2014

Effects of feeding milk replacer once, twice or three times daily on growth and performance in neonatal Holstein calves

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EFFECTS OF FEEDING MILK REPLACER ONCE, TWICE, OR THREE TIMES DAILY ON GROWTH AND PERFORMANCE IN NEONATAL HOLSTEIN CALVES.

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in Animal, Dairy, and Poultry Sciences

by
Michael Thomas
B. S., Delaware Valley College, 2011
May 2014

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Cathy Williams for not only being an exceptional major professor, but also a mentor and confidante throughout my master's program. She was the first person I met in Louisiana and was constantly supportive, patient, welcoming, and accommodating. She had sacrificed much of her free time to see that I succeed in this program and it would not have been possible without her. I would also like to thank Dr. Bruce Jenny for his greatly valued knowledge, friendship, and support throughout my career at L. S. U. as well as his support in the project organization. Dr. Thomas Bidner was a great asset to the committee his expertise and knowledge was greatly appreciate.

A special thank you to Steven Blair, Erica Chartier, Victoria Morgan, Christie Burke, and Brian Rizk for their assistance in the collection of blood and other parameters of the project. They helped me in more ways that I can state. Without them, the project would not have been so successful. I would also like to thank all other staff members of the LSU Dairy Teaching and Research Farm in Baton Rouge.

Ashley Dolejsiova and Ruth Orellana were extremely valuable assets in laboratory analysis. They have been very patient and understanding in their teaching of laboratory procedures. A special thank you to Dr. Michael Kearney for his help with statistical analysis and knowledge of the SAS program.

Thank you to Kasey Martini, my future wife, who has been nothing but supportive and encouraging throughout this time. She assisted me in any way that I ever needed and was always pushing me to do my best. This has been as much of a journey for her as it has for me. Finally, I would like to thank my parents, Dave and Pam Thomas for their unconditional love, support, encouragement, and advice while I have been away. Though we are miles apart, their impact on my life and academic progress is immeasurable.

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ABSTRACT

Sixty-three neonatal Holstein calves (45 female; 18 male) were assigned to one of 3 treatments at d 2 of age to study effects of milk replacer feeding frequency on growth, performance, and health. Treatments consisted of 1X, with total amount of reconstituted milk replacer fed 1X/day at 6:00 a.m.; 2X, with total amount of reconstituted milk replacer divided into 2 equal amounts and fed at 6:00 a.m. and 5:00 p.m.; or 3X, with total amount of reconstituted milk replacer divided into 3 equal amounts and fed at 6:00 a.m., 12:00 noon, and 5:00 p.m. Calves were housed in individual hutches and fed milk replacer until abrupt weaning at 42 d of age. Total daily amount of milk replacer offered was equal to 1.5% of birth weight and reconstituted to a total volume of 10% birth weight. An 18% crude protein calf starter was offered ad libitum beginning on d 3 and fed throughout the duration of the trial. Water was offered ad libitum on d 3. Calves remained in their hutches until d 56 to determine immediate post weaning performance. BW was determined at birth and weekly throughout the trial. Wither height (WH), hip height (HH), and hip width (HW). Feed intake, water intake, and fecal scores were recorded daily. Blood samples were collected on d 14, 28, 42, and 56 for plasma urea nitrogen, plasma glucose concentration (PUN), and β-hydroxybutyrate (BHBA). Effects of treatment, week, and their interactions were analyzed using the MIXED procedure of SAS® (Cary, NC). There was no effect ($P > 0.05$) of treatment on BW, HH, HW, WH, PUN, plasma glucose concentrations, or BHBA. There was a week effect ($P < 0.05$) for grain and water intake, with all calves increasing intake throughout the duration of the study. There was no treatment effect ($P > 0.05$) for fecal scores, with calves scoring similarly throughout the project. No treatment effects were observed ($P > 0.05$) for PUN,

glucose, or BHBA. Glucose concentrations decreased ($P < 0.05$) and PUN and BHBA concentrations increased ($P > 0.05$) as calves aged. Overall, milk replacer feeding frequency had no significant effects on growth or performance of these Holstein dairy calves fed MR once, twice, or three times daily.

CHAPTER 1
INTRODUCTION

The proper management of replacement heifers is an essential component in the dairy industry. Dairy producers want to have replacement heifers reach a proper body weight and size as soon as possible so they can be bred and become profitable (Bach et al., 2007). Attention to nutritional management of the neonatal calf is the first and most important step in successful heifer rearing. The period from birth until after the calf has adjusted to weaning represents the period of greatest stress and metabolic challenge to the young bovine (Davis and Drackley, 1998). If proper nutritional management is not met, then a delay in rumen development may occur and delay the animal's profitability.

At birth, a calf's digestive system behaves like a monogastric animal due to the underdeveloped rumen. At this time, the abomasum is the predominant compartment constituting about 50% of total tissue weight of the stomach (Davis and Drackley, 1998). During this time, the calf is fed milk replacer until the calf can utilize solid feed. Though little development occurs during the liquid phase (Tamate et al., 1962; Van Soest, 1994), it is essential to offer the calf solid feed, known as calf starter, to begin encouraging rumen development. The major factor that influences the development of the rumen is diet (Preston, 1963). If the calf is fed milk replacer longer than needed, the rumen wall and papillae will grow at a slower rate causing the rumen to be underdeveloped. With the rumen wall and papillae underdeveloped, the calf will have complications in digesting grain and forages later in life increasing the time to when the heifer can be bred. While milk replacer is essential for providing nutrients to the young dairy calf, it also has been shown that milk replacer consumption positively affects solid feed intake (Khan et al., 2007). It was initially thought that roughage consumption was essential to development of the rumen but it was discovered later that dry feed was the stimulus (Warner and Flatt, 1965).

In most feeding systems calves are fed milk replacers (MR) in two equal daily feedings. Once daily feeding systems can be utilized, but careful management is vital to their success. Calves fed once a day should receive the same amount of dry matter daily as calves fed twice daily. Due to the high total solids intake in a single feeding, incidences of scours may be increased. Therefore, young calves should be observed several times daily to monitor health (Davis and Drackley, 1998).

Previous research has shown that feeding milk replacer once a day reduces labor without affecting health, weight gain, or starter consumption of calves (Akerman, 1969; Burt 1969; Laird et al., 1969; Willet et al., 1969; Galton and Brackel, 1976; Stanley et al., 2002). However there are little data comparing the effects of feeding milk replacer 1x, 2x, on 3x daily. The few studies that do exist indicate that feeding calves milk replacer 3x a day results in increased average daily gain and hip height when compared to two times per day feeding. (Bartlett et al., 2006; Sockett et al. 2011). Therefore, more research is warranted to determine if feeding milk replacer three times per day improves performance. The objective of the research conducted was to measure growth and metabolite data for Holstein calves fed milk replacer once (1x), twice (2x), or three (3x) times daily.

CHAPTER 2
REVIEW OF LITERATURE

Background

The goal of any dairy must be to provide a continuous supply of productive heifers to replace underperforming and culled animals. Without such a supply, the dairy's future would be hindered. Furthermore, the average dairy herd has a culling rate of 25 to 30% raising the question, whether to purchase or raise replacement heifers (Herdt, 1987). In choosing to raise replacement heifers additional facilities, feed, and labor are needed. Raising replacement heifers should be considered an entirely different process than the dairy itself and the goal should be to have those heifers calve around 24 months of age. The cost of raising replacement heifers is the third most expensive process only behind feed and labor costs (Olynk, 2010). By choosing to raise the replacement heifer, the dairy manager has control over which cows will produce more efficient calves by selecting which cows should be rebred and which should be sold.

Simple and problem-free calving should be the initial concern. This is the first step to a healthy vigorous calf as well as a profitable lactation for the cow (Davis and Drackley, 1998). Proper monitoring of close-up dry cows and calving cows is essential in creating an easy delivery (Davis and Drackley. 1998). It is imperative that the cow does not gain unnecessary weight during the dry period and that the calf does not grow too large pre-parturition and causes dystocia. The cow should also be vaccinated in order to provide antibodies specific to diseases that could frequent the farm. These antibodies would be passed through the dam's colostrum. The calf should be born in a clean area and should be isolated 7-10 days before her expected calving date if possible (Davis and Drackley, 1998). The naval of the calf should be dipped with a 7% iodine solution and roughly 3-4 quarts of high quality colostrum should be provided within 30 minutes after birth followed by an additional 3-4 quarts twelve hours later. It is essential to monitor the calf closely to ensure that no additional problems arise (Davis and Drackley, 1998).

The type of liquid feed, which includes the colostrum, is available in many forms such as fresh, fermented, or frozen colostrum, whole milk and milk replacer (MR). Cost and availability will determine which liquid feed is administered. Due to increasing cost of whole milk. Milk replacers are often the feed of choice due to their effectiveness. Their use and effectiveness has increased since the 1950's because of the additional research and proper formulation of essential nutrients of the growing calf. Producers need to pay close attention to the quality and source of protein (Davis and Drackely, 1998). However attention to the total amount of energy must not be ignored.

