Louisiana State University
LSU Digital Commons

2015

Effects of Milk Replacer and Multivitamin-mineral Supplementation on Performance of Heat Stressed Dairy Calves

Steven Joseph Blair

Louisiana State University and Agricultural and Mechanical College, sblair2@lsu.edu

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Animal Sciences Commons

Recommended Citation

EFFECTS OF MILK REPLACER AND MULTIVITAMIN-MINERAL
SUPPLEMENTATION ON PERFORMANCE OF HEAT STRESSED
DAIRY 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
The School of Animal Sciences

by
Steven J. Blair
B.S., Louisiana State University, 2012
August 2015

ACKNOWLEDGEMENTS

I would like to begin by thanking Dr. Cathleen C. Williams for the years of care and support that she has shown me. Without her confidence and willingness to gamble against what were seemingly small odds time and time again, I would not be where I am. I would also like to thank Dr. Bruce F. Jenny for his mentorship and patience through many elementary explanations throughout my career to keep me up to speed in a world that was entirely new to me. To Dr. Stephanie Ward I would also like to extend my gratitude for making planning and scheduling so easy and always offering friendly advice. All of these members of my committee are exceptional not only in their field, but as people as well, and it has been my delight and privilege to spend this time working with them, not only as leaders or mentors but by my side as friends.

I would also like to give thanks to the many student workers and research associates who have made all of this work possible. To Ashley Dolejsiova and Randy Morell, I would like to say thank you for the support and advice throughout my project to make everything come together and flow properly. Gathering supplies and logistical planning is an exhausting and tedious business. Being surrounded by those who do this with such vigor and grace is truly a blessing that is too often taken for granted. To the LSU Dairy Research Farm staff thanks for being both my eyes and, in many cases, my hands in this venture, collecting data and watching out for us as a community.

A special thank you to my fellow graduate students Michael Thomas and Ruth Marisol Orellana Rivas for their aid in data collection and support as colleagues and friends. And to my other contemporaries Erica Chartier, Jake Anderson, Rachel Martin,

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Seventy-one Holstein calves were used to evaluate the effects of milk replacer (MR) feeding management alone or in combination with a multivitamin-electrolyte supplement on growth and mitigation of heat stress. Milk replacer treatments consisted of Land O'Lakes Herdmaker Supreme (20% CP, 20% fat) and Land O'Lakes Warm Front (27% CP, 10% fat). Calves received either 0 or 20 ml Palamountains Calf Boost® in MR once daily. Calves were offered treatments beginning on day 4. Calves on 27% CP : 10% fat MR were fed 2.72kg MR twice daily for the first three weeks of life, and 3.86kg twice daily until weaning. Beginning on day 42, MR feeding was reduced to 1 time per day to decrease MR intake by 50%. On day 49 calves were weaned. Water and calf starter (20% CP) were offered ad libitum beginning on day 4. Body weight, hip height, wither height, hip width, and body length were recorded weekly, and grain and water intakes were measured twice daily.

Blood was collected on days 14, 28, 42, and 56 for analysis of plasma urea nitrogen (PUN), glucose, and â-hydroxybutyrate (BHBA), as well as rumen fluid for analysis of volatile fatty acids (VFA) and pH. There was a main effect of MR, with calves fed 27% CP: 10% fat MR showing greater body weights and increased hip height, wither height, and body length ($P<0.05$). Calves fed 27% CP: 10% fat MR consumed less grain than 20% CP: 20% fat MR calves ($P<0.05$) until the end of week 7, but showed no difference at week 8). Calves fed 27% CP: 10% fat MR had greater PUN concentrations ($P < 0.05$) than 20% CP: 20% fat. Glucose concentrations decreased ($P < 0.05$) as calves aged. There was no treatment effect ($P > 0.05$) on plasma BHBA or VFA concentrations; however, concentrations increased ($P < 0.05$) as calves aged. No effects of treatment or

time were observed (P > 0.05) for rumen pH. These data indicate that MR feeding

management may improve growth performance in neonatal dairy calves, but multivitamin

mineral supplements may not provide any additional benefit.

CHAPTER I
INTRODUCTION

The dairy industry has developed many methods of rearing calves. Dairy calves are expected to double their body weight in the first sixty days of their lives (Van Amburgh, 2013). This requires an average daily gain (ADG) of about 1.5lbs per day based on the average birth weight of the Holstein breed (~90lbs). To achieve this, calves are separated from their dams and fed a combination of milk replacer (MR) and ad libitum grain based starter and ad libitum water. During the first three to four weeks of life, the calf is considered a pre-ruminant animal, unable to utilize grass or grain-based feeds due to a lack of microbes and rumen development (Tamate et al.1962; Van Soest, 1994).

MR is fed until calves can be weaned at about six weeks of age, which is long enough for the papillae found on the rumen wall to develop as a result of stimulation by volatile fatty acids. It is expected that dairy heifers grow at an exceptional rate during the neonatal period in order to be bred at 50% of their mature body weight to meet a standard calving age of 24 months. (Stelwagen and Grieve, 1992). The use of high protein MR in the daily diet of dairy calves has been confirmed to produce calves at weaning that are larger in both body weight and body size (hip height, wither height, hip width, and body length; Bartlett et al., 2006). In southeastern Louisiana, as well as across many southern states, elevated ambient temperatures and humidity are common, and the breeding programs used in the cattle industry of these areas are designed around these elevated temperatures. This means that breeding in December places calving dates in mid-August. Daytime temperatures in these areas will typically remain between 80-100° F through

October with varying levels of humidity. Elevated temperatures are known to decrease feed intake leading to lower growth performance in all ages of livestock across most species. With these stressful conditions present, the expectation of accelerated growth must be met with the use of high protein feeds that contain a decreased amount of fat to compensate for the animal's natural tendency to only consume enough to meet its energy requirement. A standard MR contains a 20:20 ratio of protein to fat, respectively, and is typically fed twice per day. The use of a twice per day feeding program would allow calves to consume more feed in a 24 hr. period, increasing feed utilization. Also the addition of a multivitamin-mineral supplement may lead to increased growth performance by fulfilling the calves' requirements for essential nutrients, and if not adding to performance, removing the possibility of deficiency.

PalaMountains' Calf Boost is multivitamin and mineral supplement that is distributed in the United States by Aragon Nutraceuticals (Charlotte, NC). Calf Boost® was designed with Omega fatty acids 3, 6, and 9, electrolytes, multivitamins, biotin, and antioxidants, and claims to promote muscle growth, control inflammation, and help joints and connective tissue heal and recover from stress. Testimonials, provided by Palamountain's selected customers, claim that the addition of Calf Boost® leads to larger calves with earlier weaning periods, calves that are observed to be healthier, and coats are reported to appear darker and thicker. There have been many field trials conducted by supporters of the company, however there has been no published scientific research to support the claims of better growth or early weaning for these calves. Land O'Lakes' Cows MATCH Warm Front MR is designed to assist producers in warm climates with heat abatement through nutritional management. A study done at Mississippi State

University by Geiger et al. in 2013 used this replacer and found the effects on heat stress

abatement to be inconclusive, due to a lack of heat stress during the length of the trial.

CHAPTER II
REVIEW OF LITERATURE

Background

    In the deep south, it is a common issue among dairy producers to view metabolic stress in cattle as not only an issue of nutrient deficiency, but also as an issue of environmental effect. In many regions of the United States the issue of cold stress is addressed nutritionally with higher energy composition in feed or increased dry matter intake for both adult animals as well as calves. In southeastern Louisiana, however, the more prevalent issue for concern is metabolic stress caused by elevated ambient temperatures and high levels of relative humidity, a common feature of the subtropical climate found across the Gulf Coast, causing significant negative effects to production in the dairy industry (St. Pierre et al., 2003). The complications brought on by this stress are vast and in many cases as unexplored as the solutions that producers in these areas desperately and often unknowingly crave. The question of the effects of higher protein and lowered energy levels along with supplementation of vitamins and minerals to already fortified milk replacers (MR) has yet to be answered in this climate condition and serves as a platform for this study.

    During the 1960's MR became a common substitution on dairy farms for waste and/or whole milk in the feeding of neonatal calves, due to its convenience, safety, and consistency after the advent of bacterial and nutritional research and its effects on the costly investment of raising replacement heifers in dairy the industry (Davis and Drackley, 1998). Since the beginning of this revolutionary practice, research has been implemented to find the most cost efficient and nutritionally productive method of feeding these animals, and has done a fantastic job of improving and specifying the

manner in which these feeds should be formulated and fed to realize the producer's and the industry's desire to raise healthy animals in a timely fashion at a reasonable cost to meet the needs of an ever expanding population.

Sadly, this mission of research and formulation has not yet been achieved to the best abilities of the scientific community. Tailoring the formulation of feeds or MR to specific climate regions, production levels, and breeds is still a work in progress. The use of 1x, 2x, and 3x per day feeding of MR has been studied on several occasions (Stanley et al., 2002; Thomas et al., 2014), and the protein levels of MR in feeding of calves have also been analyzed (Bridges et al., 2005). In these studies it was found that the use of higher levels of protein may have been economically ineffective for the producer, and the use of multiple feedings of MR has shown no significant effect on physical growth or metabolic development of Holstein calves.

The topic of accelerated MR feeding programs has become a hot button issue for those that have witnessed the effects of these frequent and substantial feedings, with champions of the programs claiming increased gains and growth leading to earlier production from our replacement heifers, and chastising conventional programs for being inadequate for and even neglectful to the needs of the growing calf. On the flip side of the coin, these claims have been met with raised brows from producers and researchers alike who have experience with raising productive animals with methods that have been accepted and promoted for nearly fifty years. These skepticisms are based around concerns of overfeeding, overspending, and retarded development that may be well founded in theory both scientifically and economically.

Normal Calf Growth and Development

The four compartments of the ruminant stomach may be clearly distinguished during 56 days post conception (Warner, 1958). At birth however, the function of a ruminant's digestive system has not yet developed in spite of the anatomical presence of the majority of the necessary tissues. Neonatal calves are classified as pre-ruminant animals, lacking the ruminal function of a more mature animal. As illustrated by Preston et al. in 1963, the development of rumen function is predominantly dictated by diet. The diet of the neonatal calf is made up of liquid milk that may be produced by the mother or fed in the form of milk replacer.

