminal pH less than 5.8. Rumen fluid was analyzed for acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate, and isocaproate acids. For volatile fatty acid analysis, 1 mL of rumen fluid was aliquoted into a 2 mL microcentrifuge tube combined with 250 μL of 25% meta-phosphoric acid and vortexed to mix. The solution was left to precipitate at 22°C for 30 minutes and was then centrifuged at 24,750 x g for 20 minutes at 22°C in an Avanti centrifuge; rotor i.d. JA 18.1 (Beckam Coulter, Indianapolis, IN). The supernatant was decanted into a new 2 mL microcentrifuge tube and frozen at -20°C for 12 hours, thawed, and centrifuged at 13,000 x g for 10 minutes at 22°C. The supernatant was then transferred to gas chromatography vials and analyzed on an Agilent 6890 series gas chromatographer with a 7673 series injector (Agilent, Wilmington, DE), with a DB-FFAP column using hexane and acetone solvents and 2-ethylbutyric acid 99% (ACROS Organics, Geel, Belgium; Smiricky-Tjardes et al., 2003; Bal et al., 2000).

3.3.5 Statistical Analysis

Due to a heat wave during experiment 1, hutch temperatures exceeded 35°C, and calves for experiment 2 were moved indoors to alleviate heat stress in a non-climate controlled, individually housed setting. As a result, experiment 1 and experiment 2 were analyzed separately.

Recorded body weights were used to determine average daily gain using PROC CORR in SAS (SAS 9.4; SAS Inst. Inc., Cary, NC). All data on nutrient intake, rumen pH, and blood metabolites were analyzed using the MIXED procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC) for a randomized complete block design according to the following model

$$Y = \mu + t_i + w_j + t \times w_{ij} + \varepsilon_{ijk}$$

where Y is the response, μ is the grand mean, t_i is the treatment effect, w_j is the time effect, $t \times w_{ij}$ is the interaction between time and treatment, and ε_{ijk} is the residual term. Temporal blood metabolite data was analyzed accounting for repeated measures through the inclusion of additional terms for time (week) and metabolite × time interaction in the model described previously. The variance-covariance structure of the repeated measures was modeled separately with an appropriate structure fitted using the lowest values of the fit statistics based on the Akaike information criteria. Data on body weight at weaning, age at weaning, and volatile fatty acid profile were analyzed using the MIXED procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC) according to the following model

$$Y = \mu + t_i + \varepsilon_{ij}$$

where Y is the response, μ is the overall mean, t_i is the treatment effect, and ε_{ij} is the residual term. Significance was declared at $P \leq 0.05$ for the means ± standard error of measurement and tendencies were declared at $0.05 < P \leq 0.10$.

3.4 Results and Discussion

3.4.1 Experiment 1

3.4.1.1 Production Performance

Over the 4 week period for the pre-weaning study, there were no differences (P = 0.33) in average daily gain across treatments with calves fed milk replacer only (PRE-M), or milk replacer, starter and forage (PRE-S) gaining 0.87 and 0.82 kg/d, respectively (Figure 3.1). This is likely due to minimal intake of solid feed by PRE-S calves where total average forage intake was 282.02 g and total average starter intake was 327.36 g indicating that nutrients for growth were mainly derived from milk replacer allotment (Appendix 2). Similar results were reported by Khan et al., (2007a,b); total solid feed intake (starter and forage combined), for the first 28 days was below 500 g/d. Roth et al. (2009) also made similar observations in calves prior to weaning. Feed efficiency was greater in PRE-S calves (1.56 ± 0.42 vs. 1.30 ± 0.19; mean ± SE respectively; P = 0.02) although average daily gain was not different between treatments. This may be a result of solid feed intake by PRE-S calves despite intake being minimal during this study.

3.4.1.2 Blood Metabolites

Plasma glucose concentration was not different between treatments ($P = 0.81$) or the time by treatment interaction ($P = 0.45$), but increased as calves aged ($P = 0.04$; Figure 3.2). This is consistent with Laarman et al., (2012b), but Khan et al., (2007a,b) observed a decrease in blood glucose as calves aged, and this decrease occurred beginning at 6 weeks of age, which is when both PRE-M and PRE-S calves were slaughtered. Therefore plasma glucose concentration could have changed if we had fed calves beyond 6 weeks in our study. Solid feed consumption was minimal in PRE-S calves, which suggest that rumination was marginal at this point in time and the shift to reliance on volatile fatty acids from ruminal fermentation had not yet happened. This is supported by the β-hydroxybutyrate concentration, which did not change with time ($P = 0.69$) or time by treatment interaction ($P = 0.72$), but was greater in PRE-S calves compared to PRE-M calves ($P = 0.04$), which is most likely due to the presence of solid feed, specifically calf starter (Figure 3.3). Laarman et al., (2012b) fed milk and forage, or milk, forage, and starter and observed no differences in β-hydroxybutyrate concentration across treatments over the course of 8 weeks, which is consistent with our study.

3.4.1.3 Rumen Parameters

Total volatile fatty acid concentration (35.6 ± 5.6 vs. 11.8 ± 5.8; mean ± SE respectively; $P < 0.01$), and propionate (23.1 ± 2.6 vs. 15.3 ± 2.7; mean ± SE respectively; $P < 0.01$) were greater in PRE-S compared to PRE-M calves and there was a tendency for the A:P ratio to be greater (2.3 ± 0.3 vs 1.6 ± 0.3; mean ± SE respectively; $P = 0.07$) in PRE-S calves as well. PRE-M calves had greater isobutyrate (5.6 ± 0.6 vs. 2.2 ± 0.5; mean ± SE respectively; $P < 0.01$), isovalerate (2.4 ± 0.2 vs. 1.7 ± 0.2; mean ± SE respectively; $P = 0.02$), and caproate (1.1 ± 0.1 vs. 0.3 ± 0.1; mean ± SE respectively; $P < 0.01$) concentrations compared to PRE-S calves. Changes in volatile fatty acid profile most likely result from ingestion of forage and starter as acetate and propionate are the main products of fermentation from these feeds (Bergman, 1990; Van Soest, 1982a,b). This is consistent with Quigley et al. (1991) where there was an increase in acetate and propionate concentrations with age and solid feed intake, although intakes were minimal in this study and rumen fluid was only

sampled once at slaughter. There were no differences across treatments for acetate (P = 0.58), butyrate (P = 0.97), valerate (P = 0.58), or isocaproate (P = 0.20) in the rumen fluid, which may be attributed to the lack of substantial starter and forage intake by the PRE-S calves to initiate excess production of acetate and butyrate. Absorption of volatile fatty acids across the rumen epithelium largely depends on development of the epithelial cells in addition to metabolic activity, which all stem from solid feed intake (Van Soest, 1982a). Solid feed intake was non-existent for PRE-M, and negligible for PRE-S in experiment 1. Low (<40 m*M*) concentrations of volatile fatty acids in both treatments may explain why rumen pH, mean, min, max, duration pH<5.8 min/d and area under curve pH <5.8 (pH*min/d) were not different between treatments again indicating that solid feed consumption during the four weeks of trial may not have been enough to induce sub-acute ruminal acidosis in PRE-M or PRE-S calves or that buffering capacity of the rumen is increased during this time period (Table 3.3).

3.4.2 Experiment 2

3.4.2.1 Production Performance

Forage intake was not different across treatments (P = 0.66), time (P = 0.90), or time by treatment interaction (P = 0.99) for POST-S and POST-B calves, but total intake increased most noticeably around 6 weeks of age, which is when milk replacer allotment was initially decreased to begin the weaning process (Figure 3.4). Starter intake had a tendency to be different across treatments (P = 0.09) and increased with time (P < 0.01), and time by treatment interaction (P = 0.02) beginning at 6 weeks where POST-B calves had a greater intake than POST-S calves (450.77 g ± 218.61 vs. 249.53 g ± 218.61 mean ± SE; respectively) suggesting that inclusion of butyrate in starter 1% (dry matter basis) affected starter consumption positively during the weaning transition and into the post-weaning period (Figure 3.5). This increase in starter intake possibly resulted in greater average daily gain (P = 0.03) in POST-B calves than in POST-S calves (0.91 ± 0.04 vs. 0.77 kg/d ± 0.04 mean ± SE; respectively) although feed efficiency was not different across treatments (P = 0.73; Figure 3.6, 3.7). Sweeney et al. (2010) made similar observations in starter intake when weaning calves over 10 days, which is comparable to the 14 days used in our study. When supplemented with butyrate in milk replacer and starter, body weight increases, which could

also be the effect we observe here where butyrate increases body weight by increasing papillae length, width and density in addition to increasing transport protein activity in the ruminal epithelium (Gorka et al., 2011; Laarman et al., 2013; Govil et al., 2017). Other butyrate supplementation studies (Gorka et al., 2009) did not report an increase in average daily gain but only an increase in body weight for the first 11 days and then a second body weight increase at weaning. In this study, butyrate concentration in the rumen fluid (P = 0.35) and β-hydroxybutyrate concentration in the blood (P = 0.65) however, were not different across treatments, which may be because the butyrate source used in this study was rumen protected butyrate. With this in mind, it is possible that supplementation of butyrate during the pre-weaning period is only necessary during the weaning transition to gain maximum benefits for body weight gains in the post-weaning period as opposed to supplementing butyrate during the entire pre-weaning period however, more research comparing time, and method of supplementation is needed to determine the optimal supplementation procedure. It must be noted that there are rumen-protected, and rumen-unprotected sources of supplemental butyrate, which can impact the absorptive quality of either the rumen or small intestinal papillae depending on the supplement. In this study, papillae were not measured, however it would be pertinent to determine the source of body weight gain whether it was from increased volatile fatty acid absorption in the rumen, or increased energy absorption in the small intestine. Since β-hydroxybutyrate concentrations were not different across treatments, and β-hydroxybutyrate is a product of rumen epithelial metabolism, we speculate that the increase in weight gain in POST-B calves may have been from greater intestinal absorption. Based on the gains we observed and as reported by Gorka et al. (2009; 2010), feeding butyrate in the starter could be a more effective than feeding in milk replacer, which tend to bypass the rumen. However, more research is needed on the proper time, amount, and method by which butyrate should be supplemented during the pre-weaning period.