The goal of the producer is to decrease the weaning time which would reduce overall rearing cost and also allow the calf to reach its genetic potential. Weaning age can vary from 3 weeks of age to just over 12 weeks of age (Heinrichs, 1992). By reducing the overall weaning time the producers would save money on labor and the cost of feed. The main expenses of a calf rearing program are feed, labor, and health care, lowering the overall weaning time, labor and feed costs could be greatly reduced. A shorter weaning time with adequate rumen development would decrease the likelihood of disease, increase growth, decrease labor, and lower overall feed costs (Quigley, 1996). Limiting MR to 10% as fed of the calf's initial body weight, offering free choice starter, and offering hay around 1 to 2 weeks after weaning are a few ways that can increase rumen development in the young calf. It would be considered a safe time to wean Holsteins calves once 681 grams are starter are consumed for 2 to 3 consecutive days.

This normally occurs around 4 to 5 weeks of age (Quigley, 1996). Like MR, the quality of the starter is a key component to proper calf growth.

In the 1960's milk replacer use increased dramatically in popularity, and in the 1970's scientific studies began to emerge regarding the frequency of feeding these formulas (Randall and Swannack, 1976). These studies strictly focused on the performance of the growing calf. Since then few studies have been conducted regarding feeding frequency. Increasing the feeding frequency adds additional labor which may be needed elsewhere on the farm or not needed at all which would save money. More recently, an interest in feeding multiple times per day has increased (Sockett et. al., 2011). Moreover, there have been great improvements in the quality of MR since the 1960's, so additional studies to evaluate times per day feeding regime on growth and metabolism are warranted.

Effects of Feeding Milk Once Versus Twice Per Day

Feeding 1x/d and weaning at 3 weeks of age was shown to decrease labor costs by 75% (Owens and Plum, 1967). Extending the use of colostrum also reduced overall feed costs as well. In 1967, these researchers performed 2 experiments using 88 Holstein calves to study the effectiveness of feeding milk 1x or 2x daily and weaning at 3 or 6 weeks. The calves fed milk 1x/d were given 3.18 kg of milk and those fed 2x/d were given 1.59 kg of milk in each feeding. There was no hay or starter offered during the study. The results showed that there was no significant difference in growth or performance. Also, there was no evidence of any adverse effects such as bloat or scours from feeding 1x versus 2x.

Studies by Khouri (1969) in New Zealand investigated feeding milk 1x versus 2x daily. All calves were fed 5 kg of milk with the 2x daily feeding being split into two 2.5 kg amounts for 5 weeks. A concentrate mix was fed to all calves for 9 weeks of age. Hay was fed ab libitum for the duration of the trial and pasture beginning at week 6. Pre-weaning and post-weaning growth data had no significant differences.

Owens and Stake (1971) at South Dakota State University compared feeding milk 1x versus 2x a day to calves living in outdoor hutches in a cold environment. Twenty Holstein calves were fed 3.65 kg of milk for 1x/day or 1.83 kg twice per day for the 2x treatment group. Hay and starter were not given during the study. There were no differences in growth or incidences of scours. The author did mention that 1x daily feeding reduced labor by 0.76 minutes per calf.

In 1973, Radmall and Adams compared the frequency of feeding milk 1x and 2x daily and the effects on growth and incidences of scours in warm versus cold milk temperatures. All diets were isocaloric. At the start of the trial the incidences of scours was greater for calves fed milk 1x and for calves fed cold milk, but overall incidences did not differ.

Effects of Feeding Milk Replacer Once Versus Twice Per Day

Burt (1968) compared the effects of feeding milk replacer to Ayrshire calves either 2x or 1x daily. The protein: fat was 20:20. There were equal amounts of DM in both 1x and 2x feeding frequency, with water being reduced with the 1x daily feeding for the duration of the project. All calves were weaned abruptly at 32 days and then provided water and concentrate mix free choice. The researchers concluded that there were no differences in weight gain, body measurements including hip height and wither height, and fecal scores. Feed intake was also not significantly different in both treatments.

Also in 1968, Perks et al. stated that feeding MR 1x/day could increase total DM intake without causing adverse health concerns. Their trial included feeding 215 Friesian and Hereford x Friesian calves MR 1x or 2x daily and weaned at 12 weeks of age. The researchers concluded that at 12 weeks of age, concentrate consumption and weight gain were similar in both treatments post-weaning. However, in this trial, the 1x feeding resulted in equal or greater weight gain and concentrate consumption in the pre-weaning phase.

Willet et al. (1969) fed Holstein calves MR at a rate of 10% of body weight. Amount fed was increased weekly according to body weight. Twenty four total calves offered free choice of hay and concentrate to a maximum of 0.91 kg/day and weaned at 40 days of age. Additional water was not offered. Calves were fed 1x or 2x daily all were weaned 40 days of age. There were no difference in weight gain, heart girth, wither height gain, or consumption of starter or hay from birth to weaning. Furthermore, they reported that feeding 1x did not cause any digestive disorders such as bloat or scours, or decrease vigor. Willet et al. (1969) recommended that calves weaned early (4 weeks of age) did not necessarily need an increase in MR. However, if calves remained on MR then more MR should be offered as the weight of the calf increases.

A similar study was done by White and Radcliffe (1970) with 32 Friesian calves fed MR 1x or 2x daily. All diets were isocaloric. Calves were weaned at 32 days of age. The amount of MR was increased weekly based on body weight throughout the study. Hay was offered ad libitum throughout the duration of the study and all calves were provided with 227 g of crushed oats daily, starting at week 4. The calves fed 1x/day all had increased weights at weeks 2, 3, 5, 6, 7, and 9. However, there was no significant difference between the two groups at weeks 5 through 9 which was the post weaning. There was also no difference in hay consumption. It is important to note that there was an increase in scours in calves fed 2x/ day which may have led to the decreased weight. White and Radcliffe (1970) concluded that lessening the volume of water in the 1x treatments led to decreased scours.

Wood et al. (1971) tested the effects of feeding 24 male Holstein calves 1x or 2x daily on body weight, efficiency of protein and energy utilization, and carcass traits with and without a 39 hour fast. There were six calves per treatment. The calves were fed MR (20% fat and 20% protein) for 5 weeks at 5, 8, 10, 12, or 14% and then 12% of body weight until they reached 125 kg. No additional water provided was for the calves. The results indicated no differences in weight gain, and longissimus dorsi was the only affected carcass trait in fasted calves. In calves fed 1x, the gastrointestinal tract weights tended to increase in the calves that were not fasted. The greater size of the longissimus dorsi muscle would also suggest that there was a treatment effect on the muscle weight distribution in the postnatal calf up to 125 kg body weight. Woods attributed this to increased glycogen storage due to the feeding rates of the 1x in non-fasted calves. There was no difference in digestible protein or energy utilization. However, fasting did demonstrate lower nitrogen retention levels than the non-fasted treatment.

In 1973, Davis and Woodward fed Guernsey and Jersey calves a 20% fat and 20% protein MR 1x or 2x per day. All calves were fed the same amount of nutrients per day regardless of feeding frequency. Treatment calves were compared to a third group fed whole milk 2x daily at 8% of body weight. All calves were weaned at 42 days of age and offered free choice calf starter beginning at one week of age. There was no significant difference in weight between the treatment groups post weaning. However, calves fed MR 1x daily had less overall weight gain than calves fed MR 2x pre-weaning, but at 90 days there were no differences in the three groups. It is worth noting that 10 calves died during the study, all of which were fed MR, but none died on whole milk. The researchers stressed that a quality MR should be used.

Randall and Swannack (1975) conducted two experiments regarding calf feeding frequency. The initial trial involved feeding Hereford or Devon x Friesian calves fed MR 1x or 2x a day at high or low concentrations. Hay, water, and concentrates were offered ad libitum beginning at 3 days of age. All calves were weaned when 0.90 kg of concentrates were consumed daily for 2 consecutive days. They reported that live weights were similar for calves fed 1x or 2x per day and no differences in body weight were reported between the 1x or 2x per day feeding of MR. However, prior to weaning calves receiving lower concentrations did have a lower overall weight as compared to the calves receiving high concentrations. There was no significance difference in post weaning weights. The second trial consisted of all Friesian heifers being fed whole milk 1x or 2x a day. As in the first trials, all calves were given free choice of hay, water, and concentrates beginning on day 5. There was no difference between treatments for calf performance. These studies demonstrated that there was no difference in weight gain or intake in calves fed MR 1x and 2x a day at high or low concentrations or whole milk 1x and 2x a day.