Once ingested the liquid feed runs down the esophagus and through the cardia. From this orifice the milk flows down the esophageal groove and through the reticular-omassal orifice into the omasum. This route by-passes the rumen altogether and therefore offers little to no introduction of digesta to the rumen environment. Due to this phenomena, rumen development is retarded and little to no development of the rumen mucosa occurs (Warner and Flatt, 1965). Proper development of the rumen mucosa generally refers to the structural development of rumen papillae. In the natural order of things, and as seen in most beef production models, the calf begins to introduce roughage to the diet to supplement its liquid feed intake. In the dairy industry, high quality starters with a crude protein value of 18-20% percent are supplemented to the calf's diet beginning between days 2 and 3 of life to simulate this natural sequence and promote rumen development (Whitelaw et al., 1961). Although many producers prefer to allow calves to receive their requirements for water from milk or replacer, the practice of denying access to ad libitum water to promote milk consumption is strongly discouraged

and frowned upon by the research community. Ad libitum intake of water promotes grain intake and thusly promotes proper development of the rumen (Kertz et al., 1984).

A survey published by the National Animal Health Monitoring System in 2007 stated that out of 2194 operations surveyed, starter was offered on day 8.5 of life, and water on day 15.3 on average. This survey suggests that the most popular methods of calf rearing are not necessarily supported by scientific study, further fueling the need for future researchers and publications that are accessible to the producer and will promote positive change in the industry.

In a normal production setting calves should double their birth weight in the first 60 days of life (Van Amburgh, 2013). By the end of these first eight weeks calves should also be weaned, eating a diet consisting of grain mix and quality hay. By 12-16 weeks of age (BW=100kg) the compartments of the calf's stomach mostly resemble that of a mature cow (Warner and Flatt, 1965). By this time the rumen should be hosting a plethora of bacteria and functioning as the site of volatile fatty acid (VFA) absorption.

Volatile Fatty Acids

Volatile fatty acids are produced as a by-product of carbohydrate fermentation in the rumen. Acetate, propionate, and butyrate are the main sources of energy in ruminant animals (Martin et al., 1959; Brown et al., 1960). By 3 weeks of age the, VFA production and metabolism have begun in the rumen. The production of these substances will increase over time with the establishment of bacterial populations (Anderson et al.,

1987). In a study by Sanders et al. in 1959, it was determined that the presence of VFAs were the major cause of ruminal papillary development.

β-Hydroxybutyrate

Volatile fatty acids are produced in the rumen as a result of bacterial fermentation of carbohydrates. Once produced, butyrate and propionate are absorbed through the rumen wall, where they are converted into ketones and used as an energy source for the ruminant. β-hydroxybutyrate is one of those ketones. Butyrate is absorbed through the rumen papillae and oxidized for energy to β-hydroxybutyrate. Approximately 27 moles of adenosine tri-phosphate (ATP) are produced in the rumen wall from the 50% of butyrate that is absorbed from the rumen (Jergens, 1997). Although physical assessment is naturally the first thought that comes to mind in the assessment of rumen development, a study by Quigley et al. in 1991 stated that the quantification of β-hydroxybutyrate levels in the calf is an acceptable measure of microbial activity in the rumen, which is low in young calves (Baldwin, 2004). Calves 0-4 weeks of age and 5-8 weeks of age differed, with calves 0-4 weeks age showing lower β-hydroxybutyrate concentrations and thus lower microbial activity (Quigley et al., 1991). Putting all of these ideas together we may conclude that the functional development of the rumen may be safely observed through the measure of β-hydroxybutyrate, without sacrificing the animal for physical examination of the digestive tract.

Glucose Metabolism

Glucose is a monosaccharide used as the main energy source in most organisms. Glucose is stored in the body as glycogen and enters the cell through the function of insulin. Insulin is a hormone released by the pancreas and binds to receptors on the cell to allow glucose to pass through the cell membrane. The main site of glycogen storage is the liver, however this is not the only place that insulin acts. After a meal there is a primary release of insulin that lasts approximately 5 min. Within 10- 20 min after the first release, there is a secondary release of insulin lasting $1 - 1.5$ hrs. Inside of the cell, there is a thermogenic reaction resulting in the production of adenosine triphosphate (ATP). This is the energy currency that is stored and fuels the majority of the biochemical reactions in the body. As a result of falling glucose levels in the blood, due to insulin activity, the liver releases the hormone glucagon. Glucagon causes the release of glucose stores from the liver through glycogenolysis and initiation of gluconeogenesis resulting in the increase of blood glucose levels. This is a dynamic system and fluctuations in hormone concentrations take time to return to a basal level (Guyton and Hall, 1996).

When blood glucose levels are depleted the body turns to fat and glycogen stored in muscle for energy. Fat stores have the highest level of stored energy. When blood insulin concentrations are low, lipase enzymes are activated to breakdown fat stores for oxidation to produce required energy. Since glucagon prevents the liver from removing fat, amounts of adipose tissue increase during periods of elevated insulin concentration.

As previously discussed the main source of energy for the ruminants are the VFAs produced by microbes in the rumen. Carbohydrates that would normally be broken down and absorbed through the small intestine are fermented in the rumen to form

9

VFA's. For this reason blood glucose levels are lower in ruminants than in other animals. Hsu and Crump (1989) stated that ruminants are in a constant state of gluconeogenesis. Blood glucose concentrations are lower in ruminants (60-80 mg/dl) than non-ruminants (80-120 mg/dl). However, in the pre-ruminant, neonatal calf blood glucose levels resemble that of a non-ruminant more than an adult cow.

Quigley and Bernard (1992) observed that calves absorb the majority of their nutrients through the small intestine. Post-weaning, the rumen has the opportunity to begin functioning at a higher capacity and progresses toward normal adult function. This is when the absorbed VFAs begin to take over as the primary source of energy production. As the rumen develops the blood- glucose level of the calf will start to decline. Once this process begins, propionate produced in the rumen become essential due to its role as precursor to glucose production (Lyford and Huber, 1988).

Rumen pH

In a mature ruminant animal the optimal pH range lies between 5.8- 6.8, with 6.7 being optimal for microbial growth (Van Soest, 1994). Rumen pH is determined by two main factors. The physical form of feed adjusts pH through microbial interaction with feed containing more surface area (smaller particle size) and those interactions resulting in digestion, causing the release of VFAs and other by-products, lowering the pH of the rumen fluid (Beharka et al., 1998). In a study by Hibbs et al. (1956), rumen pH was measured as a resulting factor of feeding various hay:concentrate ratios to 21 Jersey and Holstein steers. Rumen pH increased with advancing age, but was maintained at a lower concentrations in the calves fed larger amounts of grain (Hibbs et al., 1956). As microbial

populations increase in number, the production of acetate, propionate, and butyrate will lower the pH of the rumen, and changes the environment, thereby altering the microbial population.

Plasma Urea Nitrogen (PUN)

Urea is used as a potent source of non-protein nitrogen (NPN), with CP values approaching 280% (Kellems & Church, 2002). Carbon dioxide, methane, and NH_3 are the primary products of fermentation of feeds high in protein in the rumen. Hayashi et al. (2006), reported that free NH_3 in the rumen is used for the synthesis of microbial protein or absorbed and sent to the liver for packaging into urea. Urea formed in this way will then be secreted into saliva and reabsorbed in the rumen for later use in the same fashion, known as the urea cycle (Obara & Shimbayashi, 1980; Roseler et al., 1993). When microbial proteins are produced, they are stored in the microbes until they pass into the abomasum where microbes lyse and amino acids are digested and absorbed in the small intestine (Hayashi et al., 2006).

As the rumen develops, the rumen concentration of urea nitrogen should increase, while a steady decrease in NH_3 should also be observed (Davis and Drackley, 1998). After weaning, urea nitrogen concentrations should continue to rise due to both increased microbial activity and anatomical development of the rumen. Plasma urea nitrogen is indicative of the amount of protein degradation occurring in the body. Concentrations of PUN should rise and fall slowly in response to changes in amount and quality of the protein in the diet. Decreases in PUN values may be observed as a result of low nitrogen concentrations in the rumen, resulting in increased amounts of rumen escape protein,

calling for the recycling of the urea present in the blood. Increases in PUN values could be the result of high quality protein or increased protein supplementation (Van Soest, 1994).

Heat Stress and Related Complications

Reducing heat stress has been a long term struggle for many farmers around the world. The use of sprinklers, cooling ponds, and shade sources are necessities in the livestock industry. The Temperature Humidity Index (THI) was established to account for the effects that humidity and temperature have on animals (Ravagnolo and Misztal, 2000; Kadzere et al., 2002). Heat stress has been shown to have an inverse effect on dry matter intake and is strongly correlated with the amount of water consumed (Thibeau, 2011). This would obviously affect the amount of nutrients consumed by calves in the warmer periods of the year. It is expected that calves born during this period will not perform as well as those developing in more favorable environmental conditions.

A 1986 study by Beede and Collier pin-pointed three strategies for alleviating the adverse effects of heat stress on the animal. The first strategy is to alter the animal's physical environment. In a study done by Thibeau et al. in 2011, the effects of heat stress on Holstein calves were significantly lowered when housed in a shaded area compared to those in hutches left in direct sunlight. In milking herds shade tarps, cooling ponds, and sprinklers are also frequently used for heat abatement. The second strategy for alleviating these adverse effects is developing the genetics of heat tolerant cattle such as *Bos indicus* breeds. However, currently popular selection for production selects away from heat tolerance (Ravagnolo & Misztal, 2000). The third strategy is to improve upon the

12

nutritional management of the animal itself. Although the National Research Council's Requirements for Dairy Cattle (2001) makes no acknowledgement towards the additional requirements of heat stressed cattle, it is clear that cold ambient temperatures are in fact a concern for producers due to metabolic stress and may be compensated for through adjustments in nutritional management. This same principle may be put into action for heat related stress. Cold stress is typically addressed by adding energy to the ration of the animal, however during elevated temperatures dry matter intake (DMI) will decrease. Therefore, the ration must be lower in energy and high in protein in order to sustain a suitable level of intake of the concentrated feed, to compensate appropriately (Beede and Collier, 1986).

Considering that the average cow in the U.S. spends 14.1% of its annual hours under heat stress, and cattle raised along the gulf coast spend approximately 50% of their annual hours under heat stress (St. Pierre et. al., 2003), the nutritional requirements of these southern animals are elevated and should be considered in diet planning. Consideration should also be given to the issues caused by lack of physiological adjustment during spontaneous heat stress. Weather in the coastal region is as unpredictable as anywhere else, but the variations in temperature are significant between one day and the next, particularly during spring and fall. This lack of a transitional period may result in spontaneous heat stress without allowing for physiological compensation as discussed by Beede and Collier (1986).

Effects of heat stress may be observed and quantified through measurements of blood pH, partial pressure of CO_2 (pCO_2), measurement of growth (body weight, hip height, wither height, and hip width), DM and water intake, and hematocrit. Partial

pressure of CO_2 and pH can indicate respiratory alkalosis and compensatory metabolic acidosis after elevated THI when panting is used for heat abatement (Sanchez et al., 1994). This may cause ruminal acidosis due to the lack of saliva production for buffering in the rumen. Hematocrit levels will reflect water intake of the animal. Water intake will directly affect DMI, and DMI will directly affect growth. Although these measurements may seem to be feeble indicators on their own, together they paint an overall picture that points directly towards heat stress.