3.4.2.2 Blood Metabolites

Plasma glucose concentrations were not different (P = 0.88) between treatments. However, across treatments, glucose concentration decreased (P < 0.01) and there was a time by treatment interaction (P = 0.04) after week 6 of life, which is consistent with observations

by Khan et al., (2007a,b) and Baldwin and Jesse, (1992; Figure 3.7). Due to a drastic increase in solid feed consumption beginning at week 6, we expected a decrease in glucose concentration in response to rumen epithelial development and increased reliance on volatile fatty acids compared to glucose as an energy source. Although we did not measure volatile fatty acid concentrations weekly, the change in plasma β-hydroxybutyrate concentration is suggestive of such changes ($P < 0.01$) where there was no difference across treatments ($P = 0.65$) or time by treatment interaction ($P = 0.20$; Figure 3.10). Khan et al. (2007 a,b) made similar observations. During week 6 to 8 for when butyrate was supplemented in calf starter for POST-B calves, we expected to see an increase in β-hydroxybutyrate concentration however; there were no differences across treatments. This is likely because the supplemented butyrate was rumen protected and was not metabolized by the rumen epithelium to β-hydroxybutryate, but was instead absorbed in the small intestine. This likely contributed to the increase in average daily gain for POST-B calves compared to POST-S calves however site of absorption was not measured in this experiment.

3.4.2.3 Rumen Parameters

Of all volatile fatty acids analyzed, only propionate tended ($P = 0.05$) to have a greater concentration (34.9 ± 2.7 vs. 27.9 ± 2.7 mean ± SE; respectively) for the POST-B compared to POST-S calves (Table 3.4). There was also a tendency for the acetate to propionate ratio to be greater ($P = 0.07$) in POST-S compared to POST-B calves (2.3 ± 0.3 vs. 1.6 ± 0.3 mean ± SE; respectively; Table 3.4). This is likely due to the greater intake of starter for POST-B calves, which led to greater production of volatile fatty acids. The increase in propionate concentration could explain the decrease in acetate to propionate ratio, as well as ruminal pH for POST-B calves where mean ruminal pH was lower than that of POST-S calves (6.39 ± 0.19 vs. 5.83 ± 0.18 mean ± SE; respectively; $P = 0.05$; Table 3.5). There were no differences across treatments ($P > 0.05$) for min pH, max pH, standard deviation or area under the curve pH<5.8 (pH*min/d) but there was a tendency ($P = 0.10$) for duration pH<5.8 (min/d) to be greater in POST-B calves than POST-S calves (730 ± 199 vs. 209 ± 213 mean ± SE; respectively). The amount of time spent at a pH less than 5.8 was 3 times greater for POST-B than POST-S calves possibly because ruminal pH was measured in the 7 days post-weaning where supplementation occurred during the 2 weeks prior, and starter intake was still

increasing until slaughter. The threshold for sub-acute ruminal acidosis is pH of less than 5.8 for at least 180 min/d, which was reached across treatments. While many thresholds for sub-acute ruminal acidosis exist; 5.5 (Krause and Oetzel, 2006; Nocek et al., 1997), 5.6 (Gozho et al., 2005; Kuenen et al., 2002), and 5.8 (Aschenbach et al., 2011), we used 5.8 as our threshold for this study. At a pH of less than 5.8, fermentation patterns begin to shift in favor of lactate using bacteria, propionate concentrations increase, microbial activity begins to change due to decreased cellulolytic bacteria and protozoa, and the rumen epithelium can become inflamed (Aschenbach et al., 2011). Laarman and Oba (2011) looked at the effects of starter on ruminal pH at weaning and observed similar results where there were no differences in the min pH, mean pH, max pH, or duration pH<5.8 (min/d) but they did observe a breakpoint of forage inclusion at 80 g/d for the area under the curve pH<5.8(pH*min/d). This shows that forage inclusion does in fact minimize the severity of sub-acute ruminal acidosis, which could explain in this study why POST-S calves and POST-B calves had minimal incidence because forage intake increased with age just as starter intake did. Inducing acidosis with high grain diets in cows where intake directly correlates with pH decrease causes a decrease in dry matter intake, which was not observed in this study (Steele et al., 2009). At a lower pH in the range of 5.5 to 6.0, there is better uptake of volatile fatty acids, specifically acetate, propionate and butyrate where ruminal pH between 5.57 and 6.67 increase uptake of acetate and butyrate specifically but as pH declines below 5.8, uptake declines along with it (Dirksen et al., 1985; Penner et al., 2009). In this pH range however, bicarbonate uptake also increased, which could explain why POST-B calves had greater average daily gain compared to POST-S calves. The POST-B conditions for pH and intake are similar to both Dirksen et al. (1985) and Penner et al. (2009) suggesting this pH range may in fact be optimal for uptake of volatile fatty acids during the weaning transition.

3.5 Conclusion

Supplementing butyrate 1% inclusion of calf starter (dry matter basis) during the weaning transition increased average daily gain and starter intake, and had no effect on forage intake, feed efficiency, plasma glucose or β-hydroxybutyrate concentration across treatments. Mean ruminal pH had a tendency to be lower, and duration pH<5.8 (min/d) had a tendency to be greater in supplemented calves indicating that incidence of sub-acute ruminal acidosis was

greater for this treatment. This demonstrates that inclusion of supplemental butyrate during the weaning transition has a positive effect on weaning success through increased weight gain and starter intake, but increases the risk of sub-acute ruminal acidosis in the weaning and post-weaning period

Chapter 4

Summary

Weaning is one of the most stressful times in a young calf's life where reliance on liquid feed must end, leaving solid feed as the only source of nutrition. Calf starter is the primary solid feed of interest due to its rapid fermentation to propionate and butyrate, which are known to increase papillae length, width, and density. However, with such rapid fermentation, ruminal pH becomes variable and tends to remain at a pH of less than 5.8, which is the threshold for sub-acute ruminal acidosis (Aschenbach et al., 2011; Laarman and Oba, 2011). Therefore, the objectives of this research were to investigate the impact of limit-fed forage prior to weaning, and the impact of supplemental butyrate in calf starter during weaning on rumen pH, body weight, and animal performance.

4.1 Study Summaries

The first study investigated the effect of limiting forage intake to pre-weaned calves on ruminal pH. Despite limiting forage provision, starter intake, minimum ruminal pH, and maximum ruminal pH were not different between treatments. Body weight at slaughter tended to be lower for *ad libitum* calves, which is likely due to the tendency for age at weaning to be lower as well, meaning *ad libitum* calves had less total time for growth. Yet, by feeding 90g/d of forage, productivity is not compromised in limit-fed calves because there were no differences across treatments in feed efficiency. Ruminal pH however was different across treatments where mean ruminal pH had a tendency to be lower in limit-fed calves, and duration and intensity of sub-acute ruminal acidosis were much greater in the limit-fed calves showing that 90 g/d of forage is not sufficient to control sub-acute ruminal acidosis in pre-weaned calves.

In study two, calves were split into two experiments. In experiment one, calves were fed either milk replacer only (PRE-M) or milk replacer, starter, and forage (PRE-S) and euthanized at 6 weeks of age prior to weaning. In experiment two, calves were fed either milk replacer, starter, and forage (POST-S), or milk replacer, starter, forage, and supplemental butyrate during the two-week weaning period in weeks 7 and 8 (POST-B). Experiment 1 calves, PRE-M and PRE-S, had no differences between treatments for average daily gain,

plasma glucose concentration, or ruminal pH parameters. PRE-S calves had greater feed efficiency, plasma β-hydroxybutyrate concentration, total volatile fatty acid concentration, acetate to propionate ratio, and concentration of propionate, which is likely due to the presence of calf starter in the PRE-S treatment. Between POST-S and POST-B, there were no differences for forage intake, feed efficiency, plasma glucose, and β-hydroxybutyrate concentrations, volatile fatty acid concentration, or ruminal pH parameters. Starter intake, and average daily gain increased for POST-B calves during weaning when butyrate was supplemented in calf starter. There was a tendency for propionate to be greater and the acetate to propionate ratio to be lower in POST-B calves, which is likely due to the increase in starter intake.

4.2 Role of Forage Provision Pre-Weaning

Our first study demonstrated that forage inclusion is important for pre-weaned calves to alleviate sub-acute ruminal acidosis, and feeding limited forage does not compromise starter intake or weight gain as was previously thought. This study shows that there needs to be further research on the amount of forage included in the diet to alleviate the symptoms of sub-acute ruminal acidosis. Laarman and Oba (2011) found the breakpoint for sub-acute ruminal acidosis was 80 g/d of forage intake however; this study shows that ruminal pH is still much too variable even at 90 g/d of forage. An incremental study could potentially determine if a single allotment, multiple feedings, or a ration inclusion percentage would be the optimal method for providing limited forage quantity while controlling sub-acute ruminal acidosis incidence in calves.

4.3 Butyrate Supplementation in Young Calves

From this study we now know that supplementing butyrate solely over the course of weaning does not decrease the incidence of sub-acute ruminal acidosis but it increased starter intake and body weight. Studies have been completed on supplementing butyrate in milk replacer and calf starter during the pre-weaning period but it would be vastly beneficial to measure ruminal pH with this supplementation strategy while also incorporating different times of supplement such as during weaning, post-weaning, or different concentration and methods of administration. Since butyrate is a volatile fatty acid and its nature is to lower the

ruminal pH, measuring pH over the entire pre-and post-weaning period would be valuable to determine if supplementing butyrate would be feasible for producers. The supplemental butyrate used in this study however, was a rumen protected butyrate source (Appendix 4). Therefore, it is unknown how much of the supplement affected the rumen versus the small intestine and where the true site of butyrate absorption was. Although we observed increases in starter intake and body weight when calves were supplemented, there is no way to tell from this study what effect this specific supplement had on the rumen. The first ingredient on the feed label is vegetable fat, which indicates the butyric acid in the supplement is mostly encased in fat, which will cause the butyrate to bypass the rumen. This is why we believe Ultramix C would have a greater effect in the small intestine whether it would be used for stimulation of papillary growth, or as an energy source. More research is needed to draw any further conclusions from this study and the supplementation of Ultramix C during the weaning period.