Galton and Brakel (1976) concluded that there was a 39% decrease in labor when comparing MR fed 1x or 2x per day feeding. These researchers tested 50 cows which consisted of Ayshire, Guernsey, Holstein, and Jersey. An equal amount of MR was given to each treatment group as a percentage of body weight and adjusted weekly until weaning. At 39 days of age all calves were weaned. Water, roughage pellets and concentrate pellets were provided ad libitum. There was no significant difference in growth, feed efficiency, and water consumption between the breeds.

Stanley et al., (2002) conducted a trail with 18 Holstein and 15 Jersey heifer calves to determine the effects of feeding calves MR 1x versus 2x daily on growth and glucose metabolism. A MR contained 22% protein and 15% crude fat and was reconstituted at 15% DM and fed at 10% of initial body weight. Ab libitum calf starter was offered to a maximum of 2.64kg/d and water was also given throughout the study ad libitum. All calves were abruptly weaned at six weeks of age. There was no significant difference in body weight or starter intake between the two groups. Furthermore, there was no significant difference in urinary or plasma glucose, plasma NEFA, insulin, and glucagon concentrations. Plasma glucose was numerically higher in Jersey versus Holstein calves.

In 1975, Leibholz argued that feeding calves 1x daily was depriving them of the proper nutrition. This concept was defended by research of Blome et al., (2003) that suggested that nutritional flow was improved in calves fed MR or milk 2x daily compared to 1x/ day. These researchers indicated that the time a calf is dependent on stored energy versus when energy is supplied from the feed is the same regardless of feeding frequency. This was made evident by measuring the respiratory quotient of 25 Friesian calves. The results indicated that as feeding frequency increased so did the respiratory quotient. When the respiratory quotient increases, this indicates increased utilization of fatty acids or proteins. The respiratory quotient demonstrates the utilization of carbohydrates for energy. The respiratory quotient of calves fed 1x did not fall below 0.70 meaning that even with a 1x feeding almost all energy was supplied from the gut. Furthermore, energy retention and live weight gains did not seem to be affected by the frequency of feeding, meaning that increased feeding frequencies did not increase nutrient utilization.

Effects of Feeding Milk or Milk Replacer Once, Twice, or Three Times Daily

Bartlett et al. (2006) randomly assigned 48 Holstein bull calves to 2x or 3x per day feeding frequencies. The calves were fed equal amounts of MR (10% BW) and all diets were isocaloric. Water was offered ab libitum and no dry feeds were fed such as calf starter or hay.   The calves were fed a 28% protein-20% fat MR for 49 days at which time they were slaughtered to measure internal growth data. Internal measurements included gastrointestinal tract weight, liver, heart, and kidney weight. The 3x calves had greater average daily gain, and overall weight gain, larger heart girth, and hip heights when compared to the 2x daily.

In 2011, Sockett et al., conducted an experiment with 70 Holstein calves 2x or 3x daily. A MR consisting of 28% protein and 20% fat was fed for 55 days until all the calves were weaned. All calves were fed at 8:00 am and 9:30 pm and the 3x group received the additional meal at 2:30 pm. All calves were fed equal amounts of MR daily split into the respective feeding times. All calves were offered starter free choice beginning on day 3. The calves fed 3x had greater hip height, greater overall weights, and increased feed efficiency compared to those fed 2x/ day. Furthermore, the 3x calves consumed 26 percent more starter than the 2x daily treatment group. It was also noted that the 3x calves displayed increased vigor when compared to the 2x.

Glucose Regulation and Metabolism

Glucose is a simple monosaccharide sugar that serves as the main source of energy and is an important metabolic substrate for most living organisms. It is stored as glycogen. In response to a rise in blood-glucose after a meal, the pancreas releases pre-stored insulin within the first 5 minutes. This dramatic increase in insulin is followed by a decrease back to normal levels within 10 min. This leads to a second surge of pre-formed insulin as well as newly created insulin. The cells that require glucose have specific insulin receptors on their surface. This encourages glucose to enter the cell so the cell can utilize it. The second rise of insulin will increase and decline at a notably slower rate than the initial surge. Once inside the cell, the glucose is metabolized, generating heat and the formation of adenosine triphosphate (ATP). Adenosine triphosphate can be stored or utilized as required. During the decrease of glucose concentration, glucagon will begin to increase. This causes the liver to release stored glucose concentration into the blood stream. This additional glucagon causes an increase in gluconeogenesis and glycogenoylsis. Gluconeogenesis is the synthesis of glucose from broken down proteins and fat. Glycogenolysis is the destruction of stored glycogen to remake glucose (Guyton and Hall, 1996).

When blood glucose concentrations levels are high, insulin promotes the utilization of carbohydrates as an energy source. However, when glucose concentrations are depleted, fat and muscle glycogen become the primary energy source. This is especially true for stored fat in times of starvation. Fat has the highest amount of stored energy. The low blood-insulin levels activate lipase enzymes to breakdown fat so that it can be oxidized for energy. Adipose tissue is increased when there are increased glucagon concentrations due to the fact that increased glucagon prevents the liver from removing fat (Guyton and Hall, 1996).

Gastrointestinal development also plays a role in glucose metabolism. In an adult ruminant, most glucose obtained from feed is not passed into the small intestine. Rather, it is fermented to short chained fatty acids by rumen microgorganisms. Due to the production of the short chained fatty acids, ruminant blood-glucose level are much lower than a non-ruminant animal at around 60-80 mg/dl as compared to the a non-ruminant at around 80-120 mg/dl. Ruminants are in a constant state of gluconeogenesis (Hsu and Crump, 1989).

Pre-ruminant calves have a gastrointestinal tract similar to that of a non-ruminant. Like the non-ruminant, calves absorb all of the nutrients through the intestine prior to weaning (Quigley and Bernard, 1992). Once weaned, the rumen is allowed to fully develop and production, and absorption of volatile fatty acids (VFA) can begin and these VFA become the primary energy source for the growing calf (Quigley and Bernard, 1992). In the pre-ruminant stage, blood-glucose concentrations of the calf will be similar to that of non-ruminants, but as the rumen begins to develop, blood-glucose concentration will begin to resemble that of an adult ruminant. Once fermentation begins, the dependency on glucose metabolism will decrease and the production of propionate, a VFA produced by fermentation, will begin to become essential. Propionate acts as a precursor for glucose synthesis (Lyford and Huber, 1988).

Though glucose is essential in non-ruminant animals for the release and utilization of insulin, the same cannot be said for ruminants. Carbohydrates obtained from feedstuffs are fermented in the rumen and converted to VFA which include acetate, butyrate, and propionate. The production of butyrate and propionate cause a release of insulin in the adult ruminant (Manns and Boda, 1967). In a study by Owens et al. (1986), it was reported that calves fed a diet of MR only did not have the gluconeogenic pathways required to release insulin while calves fed MR with grain had gluconeogenic pathway. This indicates that grain is essential not just for rumen development but also for the development of metabolic pathways.

Quigley et al. (1991a) noted that glucose levels of calves after a meal and weaned at 25 days were greater than those calves weaned at 56 days. This was attributed to glucose being absorbed directly through the intestinal wall reaching and the blood at a faster rate than carbohydrates fermented to propionate and used in the gluconeogenic pathway.

As calves age, the dependency of plasma glucose becomes less important due to the ability to ferment carbohydrates into glucose precursors. Calves change from a primarily milk diet providing large amounts of dietary glucose to a diet where the primary energy sources are the end products of microbial fermentation (Reid, 1953). It is imperative that the rumen develops properly in order for the newly introduced dry feedstuffs to be fermented properly. These changes are necessary for the establishment of a healthy microbial population and productive future lactations.

Stanley et al. (2002) conducted a studying comparing calves fed MR 1x and 2x daily and its effects on glucose metabolism. Calves were fed a MR containing 22% crude protein and 15% crude fat. Calves were fed MR reconstituted at 15% dry matter and fed at a rate of 10% body weight. Results indicated that there were no significant difference in plasma glucose concentrations following a meal in calves fed 1x or 2x daily.

β-Hydroxybutryate

The microbial population in the rumen ferments carbohydrates into volatile fatty acids (VFA) which include acetate, butyrate, and propionate. These VFA are the main energy source for ruminant animals (Brown et al., 1960). Butyrate is absorbed across the rumen wall and oxidized into ketones. An example of these ketones is β-hydroxybutryate (BHBA). This provides energy to the animal. Up to 50% of the butyrate is converted to

BHBA and can produce up to 27 moles of ATP (energy) (Jurgens et al. 1997). BHBA is an indicator of rumen development especially in young ruminants (Quigley et al. 1991b). Increased BHBA production is a sign that the bacterial flora is developing. Due to the fact that young ruminants have little microbial fermentation occurring, smaller amounts of ketones are produced, leading to less BHBA.