Milk Replacer Composition: Protein and Fat Content

Milk replacer formulation has developed over time, much like other aspects of the industry. In the past, MRs typically included dried skim milk as the main ingredient. However, in the more recent past, the price of dried skim milk has risen, and the uses of low-temperature ultrafiltration and quality whey concentrates have led to those concentrates becoming the predominant ingredient in MR today (Davis and Drackley, 1998). The NRC stated in 2001 that the growth of calves less than three weeks of age is best supported by MRs containing all- milk proteins. Although other protein sources such as soy protein isolates and concentrates do exist along with animal plasma and modified wheat gluten, the amino acid profiles of these sources are not as effective in promoting growth as milk proteins and will only contribute a fraction of the actual requirements of the calf (Davis and Drackley, 1998).

The ideal dairy calf is thought of as a heifer that is raised from birth through weaning and to breeding while living at the lowest possible cost to the producer until calving. The aim of most research in the past has been to elucidate the most cost effective

way to feed protein while maintaining normal growth of the calf. Although this has been the case in the past, trials were performed by Lassiter et al. (1963) to determine the proper level of protein for optimal absorption and use by the calf. The first trial involved 32 calves fed MR containing various levels of protein, (15.2-30.9%) alongside a 16.5% CP starter and hay beginning at week 1. In this trial calves fed MR at 15.2% CP were observed to grow slower than those fed 18.7% or higher.

In the second trial by Lassiter et al. (1963) 40 calves were used in similar circumstances and five treatments were designated. Treatments were milk replacer containing 16.6, 19.6, 23.5, and 29.7% CP. Results were found to be similar to those of the first trial. Between 2-49 days of age, the growth of calves fed MR containing 16.6% CP was found to occur more gradually than those fed higher protein MR. The third trial focused on the metabolism of milk replacers used in trial one. This trial showed that the lowest level of protein that may be fed, before growth is slowed, was 19%, and CP levels above 24% may be in excess to the calf's requirement.

Although appropriate CP concentrations were studied in previously mentioned trials, the feed efficiency of calves has not been. A trial was conducted in 2001 by Diaz et al. to determine such compositions of the growth that was observed in previous trials. Holstein calves were fed MR containing 30% CP and 20% fat at 1, 3, and 4% percent of body weight. Milk replacer for treatments 1 and 2, fed at 1 and 3% of BW respectively, were reconstituted to 15% DM, and treatment 3 replacer, fed at 4% BW, was reconstituted to 18% DM. Calves were fed 3X per day with individual buckets. Calves were slaughtered at 65, 85, and 105kg BW to study carcass composition at various points of gain. Treatment 3 calves required the least DM intake to reach target weights and

consumed greater quantities of MR than treatment 2 calves. From this information, it was concluded that calves could reach levels of feed efficiency nearing that of other species, including lambs and pigs.

A later study of 40 calves tested the differences in nutrient utilization, body composition, and growth (Blome et al., 2003). After a two week period for standardization, calves were assigned to a baseline slaughter group or to one of 4 MR treatment groups containing 16.1, 18.5, 22.9, and 25.8% CP. Over a six week period calves were fed MR at 12% BW with intake adjusted weekly to account for increases in BW before the calves were sacrificed. PUN levels were highest in the 25.8% MR group indicating that protein provided was not used as efficiently as it was in the other groups. It was concluded that increasing CP from 16 to 26% increased lean gain as well as frame growth (Blome et al., 2003).

Although protein is an important component of milk, fat concentrations must also be considered. Marshall and Smith (1973), studied the effects of MR containing 3, 6, or 9% fat on the growth and utilization of energy for weight gain, when fed to dairy calves ad lib. Each MR was fed both diluted and undiluted with 1.5 parts water. Calves fed undiluted MR showed more substantial gains than calves fed diluted MR, and those in the undiluted 3% treatment group were observed to have fewer occurrences of digestive upset.

Kuehn et al. (1994) used120 Holstein calves at 3 separate locations (40 at each) to study the effects of MR fat levels in calf starter on growth and performance. All calves were fed high fat MR at 10% of body weight from day 5 to day 13 before being sorted into treatments of 1) 21.6% fat MR/ 7.3% fat starter, 2) 21.6% fat MR/ 3.7% fat starter,

3) 15.6% fat MR/ 7.3% fat starter, and 4) 15.6% fat MR/ 3.7% fat starter. From days 14-35 all calves were fed MR at 8% of birth weight per day and 4% per day from days 36-42. All calves were weaned on day 43 and continued on feed to day 56. High fat (21.6%) MR lowered dry matter intake both before and after weaning, while high fat (7.3%) starters caused lowered intake post weaning. Prior to weaning, calves fed low fat 15.6% MR gained more, as did calves fed low fat 3.7% starter post weaning. At the conclusion of the study, low fat MR and starter led to greater gains in calves.

It was concluded by Tikofsky et al. (2001) that the addition of fat to MR does not promote lean tissue gain. These researchers used 32 Holstein bull calves across 3 treatments to investigate the effects of MR fat concentrations on fat deposition. Treatments were isocaloric and isonitrogenous, and fat levels of treatments 1, 2, and 3 were 14.5, 21.6, and 30.6% respectively. Baseline calves were sacrificed on day one when all calves started their respective treatments. All calves were between 2 and 6 days of age at the beginning of the study and were raised to 85kg before being slaughtered. When substituted for carbohydrates (CHO) as an energy source, dietary fat was used for fat deposition rather than encouraging retention of additional protein (Tikofsky, 2001).

A study conducted by Brown et al. (2005) measured the effects of protein and energy on mammary growth using 49 Holstein heifers. Calves were split into four treatments, by time period and feed composition. Treatments were broken into early (weeks 2-8) and late (weeks 8-14) time periods, and high and low protein and energy treatments. Treatments during the early period included a low group being fed 21.3% CP and 21.3% fat MR at 1.1% body weight and a 16.5% CP starter, and a high groups fed 30.3% CP and 15.9% fat MR at 2.0% of body weight and a 21.3% CP starter. The late

period included groups fed either a 16.5 or 21.3% CP grain mix in the low and high groups respectively. At 8 weeks eleven calves were slaughtered and at 14 weeks 41 calves were slaughtered, and mammary tissue was collected from both groups. It was concluded that increasing protein and energy intake in Holstein calves from 2-8 weeks of age can increase the rate of development of mammary tissue (Brown et al., 2005).

Nutritional Strategies for Heat Stress Mitigation

Traditionally calves are fed MR twice per day at approximately 15% solids and 10% body weight with 20% protein 20% fat. Replacers containing a 26% CP: 20% fat have been marketed in recent years as a cold weather replacer for producers raising calves in harsh cold climates, along with MR more suitable for warm climates as well, containing a 27% CP: 10% fat. However, the use of these MRs is not exclusive to compensation for climate. These replacers are showcased in Land O'Lake's Full Potential Feeding Programs. The term "full potential feeding" has become popular among suppliers, whose phrasing carries a positive tone that must be seriously considered by all that hear it. The question one must ask is whether or not it is necessary. In the past these programs were referred to as accelerated programs because of the high levels of CP and energy and increased DMI. When DMI is increased we must feed at a rate that will allow the abomasum and, at a later point, the ruminal capacity of the calf to digest the feed appropriately for optimal growth (Van Soest, 1994).

A study by Geiger et al. (2013) utilized 44 neonatal Holstein calves to observe the effects of increasing protein with and without the supplementation of a direct-fed microbial on growth and performance during periods of elevated ambient temperature.

Although heat stress was not observed for any significant period during the duration of this study, the rectal temperature of calves supplemented with a direct-fed microbial were found to be lower than control calves fed a 20%CP: 20% fat MR and calves fed a 27% CP: 10% fat MR.

In the previously mentioned study by Blome et al. (2003) CP levels above 25% were shown to cause elevated PUN levels indicating that exceeding this level is overfeeding protein. However, this study was done on calves under normal maintenance and growth conditions. According to the NRC (1981) the nutrient requirements of the calf increase during these times of stress and it can be concluded that feeding increased amounts of CP in the presence of this heat stress may be necessary to meet all requirements. This would be particularly evident during cold stress when energy intake may be increased to allow for maximum utilization of protein.

It was concluded by Jaster et al. (1992) that increasing fat in MR also increases solids, which results in increased average daily gain (ADG) and increased fecal scores. Also, increases in protein and feeding rate led to increased body weight and frame measurements which may also result in earlier calving, according to Raeth-Knight et al. (2009). This is the leading justification for using "full potential" feeding programs that require higher input costs for feeding neonatal calves, and promise greater and earlier returns by shortening the time to first breeding and increasing mammary growth, thus leading to increased milk yield as observed by Brown et al. (2005).

Multi-Vitamin and Mineral Supplementation

Although the thought of a daily multi-vitamin sounds ideal for most humans who consume variable diets, the supplementation of dairy calves that consume well formulated and diets is largely unnecessary. Most commercial MRs and starters are fortified with essential vitamins (A, D, and E) and calves do not require additional amounts when the components of the starter and MR are both considered. However, stressful conditions such as travel or environmental changes may cause subclinical deficiencies that may require supplementation to prevent the occurrence of clinical symptoms (Davis & Drackley, 1998). Also, digestive disturbances, such as infection or diarrhea, decrease fat digestion and thus absorption of fat soluble vitamins A, D, E, and K (NRC, 2001).

Palamountains' Calf Boost® is a multivitamin and mineral supplement that contains vitamins (A, C, D, E, and K), Biotin (B7), omega (3, 6, and 9) fatty acids, assorted B vitamins (1 (Thiamin), 2 (riboflavin), 3 (niacin), 5 (pantothenic acid), 6 (pyroxidine), and 12 (cobalamin)), and minerals (K, Mg, Ca, P, and Cl). Palamountain's claim is that, along with promoting normal health and growth, Calf Boost® may assist in alleviating the stress of illness from environmental sources. In field trials, conducted by Palamountain's selected customers, calves were fed in group feeders, and, in some cases, downer cows were supplemented with Calf Boost® to act as a daily electrolyte and multivitamin and mineral supplement. Consumers claimed that results following the use of this product met expectations based on commercial advertisements. Based on these claims by Palamountains, this product may prove to be a beneficial additive to the diets

of calves experiencing heat stress due to elevated ambient temperatures and relative humidity.