4.4 Strengths and Limitations

The main strength for the first study where forage was limited to pre-weaned calves was that we effectively reported that sub-acute ruminal acidosis does exist in young calves, and that forage inclusion in a calf ration raises the ruminal pH. Unfortunately in this study we were unable to measure papillae length, width, or density, which would have made this study much more influential. Also, while it was not ideal to use grain intake as a threshold for weaning due to our slaughter dates being so variable, the high variability opens a new research opportunity. We should look into breed differences in weaning age and method because almost none of our calves were consuming enough grain at 6 weeks of age to qualify for our weaning threshold. Cold stress may have been the culprit in this instance which would lead us to perform intake studies to determine weaning time based on breed, ambient temperature, and starter intake. The greatest limitation of all was the outbreak of salmonellosis during the beginning of our study where we lost two thirds of our sample size. Not only did it decrease our statistical power, but it also compromised the health of our animals and student help. This is a massive learning experience on how crucial biosecurity is for research trials involving animals.

The main strength for the second study where butyrate was supplemented to calves during weaning was our consistency in slaughter dates as well as limiting morbidity in these calves. Housing was our main limitation in this study. For experiment 1, calves experienced heat stress for the duration of the study. There was minimal heat stress for experiment 2 however, these calves were housed indoors with nose to nose contact, which allowed rota virus to spread through half the calves. The other main issue we had was our supplemental butyrate being rumen protected. We were interested in the effect supplemental butyrate would have on the rumen epithelium and were hoping to look at transport proteins but our plans changed when we realized it was rumen protected butyrate. However, it would be very interesting to look at intestinal tissue because we speculate that this supplement targeted the small intestine so it may have had an effect on intestinal papillae integrity.

4.5 Future Directions

In the future it would be important to continue exploring both paths of research discussed in this thesis. Forage in calf rations is very important; however, a threshold needs to be determined for the quantity of forage. Studies should be completed on incremental feeding to determine if there is a set numerical threshold, such as 90 g/d of forage, or if forage needs to be added to rations according to body weight percentages. It would also be pertinent to study rumen motility in these feeding styles to determine if musculature development is a factor in reducing the incidence of sub-acute ruminal acidosis in pre-weaned calves. Another interesting route would be to determine if the acidosis threshold of pH less than 5.8 for greater than 180 min/d is the same for calves. It may be that calves can tolerate a lower pH for a greater period of time than older animals. It would also be beneficial to study the effect of feeding time of forage and the amount of forage fed on sub-acute ruminal acidosis in calves as well as the effect handling stress has on intake in these instances. In regards to supplementing butyrate, performing a study where butyrate is supplemented either in the milk or starter, and adding a time component where calves were only supplemented for the first two weeks of life, or during weaning would be beneficial to determine the time point in which butyrate is most effective. It would also be interesting to compare the effects of supplementing propionate or butyrate because they are both known to increase papillary development. Overall, there are multiple studies that should be completed to increase our knowledge on these topics.

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Tables and Figures

Table 2.1. Nutritional profile of feeds provided to calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks.

Feed	Nutritional Profile % DM
Milk Replacer	1,200 g/d (28% crude protein and 18% fat)
[1]Energy Supplement	160 g/d (7% crude protein and 60% fat)
Calf Starter	CP – 21.1
	NFC – 55.9
	Starch – 36.5
Alfalfa Hay	CP – 17.7
	ADF – 37.6
	NDF 44.5

[1] Added when the ambient temperature was below 0 °C

Table 2.2. Volatile fatty acid profile of rumen fluid collected at slaughter 2 hours post feeding from calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks.

Volatile Fatty Acid	Ad-Libitum	Limit-Fed	*P*-value
Total m*M*	87.7 ± 13.1	62.1 ± 16.7	0.16
Acetate % total	64.0 ± 3.7	63.5 ± 4.9	0.90
Propionate % total	16.7 ± 2.7	21.6 ± 2.7	0.19
Butyrate % total	12.3 ± 2.7	5.8 ± 3.6	0.06
A:P Ratio	4.0 ± 0.6	3.3 ± 0.7	0.36
Isobutyrate % total	1.5 ± 0.3	2.0 ± 0.4	0.37
Isovalerate % total	2.6 ± 0.6	3.8 ± 0.6	0.11
Valerate % total	1.8 ± 0.2	2.0 ± 0.3	0.43
Isocaproate % total	0.1 ± 0.01	0.1 ± 0.01	0.84
Caproate % total	0.7 ± 0.2	0.7 ± 0.2	0.89

Table 2.3. Rumen pH profile of the ventral sac during the final seven days prior to slaughter of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks.

	Ad Libitum	Limit-Fed	*P*-Value
Minimum pH	4.88 ± 0.35	4.18 ± 0.53	0.17
Mean pH	6.38 ± 0.16	5.98 ± 0.23	0.09
Maximum pH	7.25 ± 0.13	7.11 ± 0.20	0.40
Standard Deviation	0.26 ± 0.02	0.29 ± 0.03	0.45
Duration pH <5.8 (min/d)	261 ± 133	796 ± 145	0.03
Area under curve pH <5.8 (pH*min/d)	60 ± 43	249 ± 47	0.02

Table 3.1. Nutritional profile of feeds provided to calves fed milk only (PRE-M), pre-weaning milk, starter and forage (PRE-S), post-weaning milk, starter, and forage (POST-S), or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B).

Feed	Nutritional Profile % DM
Milk Replacer	1,200 g/d (28% crude protein and 18% fat)
Butyrate Calf Starter	CP – 21.6
	Starch – 36.9
Calf Starter	CP – 22.1
	Starch – 36.5
Alfalfa Hay	CP – 19.8
	ADF – 35.0
	NDF – 42.2

Table 3.2. Experiment 1 volatile fatty acid profile of rumen fluid collected at slaughter 2 hours post feeding from calves fed milk only (PRE-M) or pre-weaning milk, starter, and forage (PRE-S) for four weeks.

Volatile Fatty Acid	PRE-M	PRE-S	P-value
Total m*M*	11.8 ± 5.8	35.6 ± 5.6	<0.01
Acetate % total	64.5 ± 3.0	62.5 ± 2.8	0.58
Propionate % total	15.3 ± 2.7	23.1 ± 2.6	<0.01
Butyrate % total	8.4 ± 1.7	8.4 ± 1.6	0.97
A:P Ratio	1.6 ± 0.3	2.3 ± 0.3	0.07
Isobutyrate % total	5.6 ± 0.6	2.2 ± 0.5	<0.01
Isovalerate % total	2.4 ± 0.2	1.7 ± 0.2	0.02
Valerate % total	2.2 ± 0.5	1.8 ±0.5	0.58
Isocaproate % total	0.2 ± 0.1	0.02 ± 0.1	0.20
Caproate % total	1.1 ± 0.1	0.3 ± 0.1	<0.01

Table 3.3. Experiment 1 rumen pH profile of the ventral sac seven days prior to slaughter of calves fed milk only (PRE-M) or pre-weaning milk, starter, and forage (PRE-S) for four weeks.

	PRE-M	PRE-S	P-value
Min pH	5.07 ± 0.47	5.81 ± 0.44	0.26
Mean pH	6.16 ± 0.83	7.44 ± 0.79	0.28
Max pH	6.72 ± 1.41	9.14 ± 1.33	0.23
Standard Deviation	0.20 ± 0.23	0.56 ± 0.22	0.23
Duration pH <5.8 (min/d)	485 ± 178	280 ± 168	0.42
Area under curve pH <5.8(pH*min/d)	360 ± 130	90 ± 122	0.15

Table 3.4. Experiment 2 volatile fatty acid profile of rumen fluid collected at slaughter 2 hours post feeding from calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B).

Volatile Fatty Acid	POST-S	POST-B	P-value
Total m*M*	154.3 ± 15	131.0 ± 15.8	0.23
Acetate % total	53.1 ± 2.3	58.4 ± 2.3	0.12
Propionate % total	27.9 ± 2.7	34.9 ± 2.7	0.05
Butyrate % total	9.1 ± 1.5	7.5 ± 1.5	0.35
A:P Ratio	2.3 ± 0.3	1.6 ± 0.3	0.07
Isobutyrate % total	1.0 ± 0.1	0.8 ± 0.1	0.24
Isovalerate % total	1.1 ± 0.2	1.0 ± 0.2	0.35
Valerate % total	2.1 ± 0.3	2.4 ± 0.3	0.45
Isocaproate % total	0.1 ± 0.1	0.1 ± 0.1	0.56
Caproate % total	0.3 ± 0.1	0.1 ± 0.1	0.14

Table 3.5. Experiment 2 rumen pH profile of the ventral sac seven days prior to slaughter of calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B).