Quigley et al., (1991b) conducted a study using 16 Holstein calves. All calves were fed 1.8 kg of whole milk, waste milk or both twice a day until weaning. Differences in VFA production, relative to weaning times, were analyzed. Treatment group 1 was weaned at 28 days of age and treatment group 2 was weaned at 56 days of age. The researchers concluded that VFA production, including the production of butyrate, was higher at 5-8 weeks for calves weaned early (28 days of age) compared to those weaned late (56 days of age). This can be attributed to the early weaned calves consuming more calf starter than those still be offered milk replacer. Beta-hydroxybutyrate acid production will increase as the amount of starter intake increases.(Quigley et al., 1991b). Ruminants 0 to 4 weeks of age will have lower BHBA production than those week 5 to 8 weeks of age. This means that the rumen is showing proper development through its young life. The measurement of BHBA is indicative of rumen development. The greater the concentration of the BHBA the more developed the rumen has become.

Plasma Urea Nitrogen

Urea is one of the most widely used non-protein nitrogen sources. When protein enters the rumen it is fermented, and this results in the production of ammonia (NH_3), carbon dioxide, and methane. Ammonia is used for protein synthesis or is absorbed across the rumen wall where is it converted to urea in the liver (Hayashi et al., 2006). The urea will then be recycled. It will move from the liver back to the rumen where it will be used for additional protein synthesis (Obara and Shimbayashi, 1980). The urea gets back into the rumen through the saliva and is absorbed through the rumen wall (Nolan et al., 1972). In the rumen the urea is converted into microbial protein and passes into the abomasum where it is broken down into amino acids and then passes into the small intestine for absorption (Hayashi et al., 2006).

Hayashi et al. (2006) noted that the amount of recycled urea can be used as a determining factor of rumen development. As the rumen develops, there should be a higher presence of urea and lower amounts of NH_3 present in the rumen. Moreover, the presence of urea in the blood demonstrates the utilization of crude protein that the animal is receiving. Thus the urea should increase once the animal is weaned. When higher protein diets are fed, this leads to an increase in both urea and nitrogen in the ruminant's urine. Likewise, when low protein rations are fed, there will be a decreased amount of urea in the urine and blood due to the reutilization of urea (Hayashi et al., 2006). Urea concentrations are indicative of protein utilization and the amounts are expected to increase as the calf ages and begins to utilize nitrogen more efficiently.

Economic Importance

Without the practice of precisely measuring water and milk replacer, the average calf feeder would spend between 2-4 minutes feeding each calf. In 2000, the average "cow feeder" was earning $9.20/hr (Billikopf, 2004). Using the United States Department of Labor inflation calculator, that same person would be earning roughly $12.50/hr in 2014 (USBLS, 2014).

The additional feeding times will dramatically increase labor costs. If a dairy farm has 50 calves that are being raised for replacement heifers, the average cost of labor to feed these calves one time a day would be between $20.83-$41.67. If the 2x a day feeding frequency was adopted the price would jump to $41.67-$83.34 and $62.49-$125.01 for 3x per day. There is a possible 300% increase in labor specifically for calf feeding if the 3x a day plan is implemented and 200% if the 2x a day plan is used.

In order to rationalize the additional feeding frequency, producers need to see significant data supporting the idea that increased feeding frequency will increase overall growth and enhance future lactation.

CHAPTER 3
MATERIAL AND METHODS

Animal and Dietary Treatments

An experiment was performed to assess the effects of feeding neonatal Holstein dairy calves milk replacer 1x, 2x, or 3x daily. In this experiment sixty-three calves were born and housed at the LSU Dairy Science Research and Teaching Farm in Baton Rouge, La between September, 2012 and February, 2013. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center.

All calves were separated from their dams at birth, weighed, and housed individually in a 2.5-m^2 calf hutch with a 2.8-m^2 wire enclosure. Hutches were placed on rock bedding, and all calves remained in huthces until d 56. Calves were vaccinated orally for rotavirus and coronavirus (Calf Guard, Pfizer Animal Health, Lenexa, KS) prior to receiving colostrum. For the first day of life, calves received 4-6 liters of colostrum, which was followed by transition milk on days 2 and 3. On day 4 of life, calves were given milk replacer (MR) containing Decoquinate (20% protein, 20% fat; ADM Alliance Nutrition, Inc., Quincy, IL) at 10% of their birth weight and trained to drink from a bucket. MR was mixed at 15% solids. Treatments consisted of 1X, with total amount of reconstituted milk replacer fed 1X/day at 6:00 a.m.; 2X, with total amount of reconstituted milk replacer divided into 2 equal amounts and fed at 6:00 a.m. and 5:00 p.m.; and 3X, with total amount of reconstituted milk replacer divided into 3 equal amounts and fed at 6:00 a.m., 12:00 noon, and 5:00 p.m. Water was offered ad libitum beginning on d 4 and measured daily.

On d 5 calves were offered KC Premium medicated calf starter (19.25% CP, 1.65% CF; Kentwood Co-Op, Kentwood, LA) Fresh starter was provided throughout the study in sufficient amounts for ad libitum intake., and starter intake was measured daily. Calves were abruptly weaned from the MR on d 42 of age and removed from the project on d 56.

Sample Collections

Fecal scores were recorded daily. The scoring scale was based on fecal fluidity and ranged from 1 to 4, with 1= firm, 2=soft, 3=runny, and 4=watery (Larson et al., 1977). Body weight, hip height, wither height, and hip width were measured weekly and blood was collected on d 14, 28, 42, and 56. Blood was collected via jugular venipuncture for analysis of plasma urea nitrogen (PUN), plasma glucose concentrations, and β-hydroxybutyrate (BHBA). Blood was collected in 10 ml vacutainer tubes containing potassium oxalate and sodium fluoride for glucose analysis and sodium heparin for PUN and BHBA analyses. The blood was centrifuged for 15 minutes at 600 x g and plasma was collected, protected from UV light, and stored at -20ºC until analysis.

Analytical Procedures

β-Hydroxybutyrate

Commercial spectrophotometric kits (β-Hydroxybutyrate Liquicolor ® Kit; Stanbio Laboratory, Boerne, TX) were used to analyze plasma for BHBA. (Appendix A).

Plasma Urea Nitrogen

Commercial spectrophotometric kits (Urea Nitrogen (BUN)) (Berthelote/Colorimetric); (Point Scientific, Inc., Canton, MI) were used to analyze plasma for PUN (Appendix B).

Plasma Glucose Concentrations

Commercial spectrophotometric kits (Glucose Oxidase) (Point Scientific, Inc., Canton, MI) were used to analyze plasma for glucose (Appendix C).

Statistical Methods and Calculations

Response variables measured daily were reduced to weekly means prior to analysis. All dependent variables were analyzed using the MIXED procedure of SAS® (Version 9.3). For all variables measured over weeks, the model included treatment, week and the treatment*week interaction as fixed effects. Effects involving sex were absorbed due to non-significance. Weekly averages were analyzed as repeated measures using a first-order auto regressive variance-covariance structure. When significance ($P \leq 0.05$) was found in the overall analysis post hoc comparisons were conducted with pairwise t tests of least-squares means.

Starter intake, body weight, hip height, wither height, hip width, water intake, PUN, and BHBA were analyzed including the model treatment, sex, and its interactions as fixed effects.

CHAPTER 4
RESULTS AND DISCUSSION

Least squares means for body weight and average daily gain (ADG) for calves fed MR 1x, 2x, or 3x daily are presented in Table 1. Least squares means for weekly body weight are shown in Figure 1. There were no treatment effects ($P > 0.05$) on body weight or ADG, but there was a main effect of time ($P < 0.05$). As expected, body weight increased as the calves aged. The data indicate that feeding frequency did not impact overall body weight gain or ADG from birth to 8 weeks of age.

Least squares means for hip width, hip height, and wither height are presented in Figures 2, 3, and 4, respectively. There were no treatment effects on hip width, hip height, and wither height ($P > 0.05$). Least squares means for hip width, hip height, and wither height pre and post weaning are presented in Table 2. As expected there was no effect ($P > 0.05$) of treatment on hip width, hip height, and wither height. However, as expected, skeletal growth did increase with age in all treatments.

Least squares means for average daily starter intake are presented in Figure 5. While there was no effect ($P > 0.05$) of MR feeding frequency, starter intake increased as calves aged ($P < 0.05$). This was to be expected due to the calf needing additional nutrients to continue to grow appropriately.

Least squares means for average daily water intake are presented in Figure 6. Milk replacer feeding frequency did not affect water intake ($P > 0.05$). Water intake increased after calves were weaned ($P < 0.05$). This was to be expected sinces calves no longer were receiving MR; thus all the calves liquid intake came from the water provided.

Least squares means for average weekly fecal scores are presented in Figure 7. No effect of treatment was observed ($P > 0.05$). All fecal scores were well within the range for healthy calves, indicating that MR feeding frequency had no apparent effect.