Vitamin A, also known as retinol, is a fat soluble vitamin that is added to most calf starters and MRs to ensure the consumption of this essential nutrient. Retinol has been shown to have significant effect on the differentiation of epithelial tissue (Combs, 1992). Deficiency of vitamins A & E have been associated with the depression of calves' immune systems, leading to an increase in instances of infection and illness (Roy, 1980). Combs et al. (1992) observed that retinol plays a role in increasing mitogenesis in lymphocytes, as well as the phagocytic capacity of macrophages and monocytes. It was suggested by Nonnecke et al. in 1999 that elevated levels of vitamin A may decrease the bioavailability of vitamin E.

Vitamin E, also known as α-tocopherol, is often discussed in conjunction with selenium due to their close working relationship in protecting cell membranes from degradation of various oxidizing agents through enzymes such as glutathione peroxidase (Agricultural Research Council, 1980; Davis & Drackley, 1998). In this relationship glutathione peroxidase is a selenium containing enzyme that degrades toxic peroxides, and α-tocopherol is responsible for the collection of these toxic compounds that escape degradation in the first encounter (Davis & Drackley, 1998). It is made certain in most cases that commercial feeds and starters contain more than the adequate amount of vitamin E, however those feeding whole milk may require supplementation. (Tomkins and Jaster, 1991).

It has been suggested that vitamin E plays a strong role in the health of the calf without affecting growth or development of the calf in any way; however, these claim

have yet to be substantiated by others and remain a popular debate (Reddy et al., 1985, 1986; Davis & Drackley, 1998). Common deficiencies of vitamin E include calf retardation and muscular dystrophy, of which chances are increased in physiologically stressed situations (NRC, 2001).

Vitamin D, or 1,25-dihydroxycholecalciferol, is a fat soluble vitamin that responsible for increasing absorption of other vitamins and minerals, including calcium (Ca) and phosphorus (P), essential for normal bone remodeling and muscle contraction. A deficiency in vitamin D will result in rickets which illustrates its role in skeletal development. It has been stated that the requirement of vitamin D is small in the presence of adequate calcium and phosphorus (Agricultural Research Council, 1980). The use of excess amounts are common, since the toxic dose of vitamin D has been shown to be 1,000,000 IU/kg; approximately 100 times higher than the highest recommended amount; (Davis & Drackley, 1998).

Calcium and P are macro-minerals regularly associated with one another and are often found in complex with one another in the form of hydroxyapatite. Calcium is essential in heart function, muscle contraction, and nerve function, while P is found in numerous phosphate esters such as ATP and used extensively for energy conversion (McDowell, 1992; Davis and Drackley, 1998). Although the optimal ratios these minerals is debatable, it is typically included in the diet at levels around 10 g/kg of DM. However, these must be fed in conjunction with recommend levels of vitamin D to allow for appropriate absorption and avoid instances of deficiency.

Magnesium (Mg) is a macro-mineral, and only trace amounts are required for normal development and growth. A deficiency of Mg may be caused by using whole milk

as the sole source of nutrients when rearing replacements. The ratio of calcium and magnesium stored in bone decreases from 60:1 to approximately 90:1 as the calf ages and requirements increase while availability remains the same. The required amounts of Mg in the calf's diet is typically suggested in conjunction with optimal levels of other dietary minerals (Davis & Drackley, 1998). McDowell (1992) stated that Mg plays a key role in energy metabolism, acting as an activator for enzymes involved in phosphorylation.

Potassium (K) is one of the principle electrolytes in the body along with sodium (Na) and chloride (Cl). Na and Cl are usually concentrated in extracellular fluid, while K is more concentrated within the cell. These minerals are used for many processes throughout the body including osmoregulation and nerve transduction. These minerals are typically found in adequate amounts for maintenance in whole milk, however replacers are often fortified with levels exceeding the requirements to compensate for deficiencies caused by scours, which is a common problem among calves. Supplementation is often unnecessary, however the use of an electrolyte supplement during cases of diarrhea may be advisable (Davis and Drackley, 1998).

CHAPTER III
MATERIALS AND METHODS

Animals and Dietary Treatments

Seventy-three neonatal Holstein calves (bulls, n=32; female, n=41 (mean BW= 52.96kg)) were used in an 8 week experiment to determine the effects of milk replacer composition and a multivitamin and mineral supplement on performance and mitigation of heat stress in Southeast Louisiana. All animals were born and raised on Louisiana State University Agricultural Center's Dairy Research and Teaching Farm. Calves were born between August and December, 2013. Care and use of all animals in this study were conducted in accordance with and under approval of the Institutional Animal Care and Use Committee, LSU Agricultural Center.

All calves were separated from their dams between 1 and 3 hours after birth, weighed, and navels were dipped with a 2% iodine solution to prevent infection through the umbilical stump, and vaccinated for Rotavirus and Coronavirus (Calf Guard, Pfizer Animal Health, Lenexa, KS) prior to receiving colostrum. Calves received between 4-6 liters of colostrum within the first 12hrs of life, and transition milk through day 3, being offered 3 liters during both morning and afternoon feedings. Calves were trained to drink from buckets beginning on day 4, and this method of feeding was continued throughout the study.

Calves were housed in individual calf hutches measuring 2.5m^2 with a 2.8m^2 wire enclosure on rock bedding. At birth calves were blocked by sex and randomly assigned to one of four treatments in a 2x2 factorial design. Treatments were 1) control (CON)(20% CP, 20% Fat (Herd Maker 20-20 BOV BM w/ ClariFly®); (Land O'Lakes Animal Milk

24

Products Co., Shoreview, MN)), 2) TRT2 (MR 20% CP, 20% Fat) + Palamountains Calf

Boost®, 3) TRT3 (Warm Front® Land O'Lakes® (WF) MR 27% CP, 10% Fat), 4)

TRT4 (WF MR 27% CP, 10% Fat) + Palamountains Calf Boost®. Morning and

afternoon feedings were scheduled at 6 a.m. and 5 p.m. respectively. Calves on

treatments 1 and 2 were fed 2.28kg twice daily at 15% dry matter. Calves on treatments 3

and 4 were fed according to an accelerated program of 2.72kg twice daily for the first

three weeks of life, and 3.86kg twice daily until weaning on day 42. Calves receiving

supplementation (TRT2 and TRT4) received 20ml of Calf Boost® evenly mixed into the

MR during AM feedings. All calves were weaned using a gradual weaning program,

receiving half of the original amount of MR from days 42 to 48, and only calf starter

beginning on day 49. All calves were offered water and Amplicalf (Purina Animal

Nutrition, Gray Summit, MO) (20% CP; 2.5% fat) ad libitum throughout the 56 day trial,

beginning on day 4. Intakes of MR, starter, and water intake were recorded twice daily.

Sample Collection
Growth measurements

Body weight, hip height, wither height, hip width, and body length were recorded

weekly through 8 weeks approximately 4hrs after AM feeding. Body weight was

recorded with a TruTest EZWeigh1 (Auckland, New Zealand) in lbs and converted to kg

for analysis. Hip height and wither height were recorded using an altitude stick, along

with body length, which was taken from the withers to the rear of the pins, using a

Coburn Dairy Calf Tape. Hip width was measured with a Bessey® Duoklamp and

recorded from a standard meter stick. Fecal scores were recorded daily and averaged

weekly on a scale of 1-4 (1= firm, 2=soft, 3=runny, and 4=watery) (Larson et al., 1977). Rectal body temperatures and respiration rates were measured three days per week prior to morning and afternoon feedings.

Blood Sampling

Blood was collected from all calves via jugular venipuncture tubes on days 14, 28, 42, and 56 into three separate tubes of 10mL, 10mL, and 5mL volume. These Vacutainer® tubes contained various anticoagulants including no anticoagulant, sodium heparin, and potassium oxalate and sodium fluoride respectively. All were centrifuged for 15 minutes at 600 x g and plasma and serum were collected, protected from UV light, and stored at -20ºC until analysis.

Rumen Fluid Sampling

Rumen fluid was collected by stomach tube 4hrs post-feeding for analysis of VFA concentrations and rumen pH on days 14, 28, 42, and 56. Rumen fluid was collected using a SHUCO-VAC vacuum pump by Allied Healthcare Products, INC. (St. Louis, MO) and a ¼" non-toxic esophageal PVC tube. After pH was recorded using a Thermo scientific Orion 2 Star pH benchtop by Fisher Scientific (Waltham, MA) meter, 1mL of phosphoric acid (20% w/v) was added prior to freezing. All rumen fluid was stored at -20ºC until analysis.

Lab Procedures

β-Hydroxybutyrate Concentratons

Commercial spectrophotometric kits (β-Hydroxybutyrate Reagent Set); (Pointe Scientific, Inc., Canton, MI) were used to measure plasma for β-Hydroxybutyrate concentrations (Appendix A).

Plasma Urea Nitrogen

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

Plasma Glucose Concentrations

Commercial spectrophotometric kits (Glucose Oxidase Reagent Set); (Pointe Scientific, Inc., Canton, MI) were used to measure plasma glucose concentration (Appendix C).

Total Volatile Fatty Acid

A 4 mL sample of ruminal fluid was mixed with 1 mL of 25% (wt/wt) meta-phosphoric acid containing 10 g/L 2-ethylbutyric acid, which was used as an internal standard for VFA quantification. The mixture of ruminal fluid and meta-phosphoric acid was centrifuged at 30,000 x g for 25 min. Concentrations of individual VFA were measured by GLC using a Shimadzu GC2010 equipped with a 15-m EC-1000 column that had an internal diameter of 0.53 mm and a film thickness of 1.2 μm (Alltech Associates, Inc.; Deerfield, IL). The reagent preparation procedure and temperature gradient for VFA analysis was adapted from Grigsby et al. (1992) and Bateman et al. (2002), respectively (Appendix D).

Weather Data

All weather data was collected by the LSU AgCenter using a Campbell Scientific data logger (Logan, Utah). Dry bulb temperature, and relative humidity were measures and entered into an equation developed by Mader et al. (THI = 0.8 * Ambient Temperature + ((Relative Humidity/100) * Ambient Temperature – 14.3) + 46.4); 2004) to calculate the THI index.

Statistical Analysis

Treatments were arranged in a 2x2 factorial (main effects= milk replacer and multivitamin and mineral supplementation) with gender, treatment (CON, CON+, MR, MR+), week, and THI as class variables with attention to interactions. THI was removed from the model when not significant, but was significant for intake, health, and blood metabolite measures. Data with multiple measures per calf were analyzed by repeated measures ANOVA using the MIXED procedure in SAS (Cary, NC). Comparison of data used least significant means. Significance was declared at $P < 0.05$ and tendencies were declared at $P < 0.10$.