	POST-S	POST-B	P-value
Min pH	5.28 ± 0.30	4.99 ± 0.29	0.35
Mean pH	6.39 ± 0.19	5.83 ± 0.18	0.05
Max pH	7.42 ± 0.27	6.86 ± 0.26	0.11
Standard Deviation	0.24 ± 0.02	0.21 ± 0.02	0.50
Duration pH <5.8 (min/d)	209 ± 213	730 ± 199	0.10
Area under curve pH <5.8 (pH*min/d)	77 ± 118	353 ± 110	0.12

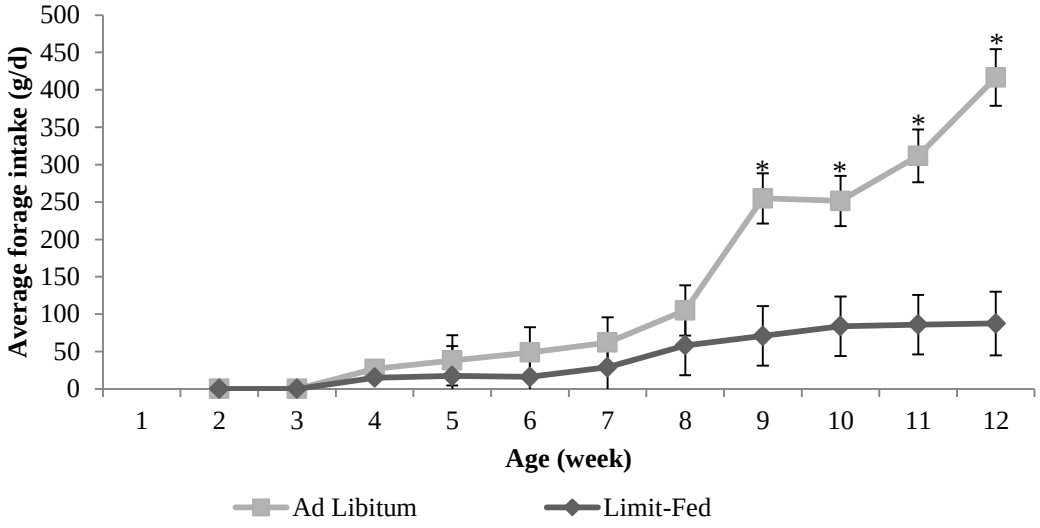


Figure 2.1. Long-stem alfalfa forage intake (g/d) of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. Forage intake was greater for *ad libitum* compared to limit-fed calves ($P < 0.01$); time ($P < 0.01$); treatment x time interaction ($P < 0.01$). The error bars reflect the SEM associated with treatment.

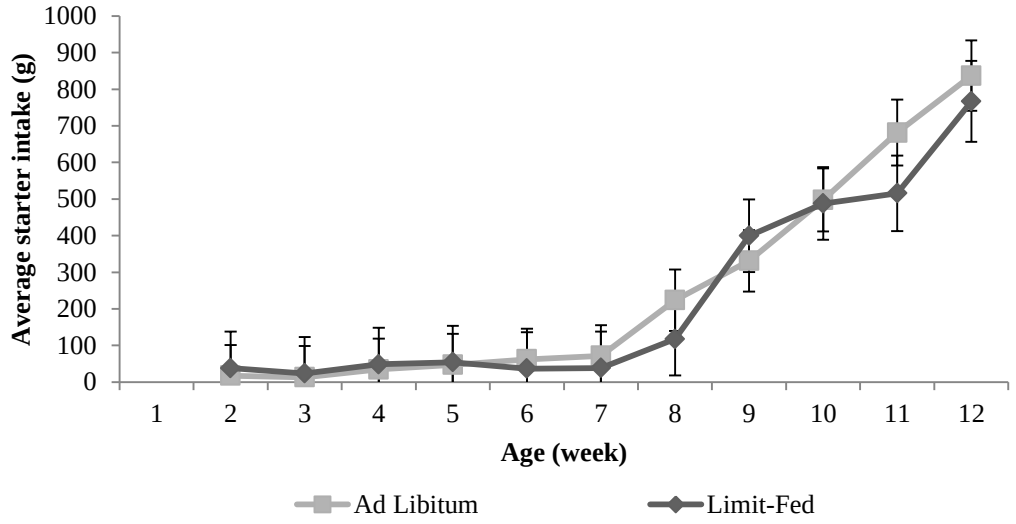


Figure 2.2. Texturized calf starter intake (g/d) of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. Starter intake was not different for *ad libitum* compared to limit-fed calves ($P = 0.67$), but time ($P < 0.01$); treatment × time interaction ($P < 0.01$) were greater in *ad libitum* compared to limit-fed calves. The error bars reflect the SEM associated with treatment.

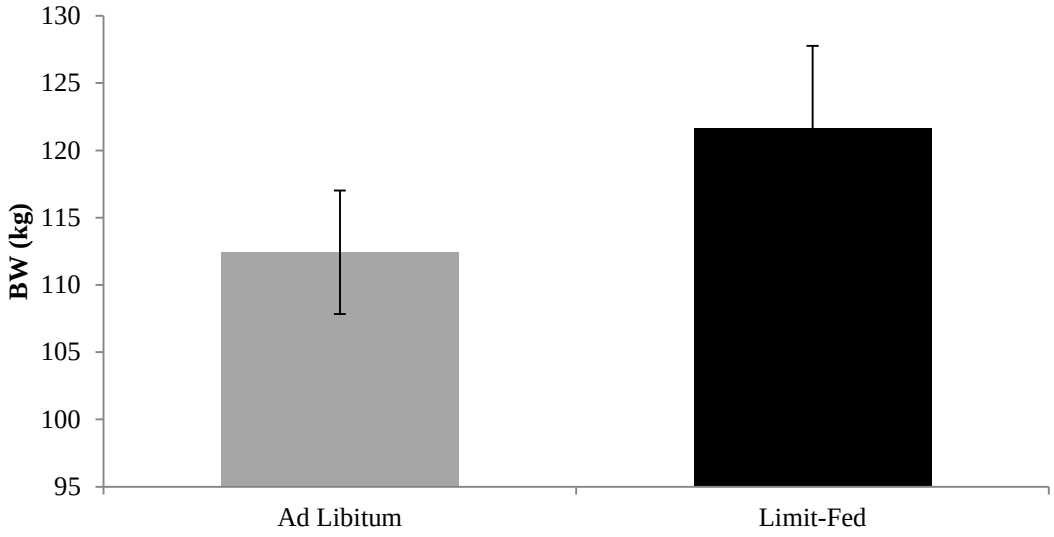


Figure 2.3. Body weight (kg) at slaughter of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. Limit-fed calves tended to have a greater weight ($P = 0.10$).

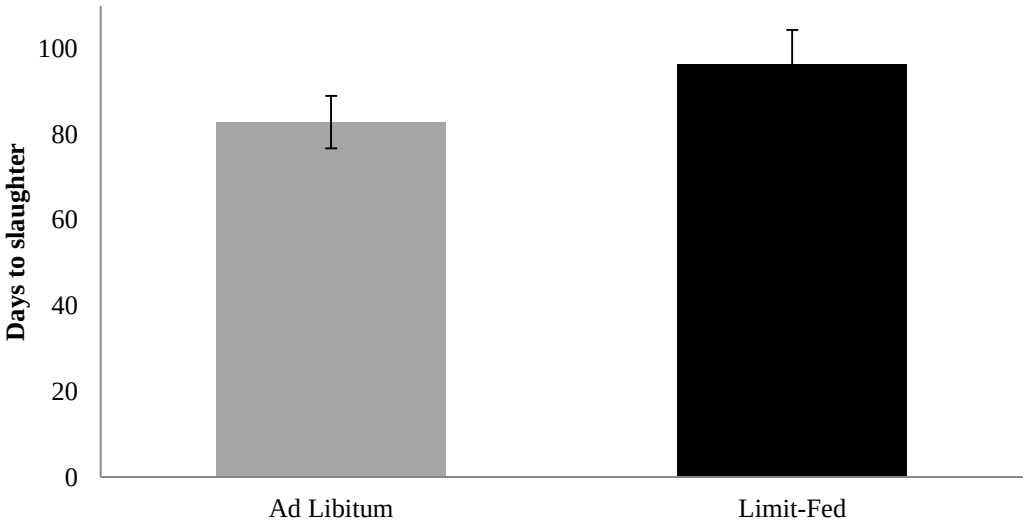


Figure 2.4. Age at slaughter (weaning) of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. There is a tendency ($P = 0.08$) for *ad libitum* calves to wean earlier than limit-fed calves.

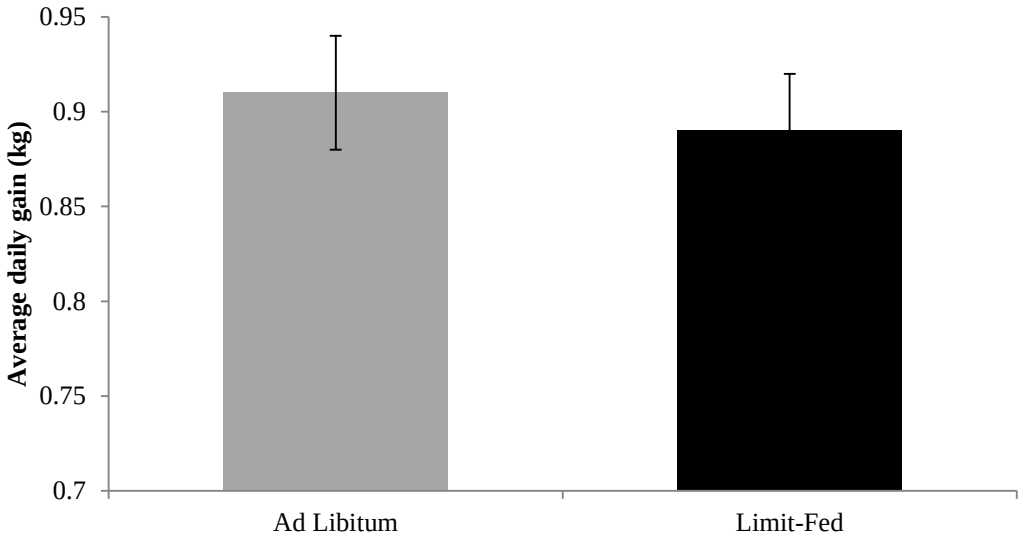


Figure 2.5. Average daily gain (kg/d) of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. Average daily gain was similar (P = 0.61) across treatments.