Feeding calves MR 1x per day will save money on labor without affecting intake or weight gain. Previous studies reported that feeding MR 1x daily as opposed to 2x daily does not affect weight gain or starter intake (Burt, 1968; Akerman, 1969; Willett et al., 1969; Stanley et al., 2002). In 1968, Perks et al. reported that calves fed 1x consumed more starter and had higher body weights in the pre-weaning phase than those being fed MR 2x daily. White and Radcliffe (1970) also reported that calves fed MR 1x daily had increased body weight during the pre-weaning phase but weights were similar during the post weaning stage.

Sockett et al. (2011) reported that growth and starter intake were significantly greater in calves fed MR 3x/d as compared to 2x/d. Calves fed 3x/d had greater hip heights, overall weights, and increased feed efficiency when compared to those fed 2x/d. Additionally, calves fed 3x/d consumed 28% more starter. This contradicts the findings of data in the current study. Sockett et al. fed the calves at 8:00 am and 9:30 pm and the experimental 3x group received the additional meal at 2:30 pm with a 28% protein 20% fat MR. The feeding times and MR composition could have attributed to the improved performance.

Table 1. Least squares means for body weight (kg) and ADG (kg) for calves fed MR once (1x), twice (2x), or three (3x) per day.

	Treatment				
Item	1x	2x	3x	SEM[1]	*P* value
Body Weight (kg)					
Preweaning, d 42	54.46	54.86	53.71	1.0070	0.8146
Postweaing, d 56	62.81	65.39	64.78	1.0070	0.3425
ADG[2] (kg)					
Preweaning,d 0-42	0.502	0.470	0.450	0.1438	0.7525
Postweaning, d 42-56	0.596	0.753	0.791	0.1438	0.2665
ADG Entire Study	0.525	0.540	0.535	0.2198	0.7536

SEM[1] = Standard Error of the Mean

ADG[2] = Average Daily Gain

Table 2. Least squares mean for hip width (cm), hip height (cm), and wither height (cm) in calves fed MR once (1x), twice (2x), or three (3x) per day.

	Treatment				
Item	1x	2x	3x	SEM[1]	*P* value
Hip Width[2] (cm)					
Preweaning, d 42	18.75	18.87	18.82	0.0824	0.7066
Postweaning, d 56	19.96	20.04	19.99	0.0899	0.8891
Entire Study	18.19	18.36	18.29	0.0915	0.7362
Hip Height (cm)					
Preweaning, d 42	85.85	85.67	86.08	0.1842	0.8576
Postweaning, d 56	88.24	88.67	88.52	0.1842	0.7542
Entire Study	83.64	84.25	84.51	0.3025	0.6034
Wither Height (cm)					
Preweaning, d 42	81.15	81.28	81.20	0.1835	0.9439
Postweaning, d 56	83.82	84.35	85.34	0.1835	0.4133
Entire Study	78.28	79.08	80.37	0.3075	0.6188

SEM[1] = Standard Error of Mean.

[2]There was a main effect of week (*P* <0.0001).

Bi-**Weekly Metabolic Data**

Milk Replacer Feeding Frequency

Least squares means for concentrations of glucose, PUN, and BHBA are reported in Table 3 and Figures 8, 9, and 10 respectively. PUN concentrations were not affected by MR feeding frequency ($P > 0.05$). However, PUN increased with age ($P < 0.05$) in all three treatments as expected. Plasma urea nitrogen concentration has been suggested to be a realistic predictor of both nitrogen utilization (Egan and KeUaway, 1971) and nitrogen intake (Nolan etal., 1970) of ruminants. As the calf ages, there is an increase starter intake. The increase in PUN demonstrated the increase amount of feed consumed and the increased amount of protein utilization of the calf. Preston et al., (1965) reported that increase blood urea nitrogen is indicative of increased intake protein digestibility.

Glucose concentrations were not affected by treatments ($P > 0.05$). Glucose concentrations did decline as the calves aged ($P < 0.05$). This is expected due to the glucose in the feed no longer passing into the small intestine but rather being utilized by the rumen microbial population to synthesize volatile fatty acids (Hsu and Crump, 1989). Furthermore, Stanley et al. (2002) reported that there were no differences in glucose concentrations when calves were fed once or twice daily. However, the researchers also observed a decrease in glucose concentrations as the calves aged.

β-hydroxybutyrate levels were not affected by MR feeding frequency ($P > 0.05$). However, there was a week effect ($P < 0.05$), indicating that BHBA levels increased as the calf aged. This was expected due to the fact that BHBA levels should increase as the rumen develops (Quigley et al, 1991). The increase in BHBA in all treatments indicated that feeding frequency did not affect the rate at which the rumen developed nor does any treatment prove to be more beneficial to ruminal development.

Table 3. Least squares means for β-hydroxybutyrate (BHBA), plasma urea nitrogen (PUN), and glucose concentration for calves fed MR once (1x), twice (2x), or three (3x) times per day.

	Treatments				
Item	1x	2x	3x	SEM[1]	*P* value
PUN, mg/dL[2]					
Preweaning, d 42	20.75	21.29	19.86	0.5470	0.5942
Postweaning, d 56	24.79	25.70	23.10	0.5668	0.3759
Entire Trial	19.69	19.94	19.64	0.6160	0.8245
Glucose Concentration, mg/dL[2]					
Preweaning, d 42	82.38	81.58	85.22	2.4128	0.7579
Postweaning, d 56	72.29	77.63	76.75	2.4128	0.3978
Entire Trial	81.17	82.42	85.16	3.0966	0.6803
BHBA, mmol/L[2]					
Preweaning, d 42	0.0618	0.0718	0.0704	0.0035	0.1765
Postweaning, d 56	0.0956	0.0929	0.0927	0.0035	0.7367
Entire Trial	0.0595	0.0615	0.0592	0.0036	0.7677

SEM[1] = Standard Error of the Mean.

[2]There was a main effect of week (P <0.0001) at weaning.

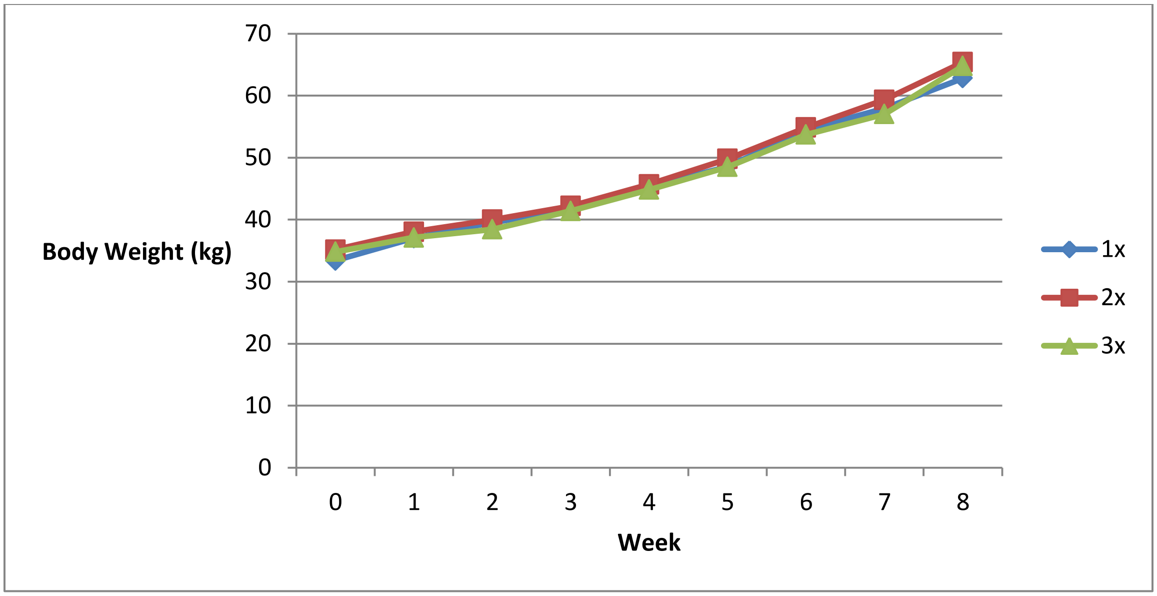

Figure 1. Least squares means for weekly average body weight for calves fed MR once (1x), twice (2x), or three (3x) time daily. SEM 3.536