CHAPTER IV
RESULTS AND DISCUSSION

Feed Intake Data

     Least squares means for MR intake are presented in Figure 1, which illustrates a treatment by week interaction ($P<0.05$), as well as a main effect of week ($P<0.05$). Milk replacer intake was greater in calves fed 27:10. Intake increased during weeks 3 through 6 as indicated in protocols for these treatments, and addition of Calf Boost® did not affect MR intake ($P>0.05$). This also serves as a clear depiction of the weaning program used in this study for both CP treatment groups.
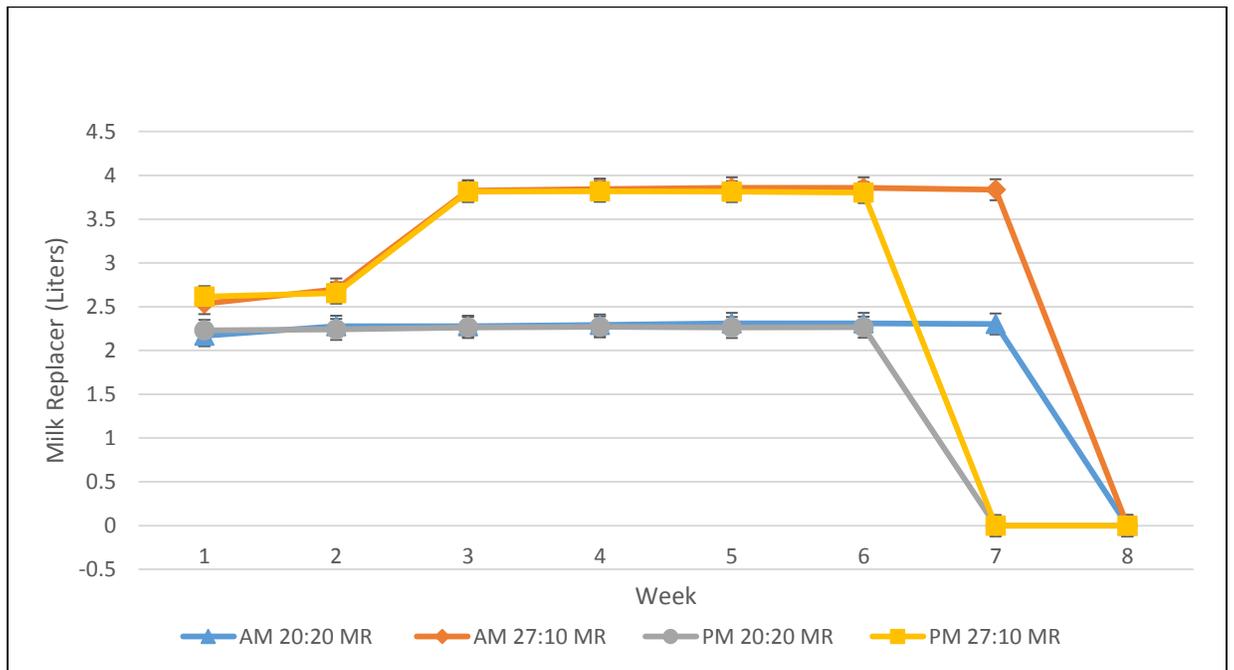

Figure 1. Least squares means of average daily MR intake in calves fed MR containing 20% CP: 20% fat or 27% CP: 10% fat with treatment by week interaction ($P<0.05$). Main effect of treatment ($P<0.05$) and week ($P<0.05$). SEM =0.120.

Interactions between MR treatment and heat stress are presented in Figure 2 and illustrate the effects on average MR intake. The differences in the 20:20 and 27:10 feeding
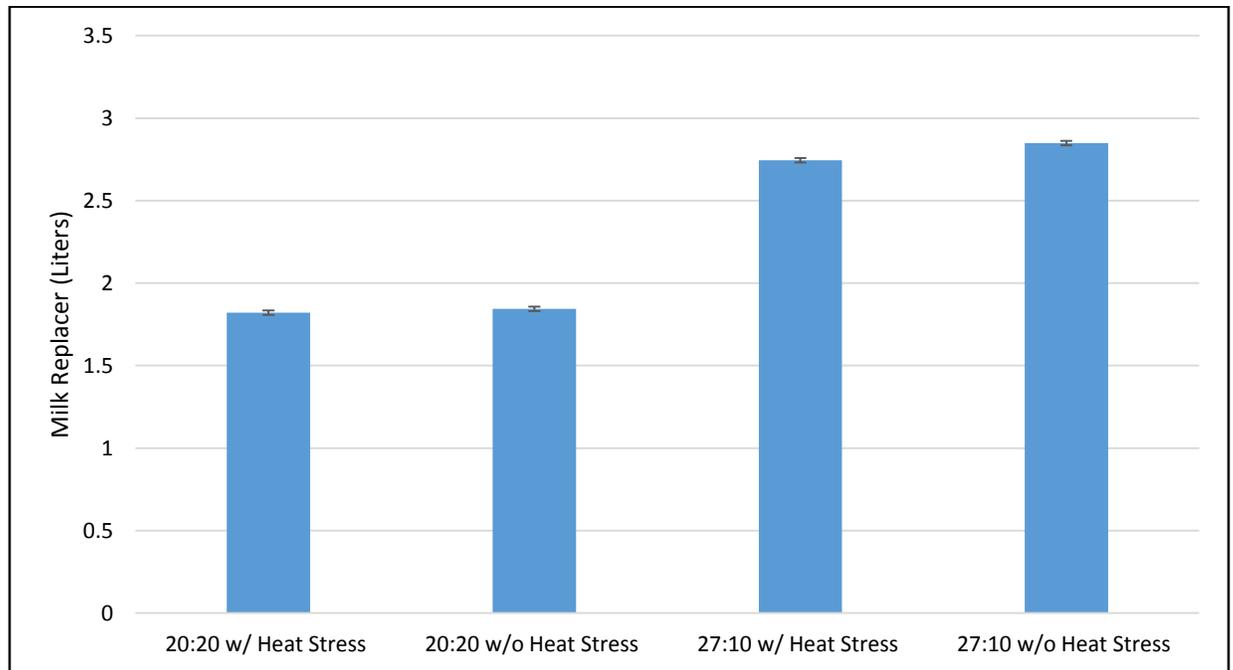
Figure 2. Least squares means of average milk replacer intake of calves fed varying MR levels with or without the presence of heat stress. Interaction of MR and heat stress (P<0.05) SEM =0.014.

programs were expected. However, the difference in the heat stressed and non- heat stressed values may be due to intake differences in feeding bull and heifer calves (P<0.05) during the first week of the trial. Heifers were observed to consume less when learning to drink from buckets and being offered the substantial amount of replacer used in the accelerated program.

Least squares means of average daily starter intake are presented in Figure 3 showing the main effect of week (P<0.05). A significant difference (P<0.05) between AM and PM feedings was observed during week 7, which is the point of weaning. Increased grain consumption is typically observed after weaning because nutrients are no

longer provided by feeding MR. Interactions between MR treatment and week are

illustrated, with increased starter intake ($P<0.05$) in calves fed 20:20 and 27:10 MR
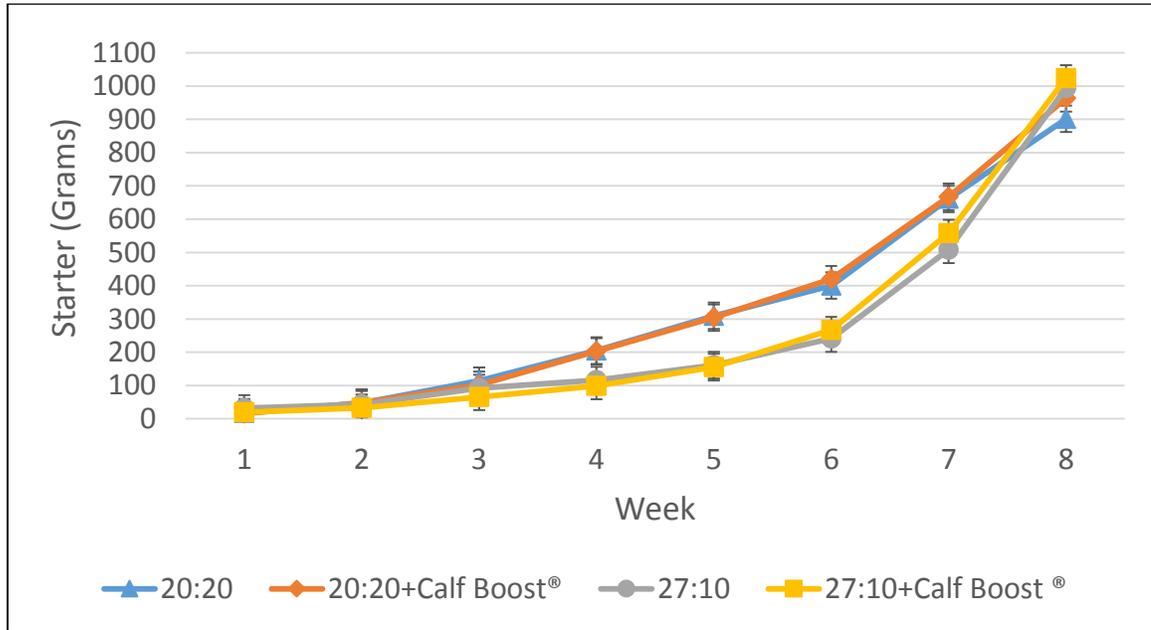
during weeks 4 through 7.