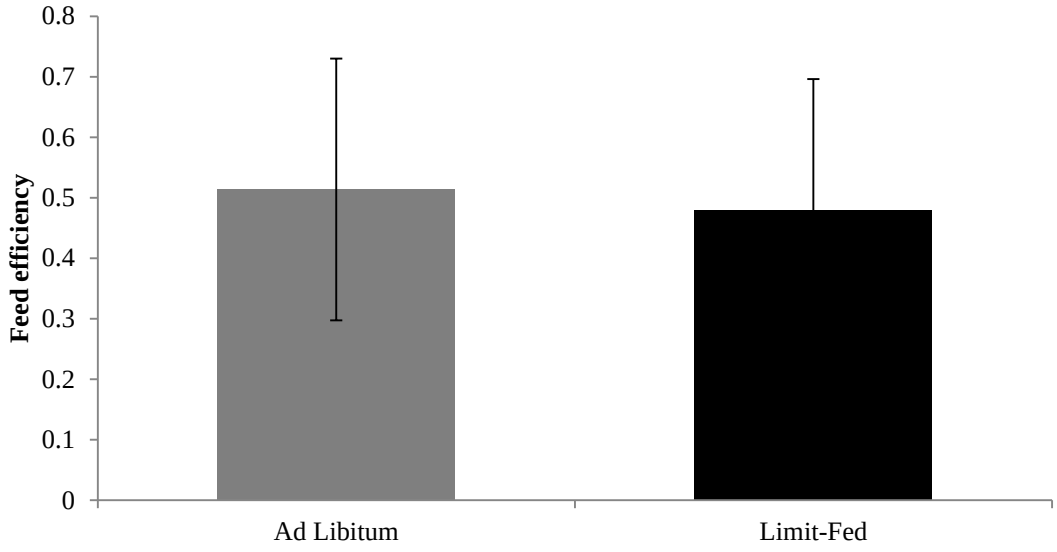


Figure 2.6. Feed efficiency of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. Feed efficiency was not different across treatments (P = 0.24).

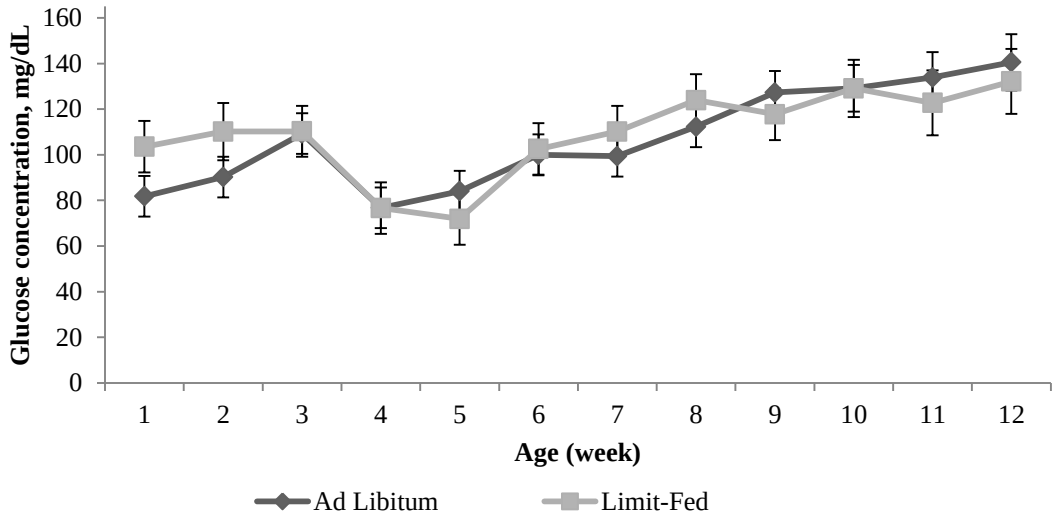


Figure 2.7. Plasma glucose concentration (mg/dL) of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. Plasma glucose was not different for *ad libitum* compared to limit-fed calves ($P = 0.43$); treatment x time interaction ($P = 0.41$). As time increased, plasma glucose increased for both treatments ($P < 0.01$). The error bars reflect the SEM associated with treatment.

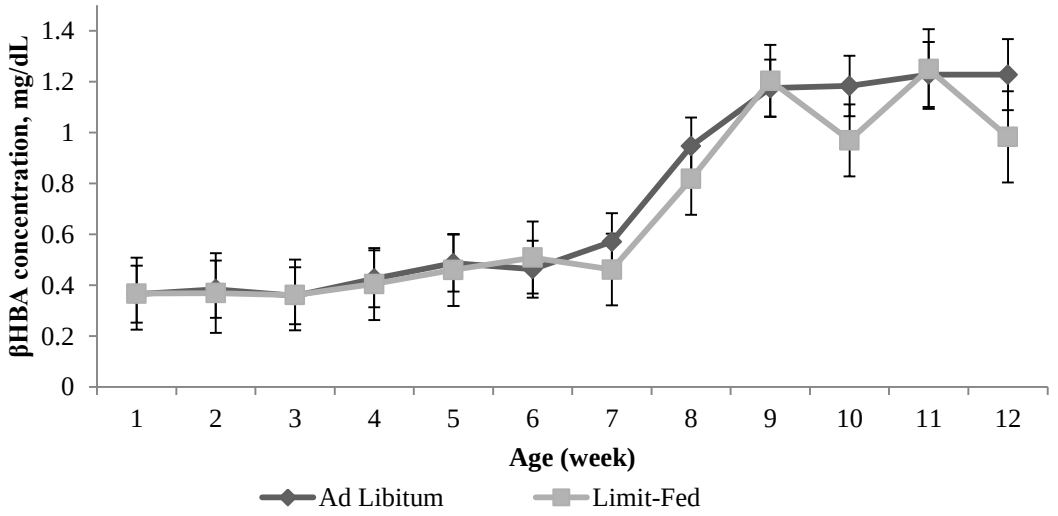


Figure 2.8. Plasma βHBA concentration (mg/dL) of calves fed either ad libitum alfalfa hay or limit-fed alfalfa hay at 90 g/d for 12 weeks. Plasma βHBA was not different for *ad libitum* compared to limit-fed calves ($P = 0.69$); treatment × time interaction ($P = 0.38$). As time increased, plasma βHBA increased for both treatments ($P < 0.01$). The error bars reflect the SEM associated with treatment.

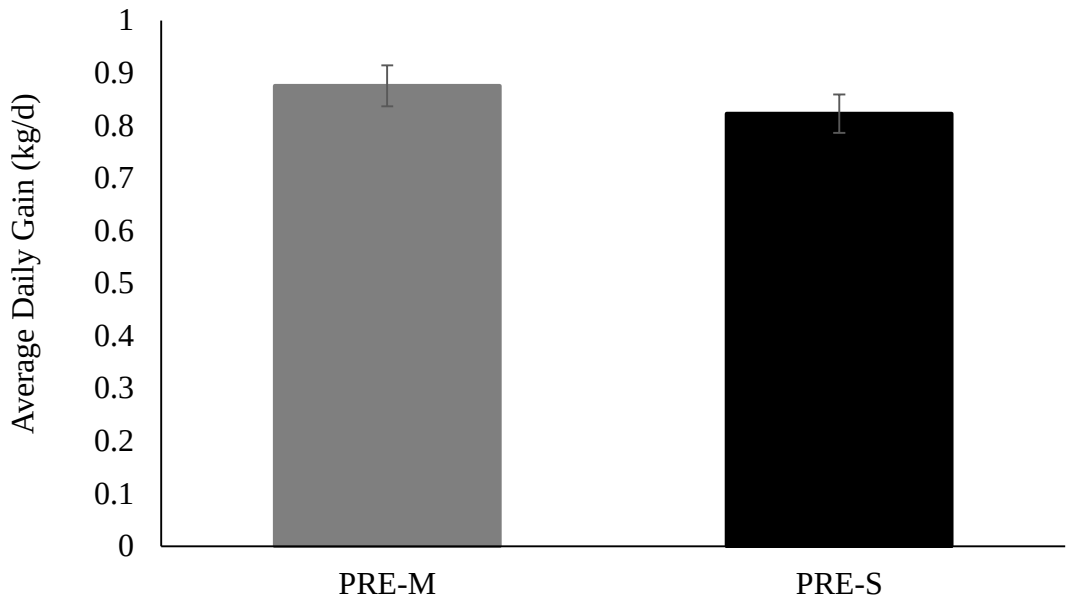


Figure 3.1. Experiment 1 average daily gain (kg/d) of calves fed milk only (PRE-M) or pre-weaning milk, starter, and forage (PRE-S) for four weeks. Average daily gain was similar ($P = 0.33$) across treatments.