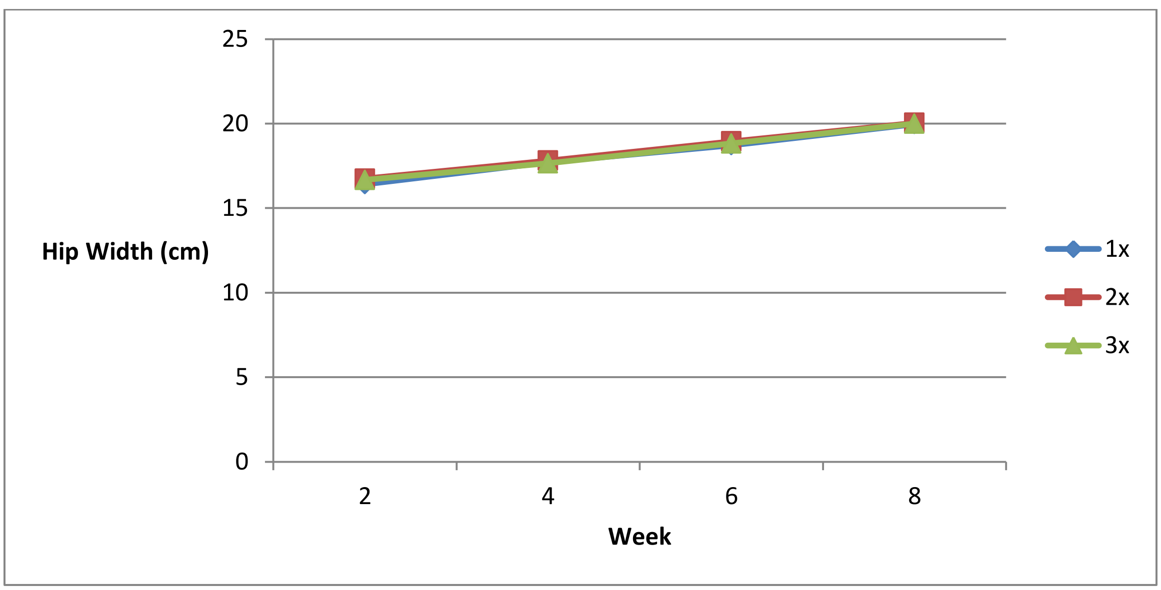

Figure 2. Least squares means for weeks two, four, six, and eight of hip width for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=0.0882

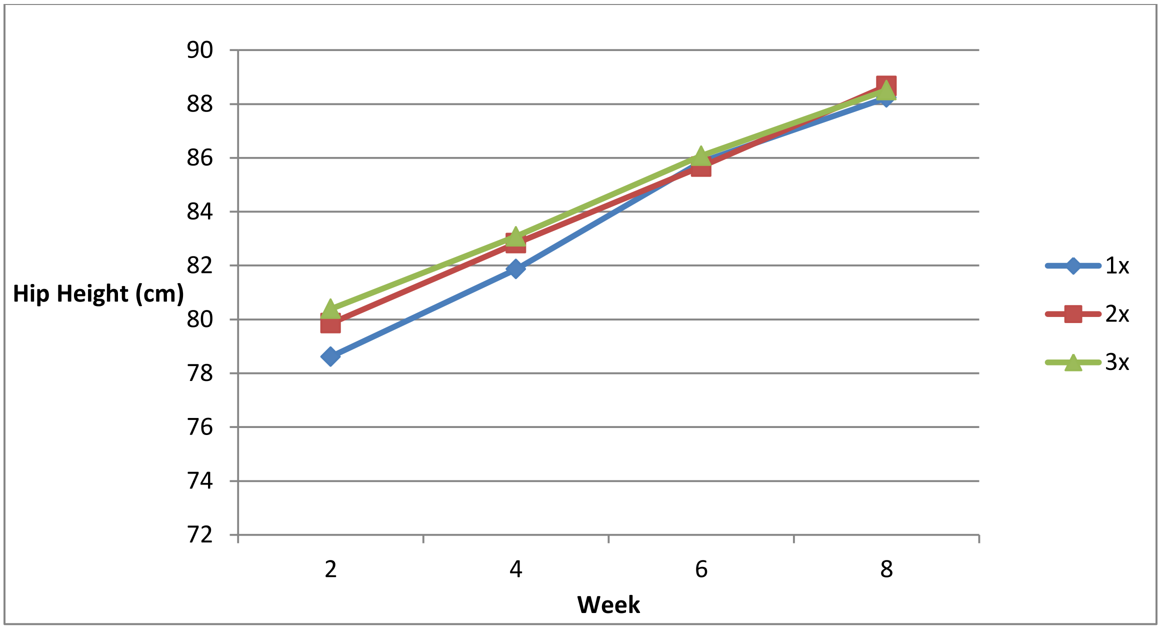

Figure 3. Least squares means for weeks two, four, six, and eight of hip height for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=0.3025

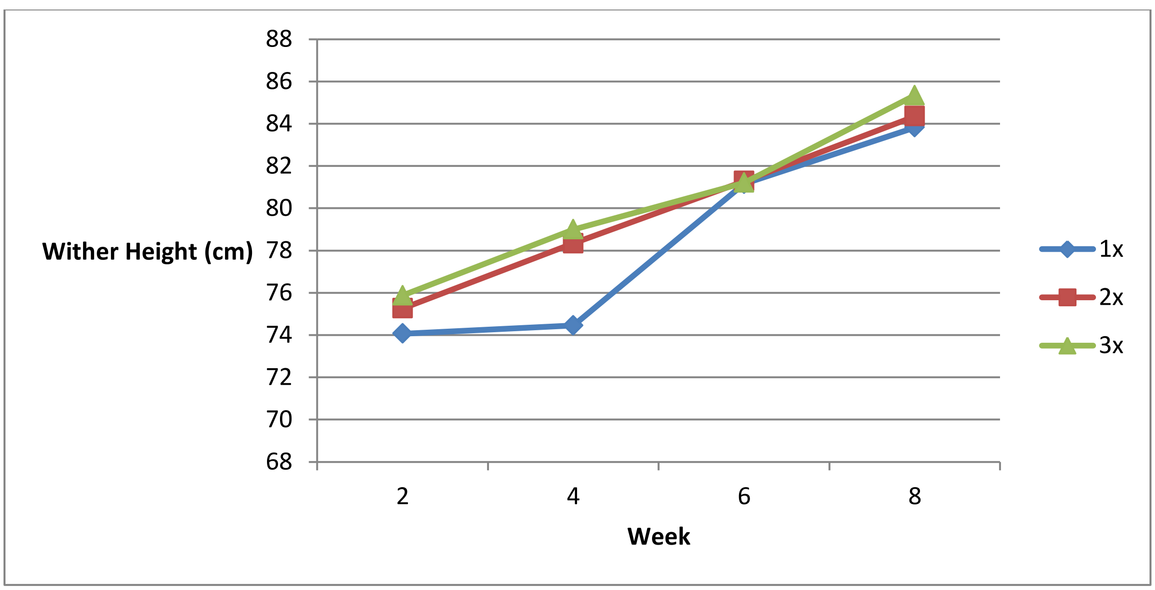

Figure 4. Least squares means for weeks two, four, six, and eight of wither height for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=0.3075

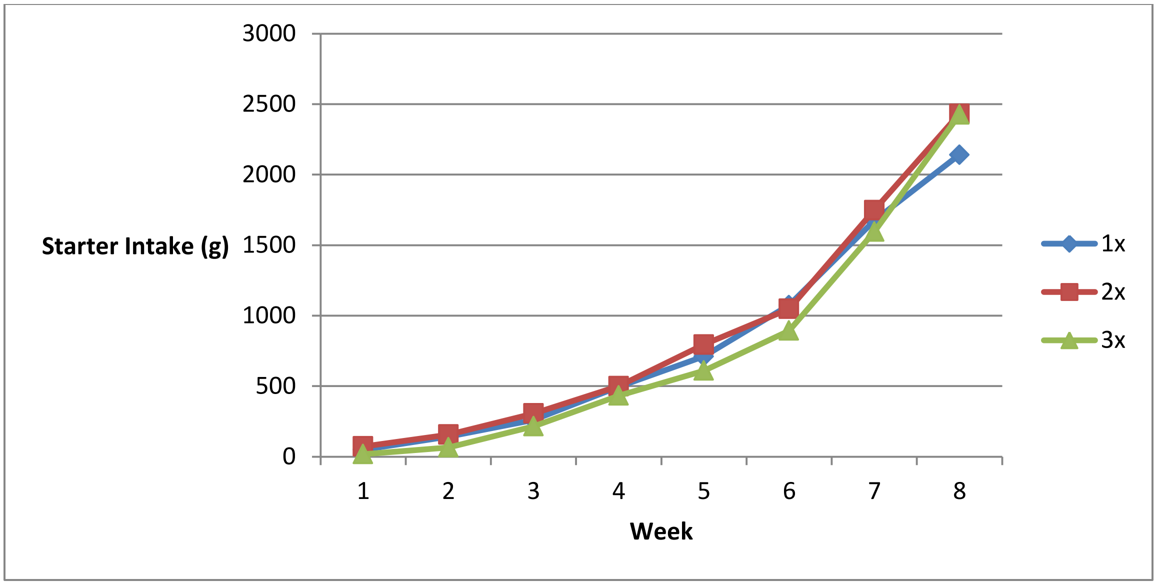

Figure 5. Least squares means for average daily starter intake by week for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=56.96