Figure 3. Least squares means of average daily starter intake for calves fed MR
containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and
27% CP: 10% fat with Calf Boost ®. Interaction of MR and week ($P<0.01$). SEM =
39.845

A difference in AM and PM starter intake during week 7 was also observed in calves fed

20:20 MR. With increases in MR for calves in the 27:10 MR treatment groups, the

differences during weeks 4 through 6 were expected until weaning during weeks 7 and 8

when values for both groups eventually converge. The significant difference observed

during week 6 is a result of normal feeding schedule changes, and the expectation for MR

feedings in the afternoon not being met. During week 7, calves normally wait until after

daylight feeding times have passed and the temperature has dropped and then consume

grain during the night time hours. Least squares means presented in Figure 4 show the

interactions of sex and week on AM and PM grain intake. A significant difference

(P<0.05) during week 7 is observed, with bull calves consuming more starter than heifers. This difference is due to the inclusion of several outliers in the study who consumed much larger amounts of grain throughout the study, and who consumed exceptional amounts of starter during the first week post weaning as part of their adjustment. These calves were also found to be larger in size than other calves, which may also contribute to this observation.
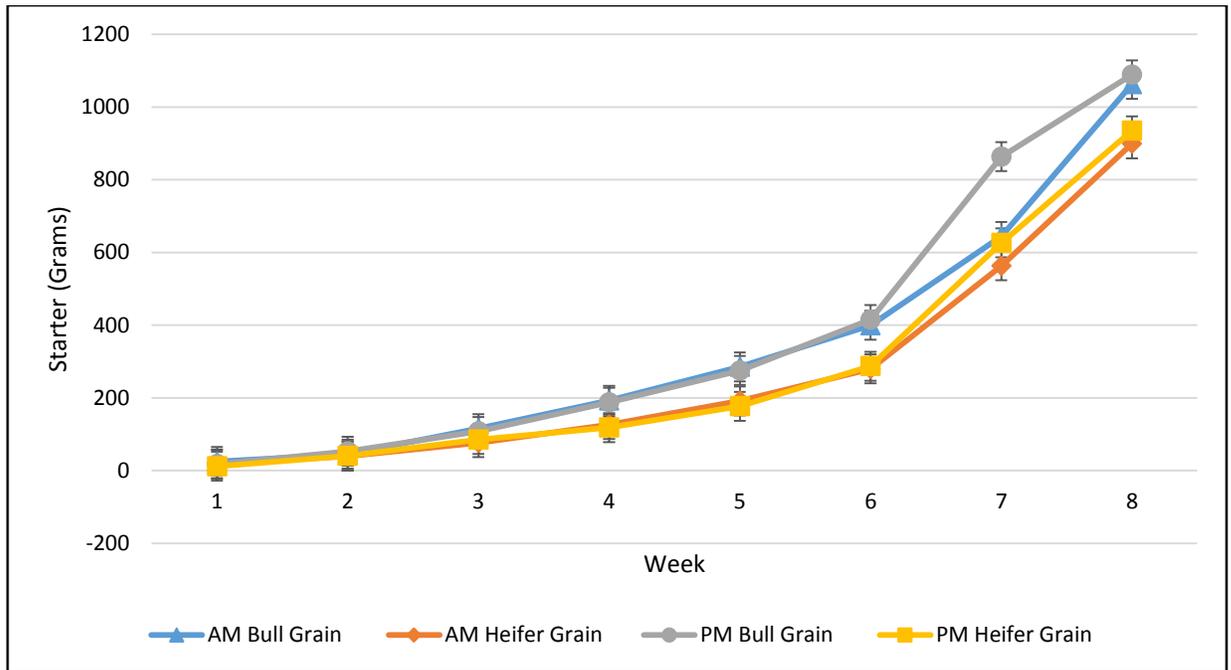
Figure 4. Least squares means of average daily AM and PM starter intake. Interaction of intake and sex (P<0.05). SEM = 39.845

Growth Performance Data

Least squares means of body weight are presented in Figure 5, and demonstrate normal growth for neonatal calves in which they double their birth weight in the first 60 days of life. A main effect of MR was observed, with calves fed 27:10 having higher average body weight than those in the 20:20 MR program (P<0.05). There was an

interaction between MR composition (P<0.05) and week, illustrating increased body weight in calves fed 27:10 MR throughout the study, with the exception of weeks 0 and 1. This may be attributed to the set minimum birth weight of 29.5kg without the inclusion of undersized twins. Beginning at weeks 2, 3, 4, and 5 respectively, body weight, hip height (Figure 6), wither height (Figure 7), and body length (Figure 8) were all found to be significantly higher in the 27:10 MR groups. These differences are consistent with the results of Geiger et al., where calves fed Land O'Lakes Warm Front Milk Replacer were observed to grow significantly larger during this first 8 weeks of life. Least squares means for body weight, hip height, wither height, hip width, and body length are also presented in Table 1 with male calves having greater (P<0.05) body weights, hip heights, wither heights, and body lengths.

Table 1. Least squares means for growth parameters, glucose, and PUN concentrations in bull and heifer calves with or without heat stress.

	Bull	Heifer	SEM[1]	P Value	Stress	No Stress	SEM[1]	P Value
Body Weight, kg	55.88	50.04	2.402	0.0001	53.24	52.69	1.870	0.2792
Hip Height, cm	85.98	84.26	0.425	0.0188	82.00	85.41	0.793	<0.0001
Wither Height, cm	79.94	78.46	0.632	0.0180	77.48	80.92	0.931	0.0003
Hip Width, cm	18.25	18.18	0.782	0.9200	17.35	19.09	1.254	0.1774
Body Length, cm	63.66	60.54	0.629	<0.0001	58.25	65.95	0.983	<0.0001
Glucose, mg/dl	100.66	93.19	2.717	0.0137	101.72	92.14	3.546	0.0057
PUN, mg/dl	17.71	13.91	0.643	<0.0001	14.76	16.86	0.973	<0.0001
# of Observations	32	41	N/A	N/A	57	579	N/A	N/A

[1]Standard error of the means.

Figure 5. Least squares means of weekly body weights for calves fed MR containing 27% CP: 10% fat, 27% CP: 10% fat with Calf Boost ®, 20% CP: 20% fat, and 20% CP: 20% fat with Calf Boost ®. Interaction of MR and week (P<0.01). SEM = 1.8794
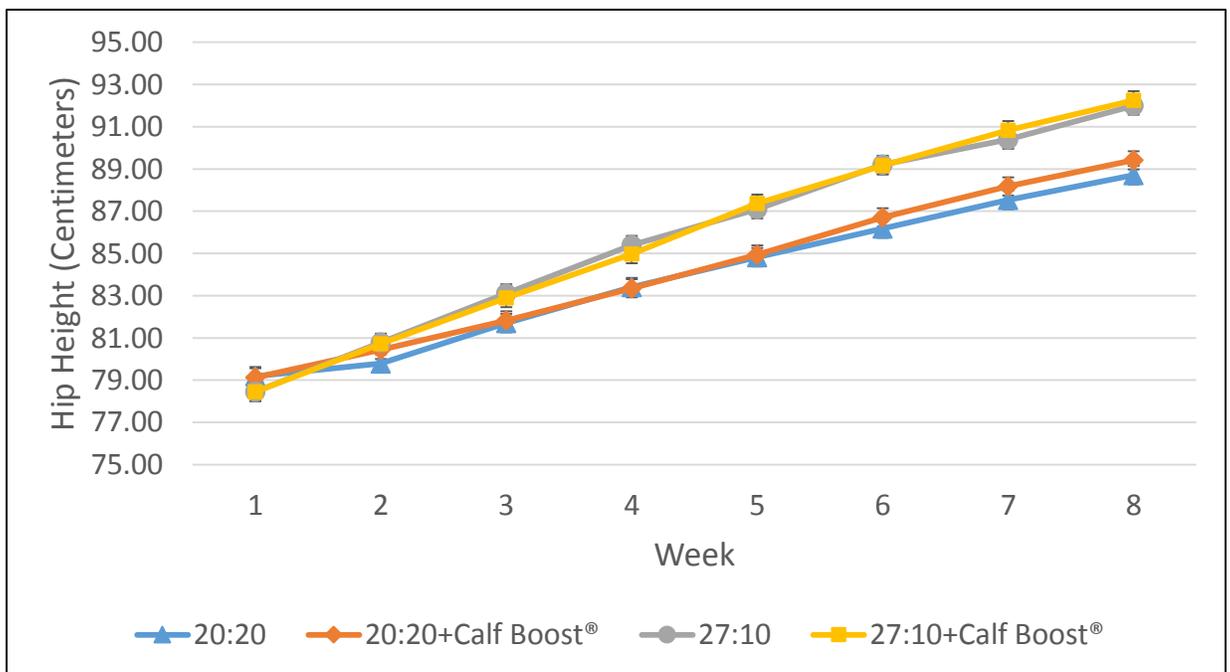
Figure 6. Least squares means of weekly hip heights for calves fed MR containing 27% CP: 10% fat, 27% CP: 10% fat with Calf Boost ®, 20% CP: 20% fat, and 20% CP: 20% fat with Calf Boost ®. Interaction of MR and week (P<0.01). SEM = 0.4346.

Figure 7. Least squares means of weekly wither heights for calves fed MR containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and 27% CP: 20% fat with Calf Boost ®. Interaction of MR and week (P<0.01). SEM = 0.604.
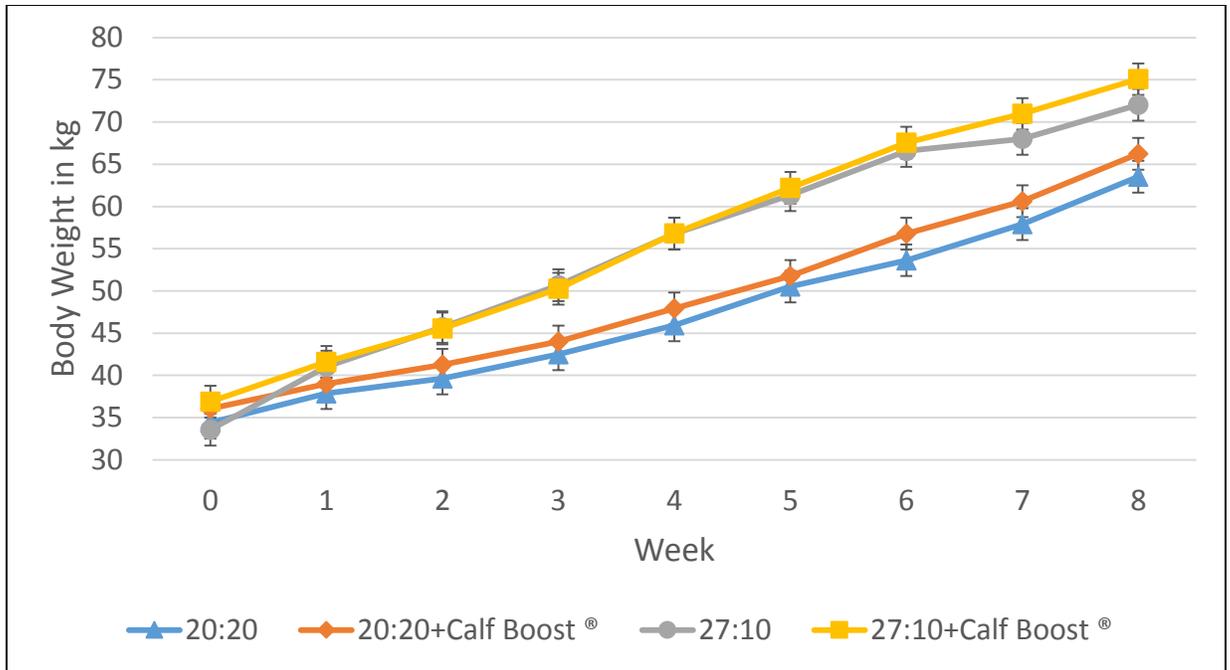