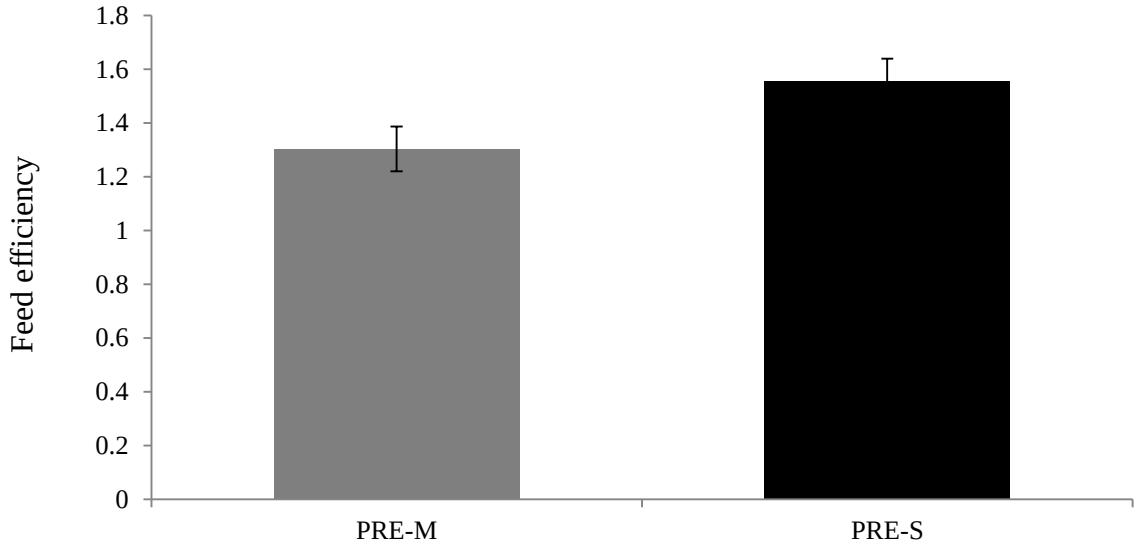


Figure 3.2. Experiment 1 feed efficiency of calves fed milk only (PRE-M) or pre-weaning milk, starter, and forage (PRE-S) for four weeks. Feed efficiency was greater in PRE-S calves ($P = 0.02$).

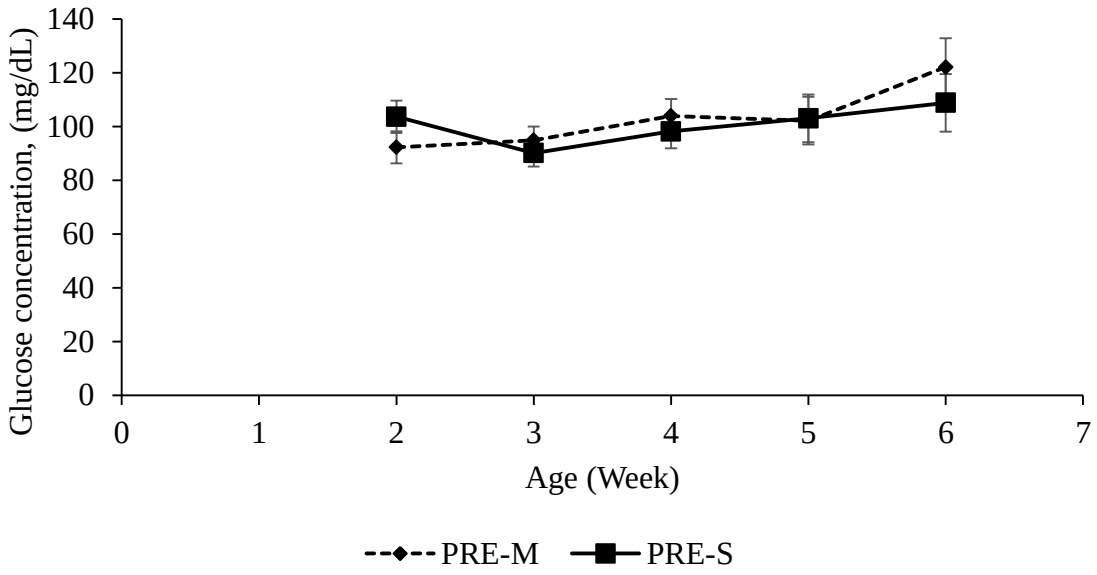


Figure 3.3. Experiment 1 plasma glucose concentration (mg/dL) of calves fed milk only (PRE-M) or pre-weaning milk, starter, and forage (PRE-S) for four weeks. Plasma glucose was not different for PRE-M compared to PRE-S calves (P = 0.81); treatment x time interaction (P = 0.45). As time increased, plasma glucose increased for both treatments (P = 0.04). The error bars represent the SEM associated with treatment.

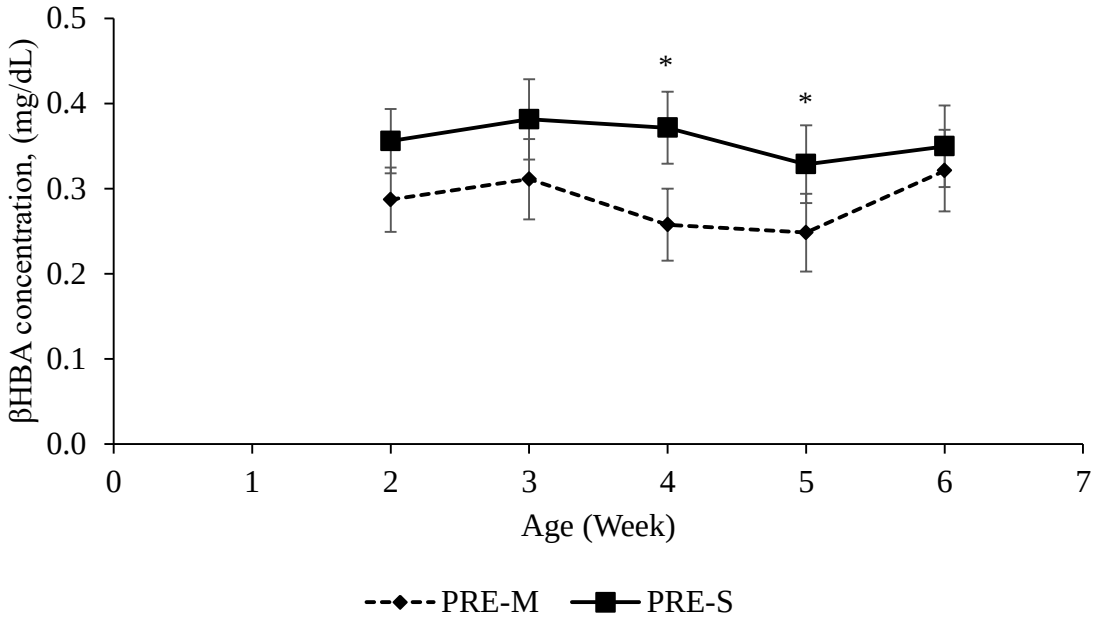


Figure 3.4. Experiment 1 plasma βHBA concentration (mg/dL) of calves fed milk only (PRE-M) or pre-weaning milk, starter, and forage (PRE-S) for four weeks. Plasma βHBA was greater in PRE-S calves (P = 0.04) but was not different for time (P = 0.69); treatment × time interaction (P = 0.72). The error bars represent the SEM associated with treatment.

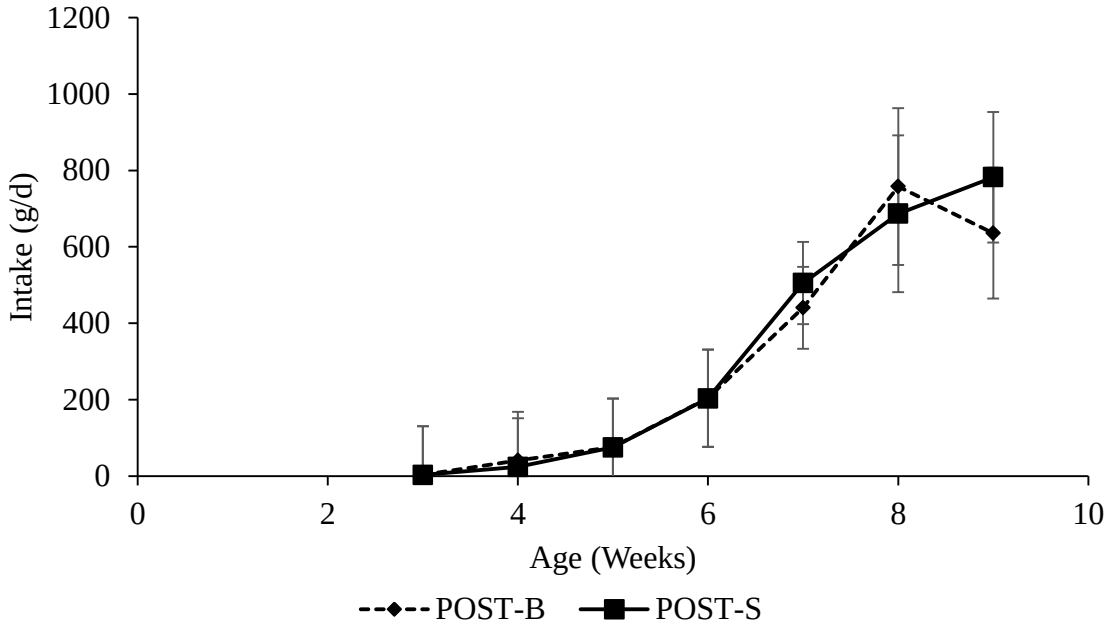


Figure 3.5. Experiment 2 forage intake (g/d) of calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B). Forage intake was not different across treatments ($P = 0.66$); time ($P = 0.90$); treatment × time ($P = 0.99$). The error bars represent the SEM associated with treatment.