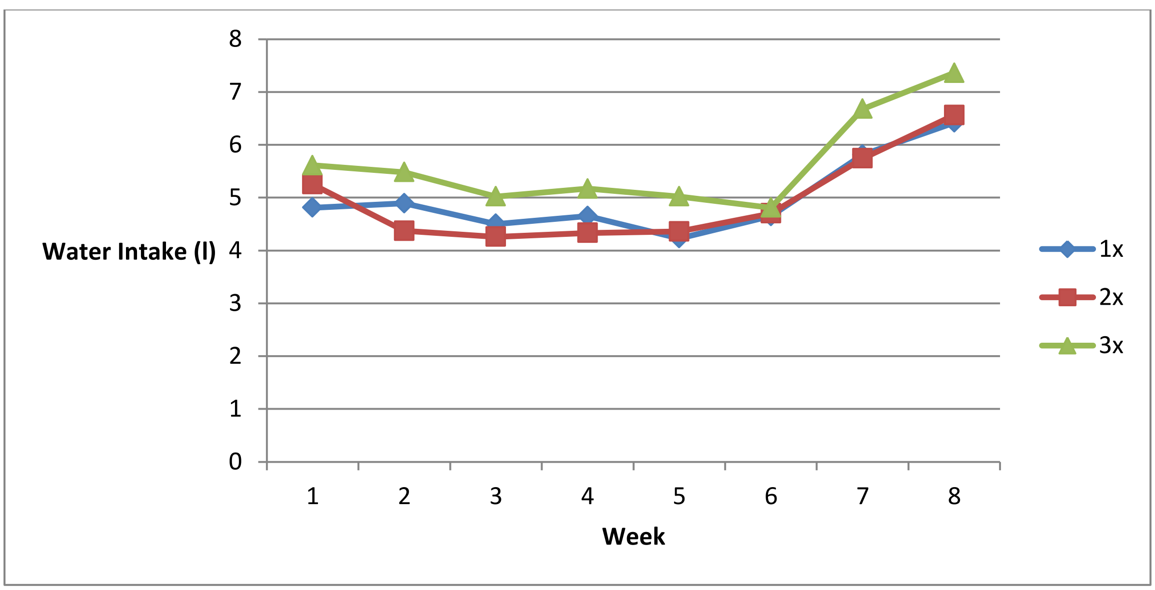

Figure 6. Least squares means for average daily water intake by week for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=0.5796

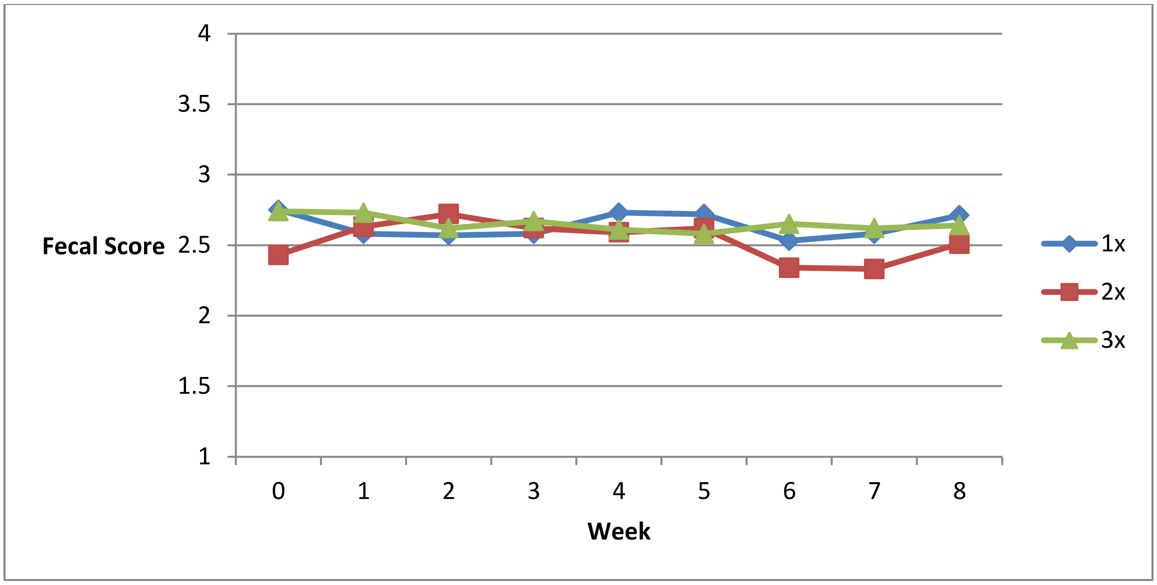

Figure 7. Least squares means for average daily fecal score by week calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=0.0519

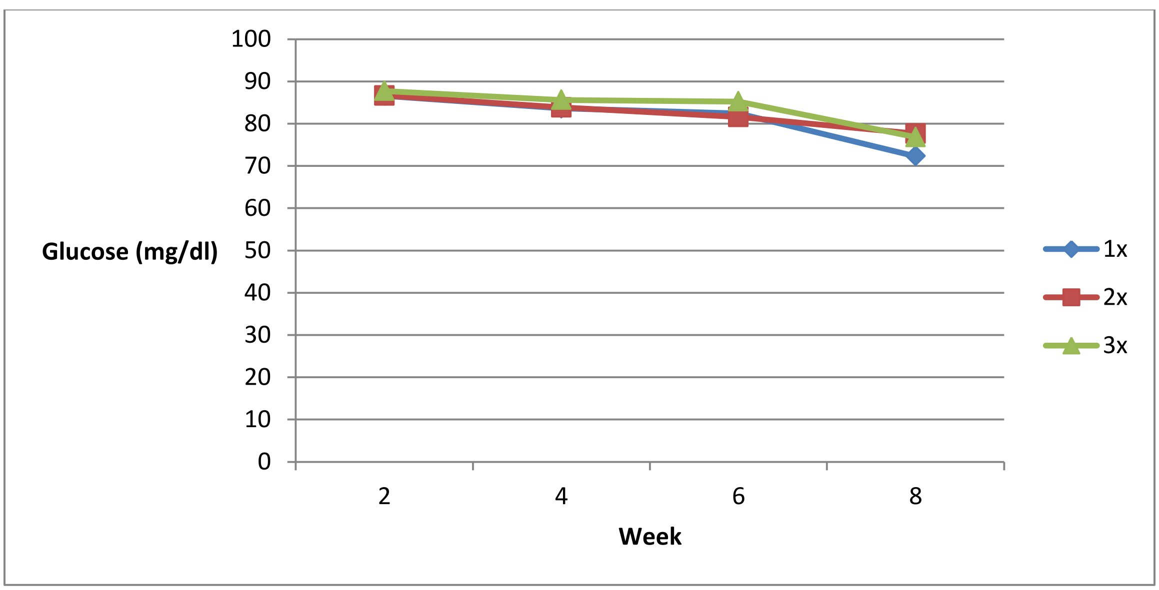

Figure 8. Least squares means for weeks two, four, six, and eight of glucose concentrations for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=3.004

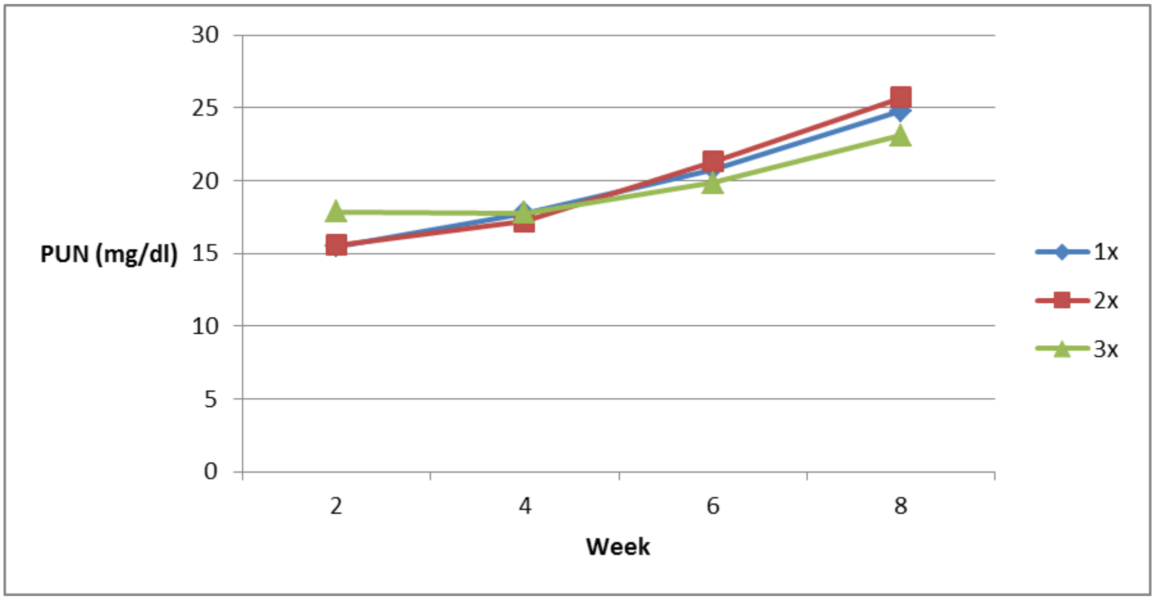

Figure 9. Least squares means for weeks two, four, six, and eight of PUN for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM=0.614