Figure 8. Least squares means of weekly body lengths for calves fed MR containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and 27% CP: 10% fat with Calf Boost ®. Interaction of MR and week (P<0.01). SEM = 0.9094.
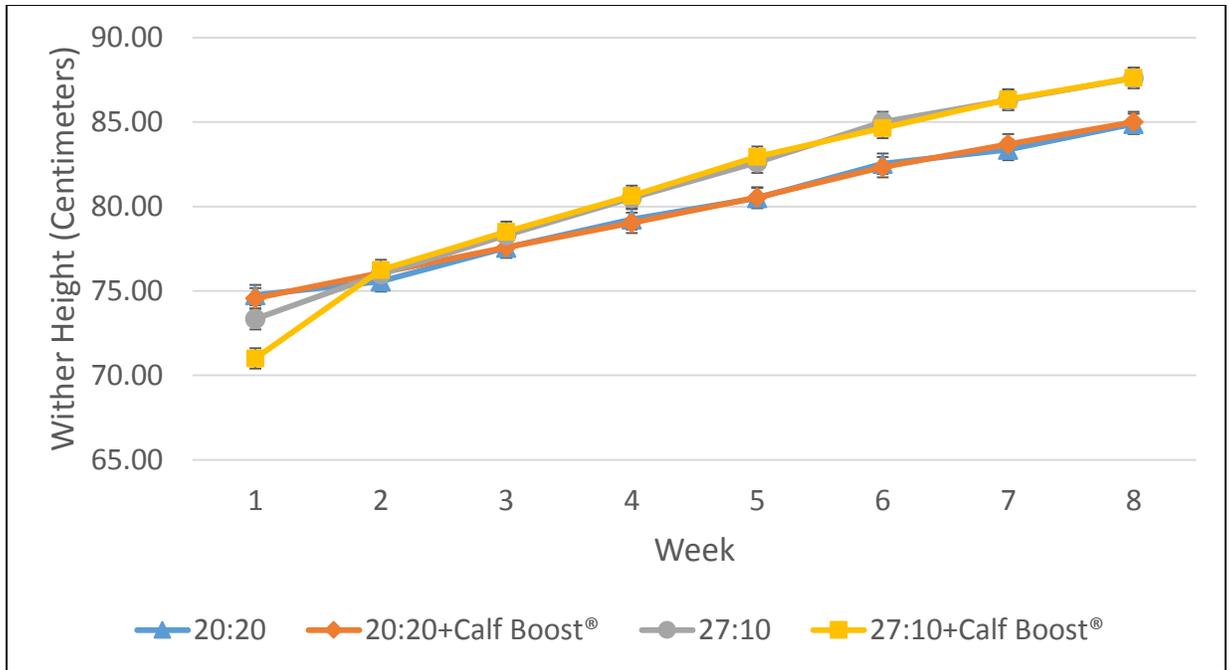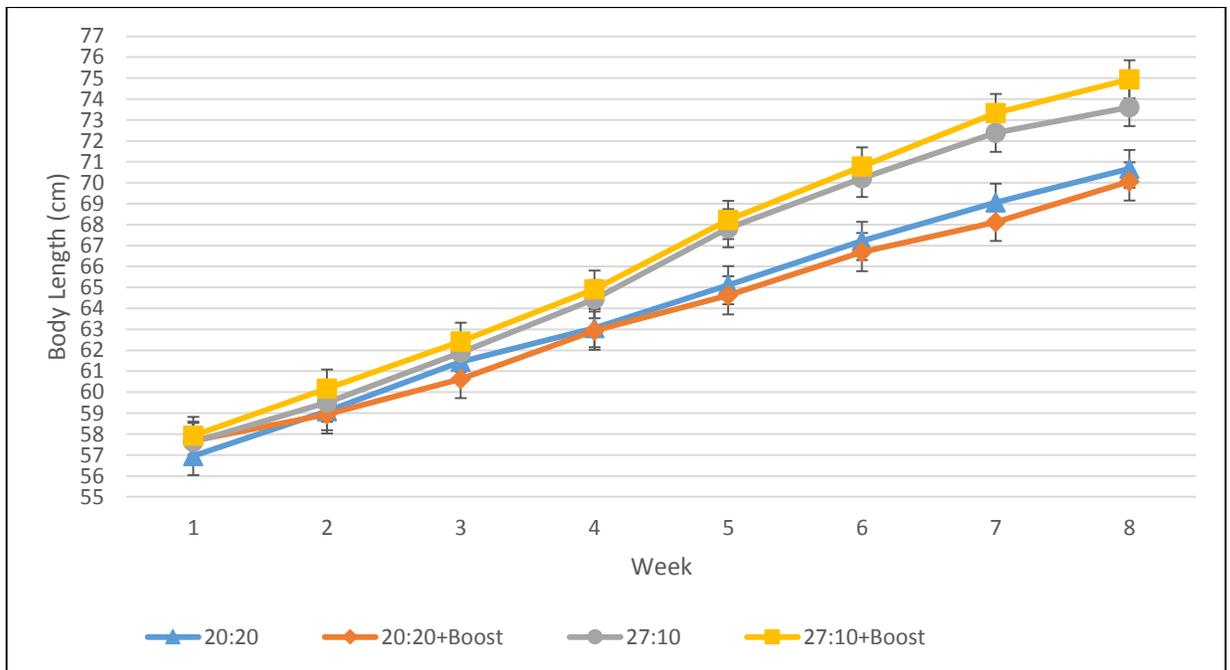
Blood Metabolite Data

Least squares means for plasma glucose concentrations are presented in Figures 9, 10, and 11. Blood glucose levels decreased over time as expected, with a main effect of week (P<0.05), particularly between weeks 2 & 4 and weeks 6 & 8. A MR by week interaction was observed (P < 0.05), with calves consuming 27:10 MR having greater glucose concentrations during week 4 as presented in Figures 9 and 10. This difference may be attributed to the difference in MR feeding regimens, with the 27:10 calves consuming more than twice the amount as the 20:20 calves during weeks 3 through 6. It is expected that during weeks 3 and 5 the levels would follow a linear pattern of decline as well, with week 3 being the peak of the curve.

Least squares means for the main effects of sex and heat stress on PUN concentrations are presented in Table 1 and represented graphically in Figure 11. Bulls were inexplicably shown to have higher blood glucose levels than those observed in heifers (P<0.05). Heat stressed calves were also observed to have higher blood glucose levels than those considered outside of our heat stress parameters (P<0.05).

A Calf Boost® by sex by week interaction was observed for glucose concentrations (P < 0.05; Figure 11). This interaction indicated that bulls fed Calf Boost® had higher blood glucose levels during week 6 than bulls not fed Calf Boost® (P<0.05). This difference is likely due to some abnormality caused by human error or random chance. This theory is supported by the lack of difference in heifer calves fed in the same manner and similar trends observed by Geiger et al. (2013). A 3-way interaction between heat stress, sex, and week was observed (data not shown) and not easily explained. There was a significant difference (P<0.05) between bulls and heifers during

week 2, when heat stressed bulls were observed to have higher blood glucose concentrations than all others. The lack of heat stress during weeks 6 & 8 is responsible for the significant change in heat stressed and non-heat stressed calves. While these significant interactions were observed, no biological implications were evident in growth and performance in the calves in this study.

Least squares means for β-hydroxybutyrate are presented in Figure 12 and support our understanding of the normal development of the calf. A main effect of week was observed with concentrations increasing over time with significant increases ($P<0.05$) over each 2 week testing period. Week 2 values were not analyzed due to the expected minute concentrations of β-hydroxybutyrate that are found in the pre-ruminant calf. A significant difference ($P<0.05$) was also observed in weeks 6 and 8 between calves in treatment group 3 (27:10) and all other calves. Calves in this treatment group were shown to have significantly higher β-hydroxybutyrate concentrations than other calves, suggesting greater rumen function, however this trend was not observed in treatment group 4 (27:10 +Calf Boost®), which suggests that this occurrence was either coincidental or hampered by the presence of Calf Boost®.

Least squares means of plasma urea nitrogen levels are presented in Figure 13. The main effect of MR ($P < 0.05$) can be explained in part by the difference in protein percentage, but more by the amount of protein that was substantially higher (more than twice as much) than the 20:20 treatment groups. A main effect of week was also observed ($P < 0.05$) in week 8, and not the previous weeks or over time. This period is post weaning and is attributed to the starter consumed in copious amounts of over 2kg per day in some cases. This period is also the point in the study where the amount of feed intake

is left entirely to the calf. With no liquid feed to fill the appetite of these calves, they are left to eat to fill their own needs, with starter intake based on their individual desire to consume dry matter. Least squares means for sex (Table 1) were shown to be significantly different (P<0.05) with bulls having a much higher average PUN than heifers. The difference in sex has no clear explanation. However, intake differences should be considered when explaining the main effect of sex, along with the main effect of sex on body weight found in Table 1, where bulls were observed to be significantly larger (P<0.05) than heifers and can be expected to consume more feed in this period to meet their individual requirements.

Least squares means of rumen VFA concentrations are presented in Figure 16 with a main effect of week (P<0.0001). Treatment means for rumen VFA and pH are presented in Table 2. The rumen pH data are similar to those reported by Geiger et al. (2013) and Williams et al. (2007). These researchers also compared accelerated milk replacer feeding programs to conventional rearing programs. However, the lack of differences in VFA concentrations in the current study are in contrast to results reported by Geiger et al. (2013) who reported greater concentrations of acetate, propionate, and butyrate in calves fed milk replacer containing 22% CP and 20% fat. Williams et al. (2007) also reported greater concentrations of acetate, propionate, and total VFA in conventionally reared calves consuming 20:20 MR prior to weaning, compared to higher protein lower fat MR, which coincided with the greater starter intake observed in their study. The concentrations molar percentages of acetate, propionate, and butyrate were observed to be in normal proportions, but molar percentages were elevated when

38

compared to those in other studies conducted at our research facilities (Williams et al, 2007; Bridges, 2009; Doescher, 2010).

Least squares means for fecal scores are presented in Figure 14 and fall well within the range of normal healthy calves and decline over time as feed changes occur. No effects of MR or Calf Boost® (P > 0.05) were present in this study. Similar results were reported by Geiger et al. (2013). However, Williams et al. (2007) reported higher fecal scores in calves in an accelerated feeding program.

Figure 9. Least squares means of blood glucose for calves fed MR containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and 27% CP: 20% fat with Calf Boost ®. Interaction of treatment and week. (P= 0.0734)SEM= 3.9325.