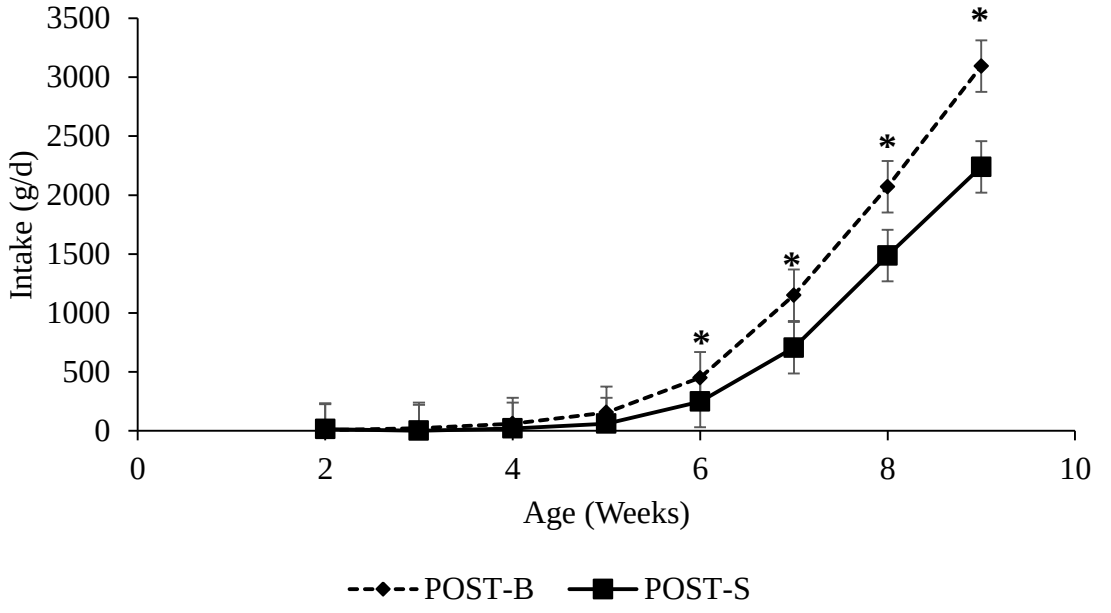


Figure 3.6. Experiment 2 starter intake (g/d) of calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B). Starter intake had a tendency to be greater for POST-B calves ($P = 0.09$), but was greater for POST-B calves for treatment x time interaction ($P = 0.02$); time ($P < 0.01$). The error bars represent the SEM associated with treatment.

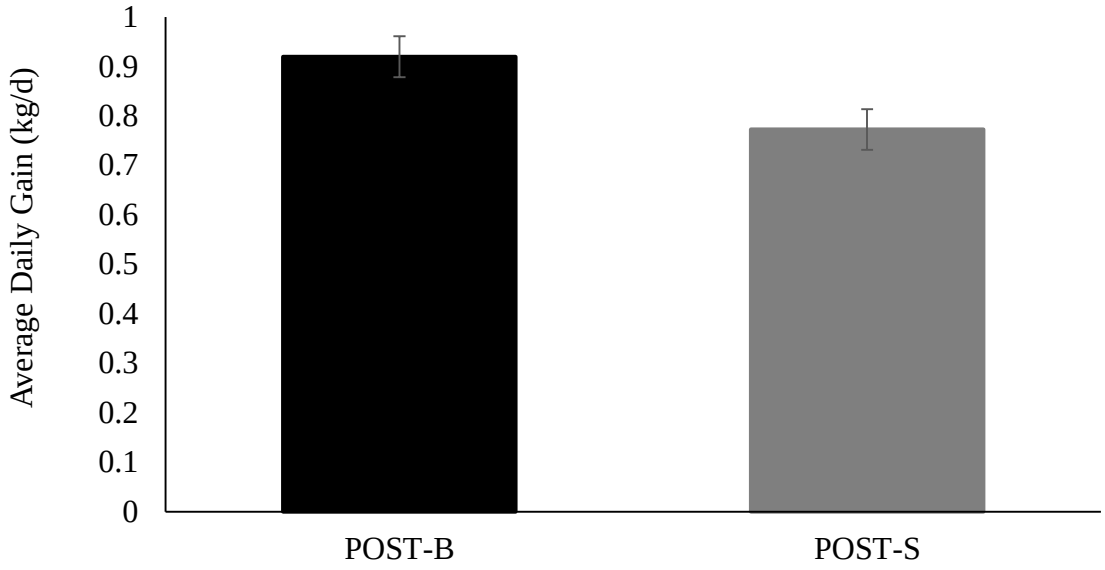


Figure 3.7. Experiment 2 average daily gain (kg/d) of calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B). Average daily gain was greater in POST-B calves (P = 0.03).

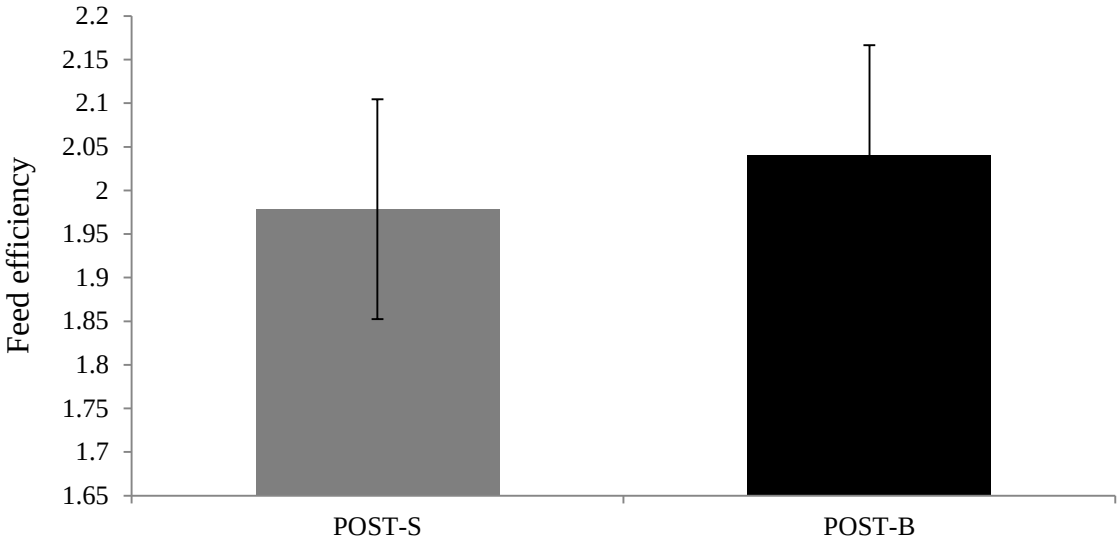


Figure 3.8. Experiment 2 feed efficiency of calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning *(POST-B).* Feed efficiency was not different across treatments (P = 0.73).

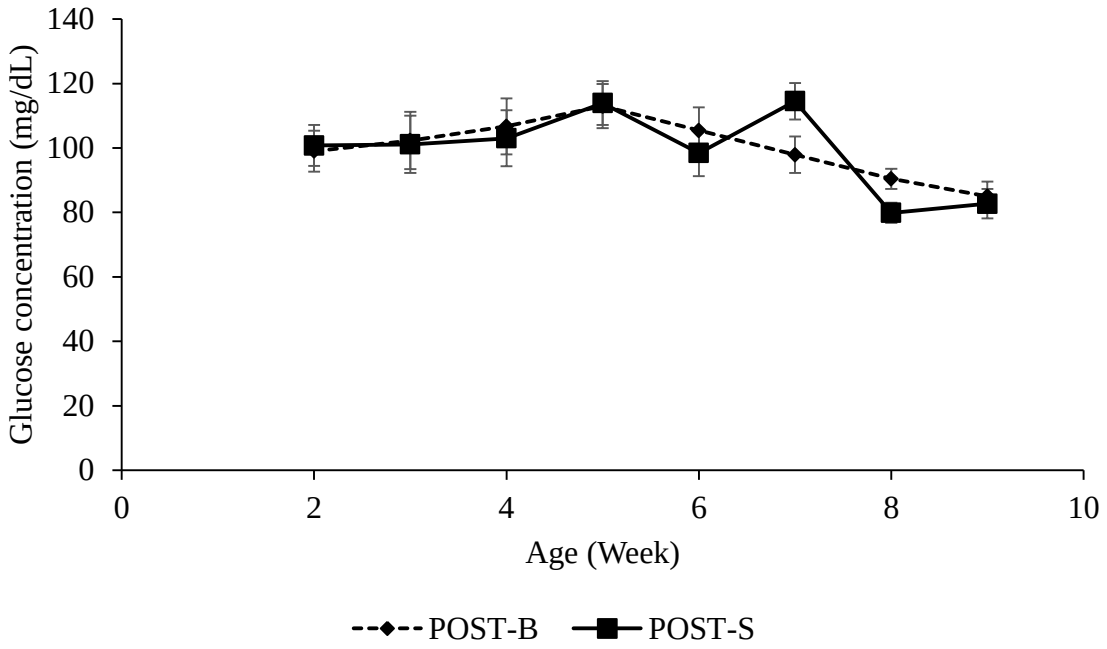


Figure 3.9. Experiment 2 plasma glucose concentration (mg/dL) of *calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B).* Plasma glucose concentration was not different across treatments ($P = 0.88$), but increased with age ($P < 0.01$), treatment x time ($P = 0.04$). The error bars represent the SEM associated with treatment.

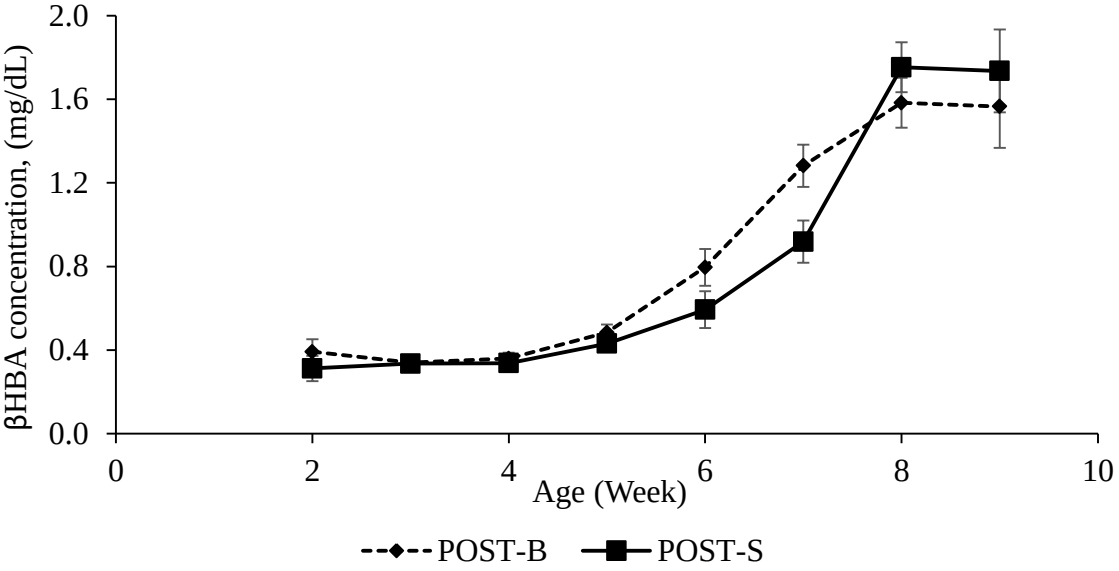


Figure 3.10. Experiment 2 plasma βHBA concentration *calves fed post-weaning milk, starter, and forage (POST-S) or post-weaning milk, starter, forage, and supplemental butyrate during weaning (POST-B).* Plasma βHBA concentration was not different across treatments ($P = 0.65$); treatment x time ($P = 0.20$). As time increased, plasma βHBA increased for both treatments ($P < 0.01$). The error bars represent the SEM associated with treatment.