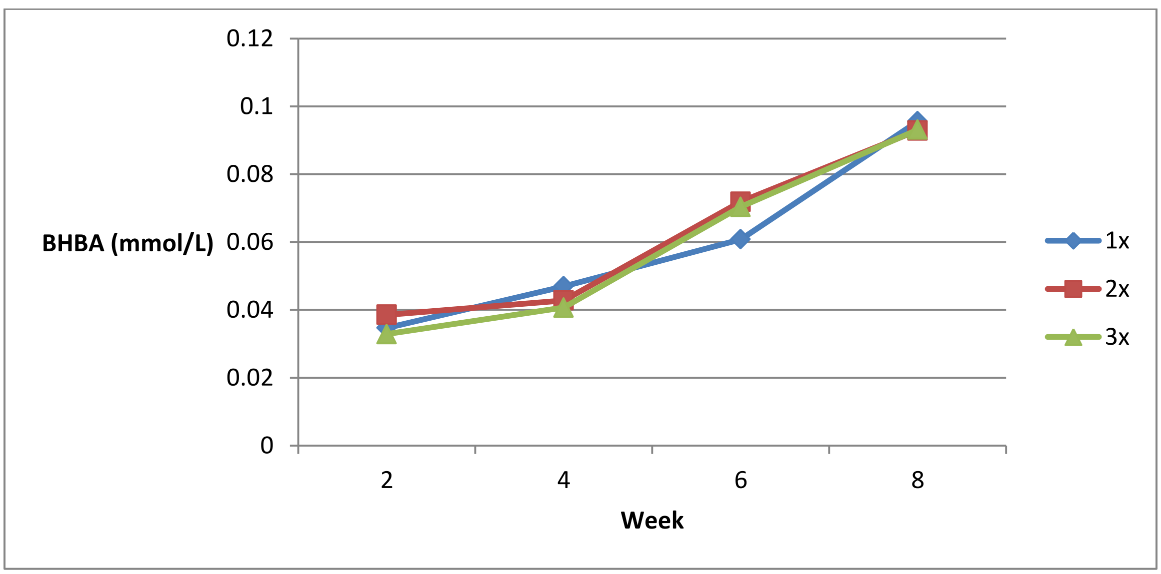

Figure 10. Least squares means for weeks two, four, six, and eight of β-hydroxybutyrate (BHBA) for calves fed MR once (1x), twice (2x), or three (3x) times daily. SEM = 0.0035

CHAPTER 5
SUMMARY AND CONCLUSION

Summary

Sixty-three neonatal Holstein calves were utilized in an 8 week study to determine if milk replacer 1x, 2x, or 3x daily affected growth and metabolite parameters. Calves were divided into three treatment groups consisting of 1x, 2x, or 3x daily feeding frequencies. Feeding times consisted of 6:00 am for 1x, 6:00 am and 5:00 pm for 2x, and 6:00 am, 12 noon, and 5:00 pm for 3x. All calves were fed milk replacer (21% CP; 20% fat) and all diets were isocaloric. Weekly body weight and bi-weekly hip height, hip width, and hip width were measured to and samples were collected bi-weekly for analysis of PUN, plasma glucose, and BHBA concentrations. Water intake, starter intake, and fecal scores were measured daily from wk 1 to wk 8.

There was no treatment effect on body weight gain, hip height, wither weight, hip width, starter intake, fecal scores, water intake, or average daily gain ($P > 0.05$). Plasma urea nitrogen, BHBA, and glucose concentrations were also not affected by feeding frequency ($P > 0.05$). As expected with normal growth and development, starter intake, body weight, hip height, hip width, wither height, average daily gain, PUN, and BHBA increased, ($P < 0.05$) but glucose concentrations decreased ($P < 0.05$) as the calves aged.

Conclusion

The data suggest that feeding milk replacer to neonatal dairy calves 1x, 2x, or 3x daily has no effect on growth or metabolism. Rumen development is also not affect by MR feeding frequency. Therefore, producers could implement which ever practice suits their labor force without compromising growth. However, feeding calves MR 1x daily reduces labor costs the most without negative effects on development.

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APPENDIX A
β-HYDROXYBUTYRATE COLORIMETRIC ASSAY

(REF: β-Hydroxybutyrate Reagent Set® Catalog No. H7587-58; Pointe Scientific, 5449 Research Drive, Canton, Michigan 48188)

Reagents:

1) Reagent 1(R1), containing β-hydroxybutyrate dehydrogenase and diaphorase enzymes
2) Reagent 2 (R2), containing NAD, INT, and oxalate
3) Standard, 1mmol/L sodium D-3-hydroxybutyrate

Procedure:

1) Prepare working reagent by mixing 10 parts R1 with 1.5 parts R2.
2) Pipette 200 μL of working reagent into each well of a 96 well plate. Incubate 10 min at 25º C.
3) Add 5 μL of standard and unknowns in duplicate into appropriate wells. Incubate 10 min at 25º C

Read and record absorbance of all wells at 505 nm. Subtract blank reading from absorbance values.

Calculation:

$$\beta\text{-Hydroxybutyrate (mM)} = \frac{\text{OD Sample - Blank}}{\text{OD Std - Blank}} \times \text{Standard Concentration}$$

APPENDIX B
UREA NITROGEN BERTHELOT/COLORIMETRIC ASSAY

Reagents:

1) Enzyme Regent (ENZYME RGT)
2) Color Reagent (COLOR RGT)
3) Base Reagent (BASE RGT)
4) Standard (25 mg/dl)

Procedure:

1) Transfer 0.5 ml of COLOR RGT to vials; unknown, control, standard, blank.
2) Add 0.010 ml (10 μL) of sample to it corresponding vial.
3) Add 0.5 mL of ENZYME RGT to all vials, mix gently, and incubate at 37ºC for five minutes. (Alternative: React to 10 minute at room temperature 2- 26ºC).
4) Add 2.0 mL of BASE RGT, mix and incubate at 37ºC for 5 min. (Alternative: React for 10 minutes at room temperature 2- 26ºC).
5) Set the wavelength of the photometer at 630 nm and zero the photometer with the BLANK. Read and record the absorbances of all vials and proceed to the Calculations with example below.

Note: For the direct read-out instrument, set read out to concentration of Standard (25 mg/dl_. Read unknown concentration directly.

Calculation:

Where A=absorbance, U= UNKNOWN, S = STANDARD, C = concentration;

$$\frac{A(U)}{A(S)} \times C(S)\ \text{mg/dl} = C(U)\ \text{mg/dl}$$

APPENDIX C
PLASMA GLUCOSE ASSAY

(REF: Sigma Technical Bulletin, #315 (December 1989), Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178, U.S.A.

Reagents:

1) Glucose Trinder Reagent: Sigma #315-500 (5x 500 mL). Store at 4ºC before and after reconstituting with distilled/deionized water; however, use at room temperature.
2) Glucose Standard: Sigma # 16- 100 (100 mL). A combined Glucose (100 mg/dL = 5.56 mmol/L) and Urea-N (10 mg/dL = 3.57 mmol/L). Store refrigerated at 4ºC.

Procedure:

1) Turn spectophotormeter (505 nm) on to warm up (~ 30 min.) Set the absorbance reading to 0.00 against distilled water.
2) Label borosilicate glass tubes (12 X 75 mm).
3) Pipette 6.25 µL (right syringe) of standards and sample, and 1,250 µL (left syringe) of the Glucose Trinder Reagent.
4) Vortex tubes and incubate at room temperature for 18 minutes.
5) Rad on spectrophotometer at 505 nm.

Note: Used the "Timed Assay Sheet" to insure samples are read on spectrophotometer exactly 18 minutes after adding Trinder Reagent.

Calculations:

$$\text{Plasma Glucose Concentrations} = \frac{\text{Abs sample}}{\text{Abs}_{\text{standard}}} \times \text{C standard} = \text{C sample (mg/dL)}$$

VITA

Michael Thomas was born in August 1988, in Bethlehem, Pennsylvania to David and Pamela Thomas. Michael graduated from Jim Thorpe Area High School in 2007 and began studies at Delaware Valley College. In 2011, Michael receive his bachelor of science degree from Delaware Valley College. In the fall of 2012, Michael began graduate school in the area of dairy nutritional physiology. In May 2014, he will receive the degree of Master of Science in the Interdepartmental Program in Animal, Dairy, and Poultry Science. Upon completion he plans to move to Dallas, Tx and work as a Nutritional and Sale Consultant for Land O' Lakes, Inc.