Appendix 1

Research Protocol Approval

University of Idaho

Institutional Animal Care and Use Committee

Date: Monday, May 16, 2016

To: Anne Laarman

From: University of Idaho
Institutional Animal Care and Use Committee

Re: Protocol 2016-16
Effect of limit-feeding hay on subacute ruminal acidosis in pre-weaned Jersey calves

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care and Use Committee on Monday, May 16, 2016.

This protocol was originally submitted for review on: Friday, April 1, 2016

The original approval date for this protocol is: Monday, May 16, 2016

This approval will remain in effect until: Tuesday, May 16, 2017

The protocol may be continued by annual updates until: Thursday, May 16, 2019

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Barry Robison

IACUC Chair

University of Idaho

208-885-7137

University of Idaho

Institutional Animal Care and Use Committee

Date: June 16, 2017

To: Anne Hermen Laarman

From: University of Idaho

Institutional Animal Care and Use Committee

Re: Protocol IACUC-2016-32 Seed Grant: Mechanism of butyrate action on gene expression in rumen tissue

Your requested amendment of the animal care and use protocol listed above was reviewed and approved by the Institutional Animal Care and Use Committee on 06/16/2017.

The original approval date for this protocol is: 06/03/2016

This approval will remain in effect until: 06/16/2018

The protocol may be continued by annual updates until: 06/02/2019

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Craig McGowan

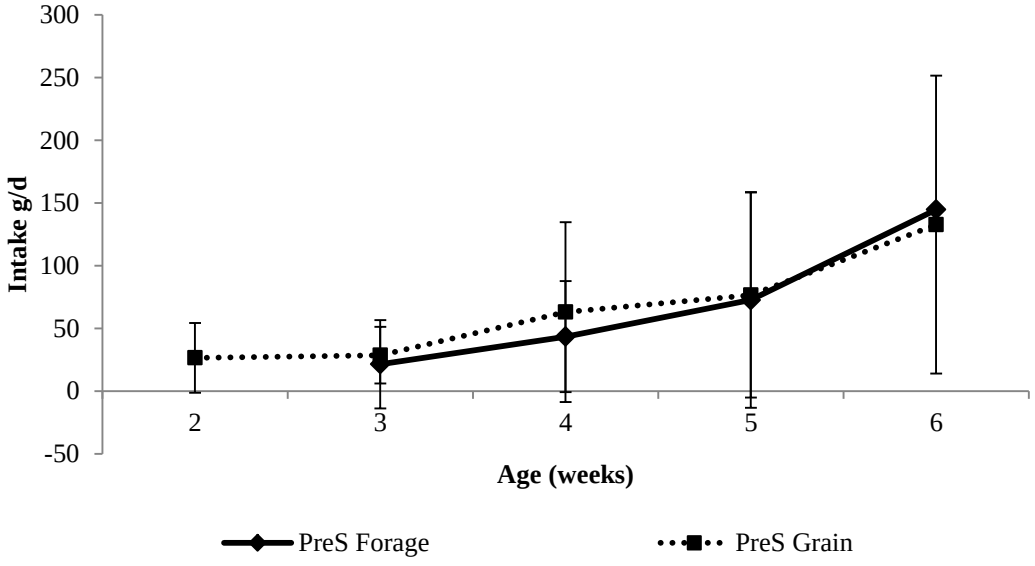
IACUC Chair

University of Idaho

208-885-6598

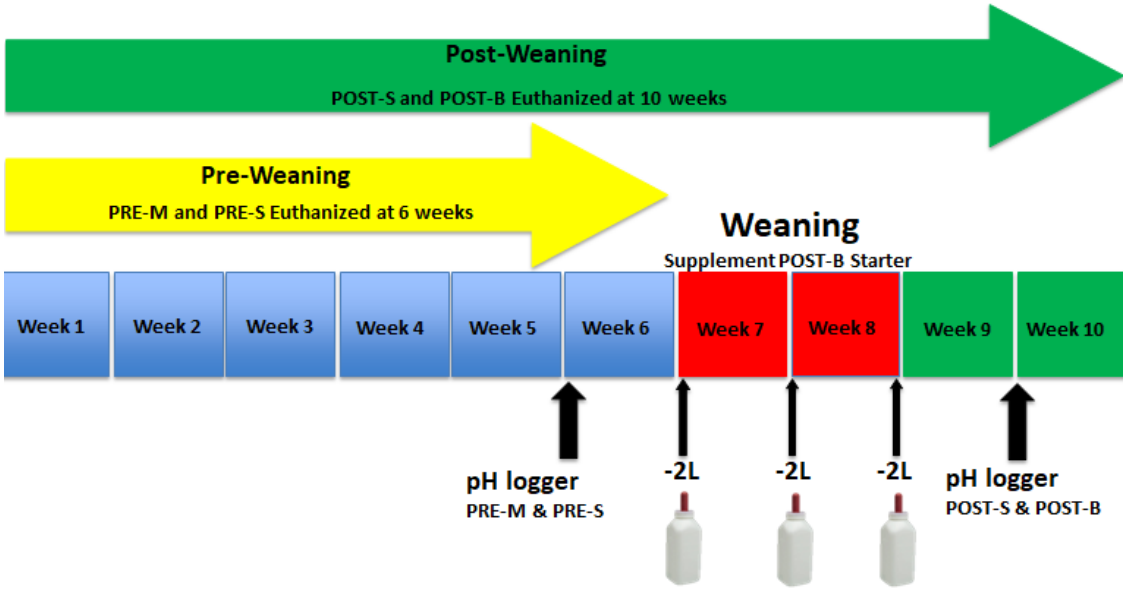
Appendix 2

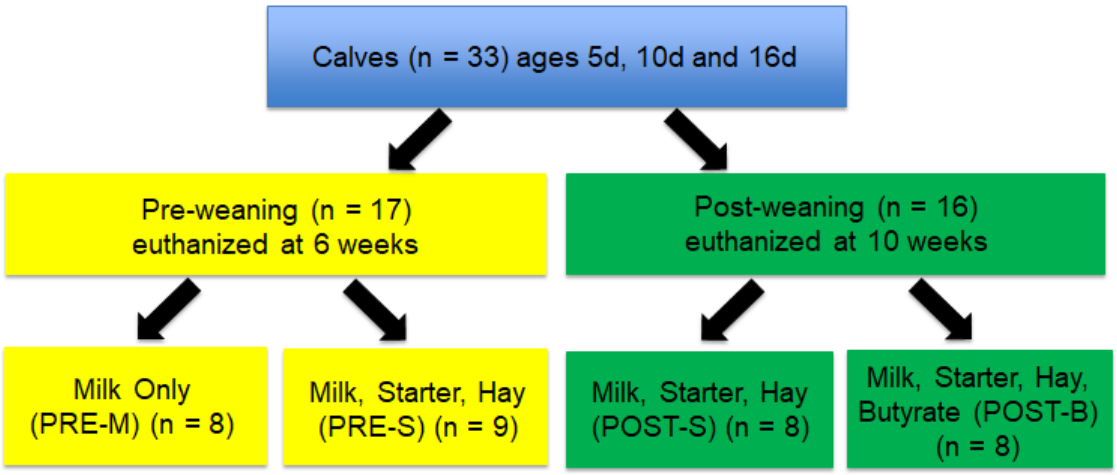
Solid feed intake of calves fed milk, starter, and forage (PRE-S) for four weeks



Appendix 3

Experimental Design of The Effects of Supplemental Butyrate on Sub-Acute Ruminal Acidosis and Weaning in Holstein Calves. Image one shows the time frame (blue), treatment groups, and pH logger dosage for experiments 1 (yellow) and 2 (green). For experiment 2 only, the weaning period (red) is when POST-B calves were given 1% supplemental butyrate in the calf starter as total milk volume was reduced by 2 L each week. Image two shows the breakdown of treatments (blue, yellow, green), age range (blue), and euthanasia dates (yellow, green) for both experiments





Appendix 4

Nutriad Ultramix C

Purpose: Dry Flavor Agent for Livestock, Poultry, and Pet Diets

Ingredients: Vegetable fat (coconut & palm), butyric acid, sodium hydroxide, calcium carbonate, silicon dioxide

Usage Rate:

Swine: 1-2 kg/tonne (2-4 lb/ton)
Poultry: 0.5-1 kg/tonne (1-2 lb/ton)
Pet: 1-2 kg/tonne (2-4 lb/ton)

Handling and storage: Store in a cool, dry area protected from sunlight. Keep container closed when not in use.

Use with adequate ventilation

THIS PRODUCT DOES NOT CONTAIN ANY ANIMAL-DERIVED COMPONENTS

Distributed by:

Nutriad Inc.
201 Flannigan Road
Hampshire, IL 60140
Phone: 847-214-4860
Fax: 847-214-4880

Country of origin: Italy