Figure 10. Least squares means of glucose concentration for calves fed MR containing 20% CP: 20% fat and 27% CP, and 10% fat. Interaction of MR and week (P<0.05) SEM=3.2804.
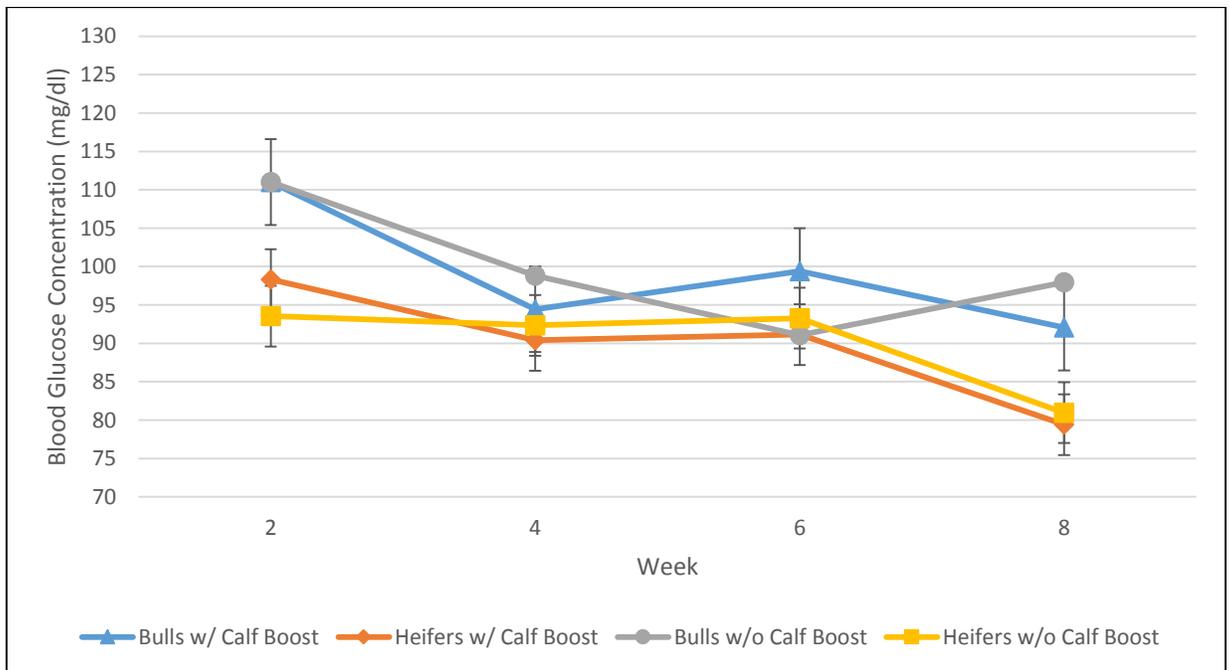
Figure 11. Least squares means of plasma glucose in Male and female calves fed MR with and without Calf Boost. Interaction Calf Boost® x Sex x Week (P<0.05) SEM=5.5916.
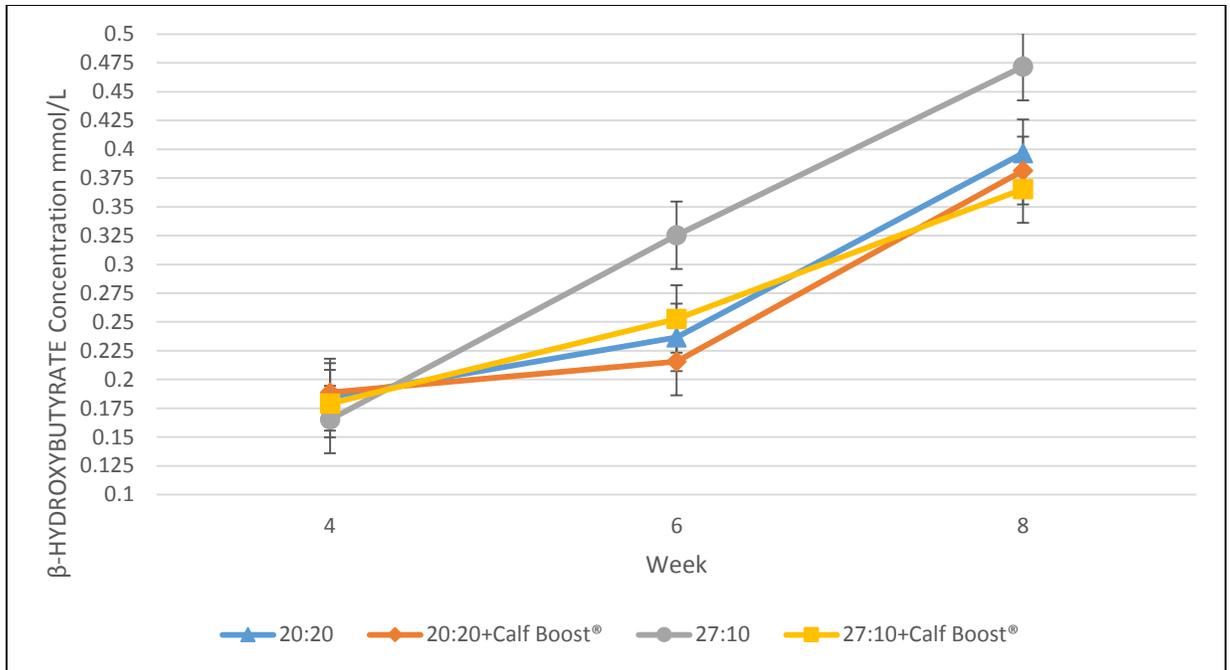
Figure 12. Least squares means of β-hydroxybutyrate for calves fed MR containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and 27% CP: 20% fat with Calf Boost ®. Interaction of MR and week (P<0.05) SEM=0.0293.
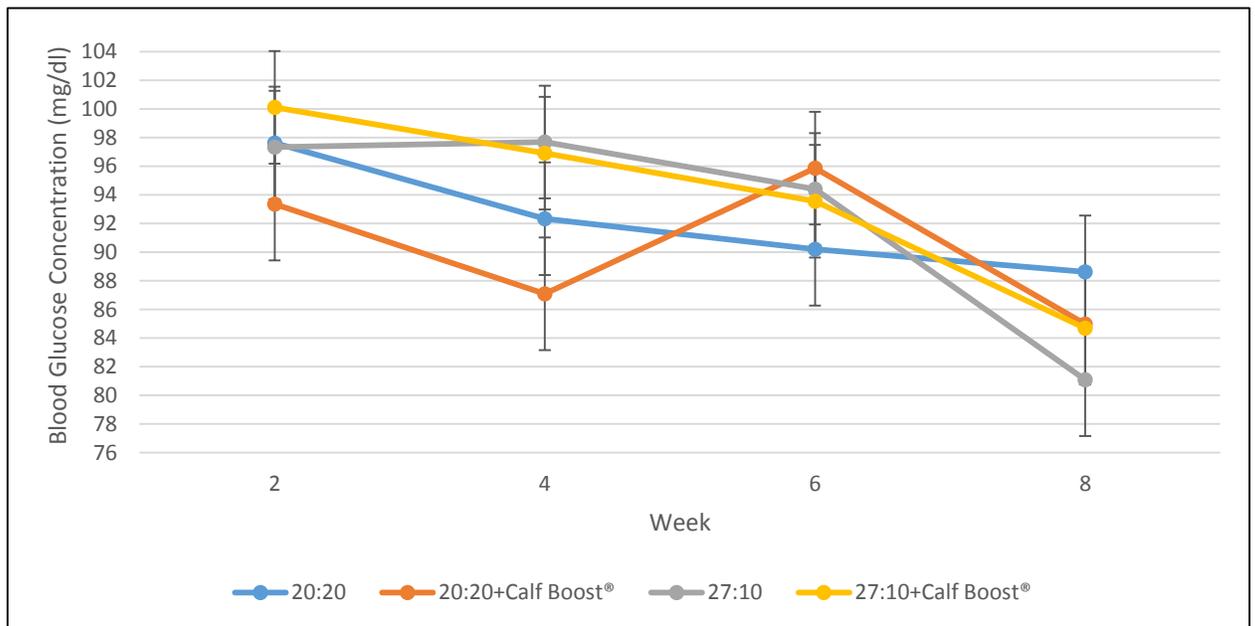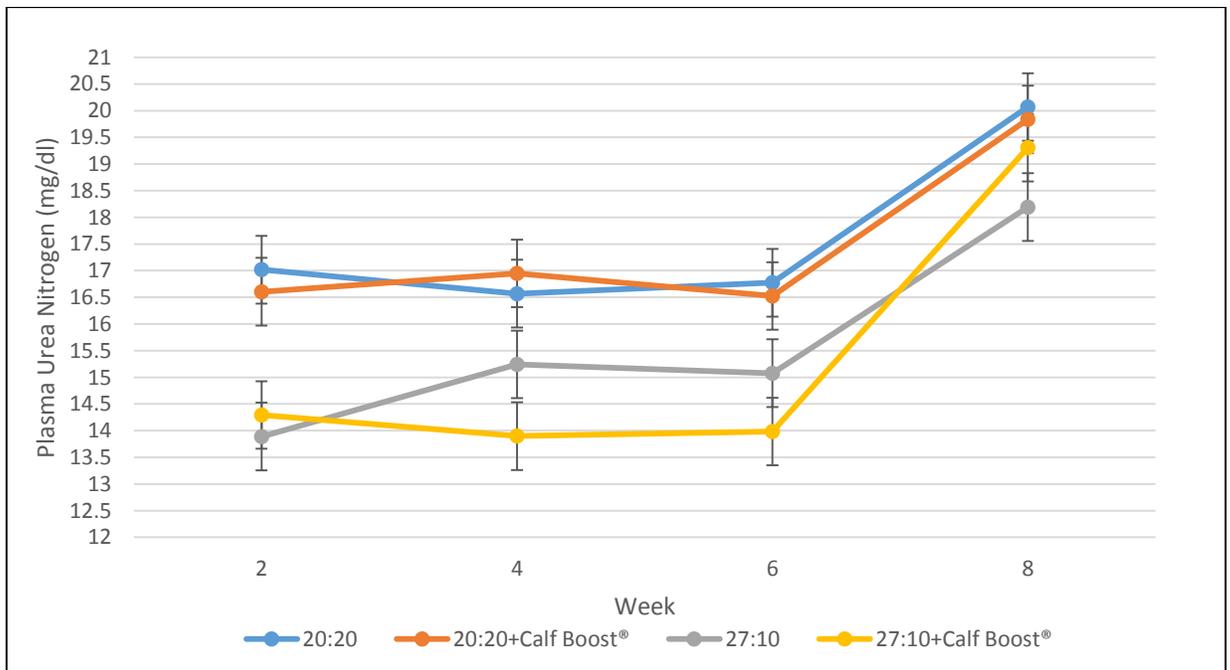

Figure 13. Least squares means of PUN for calves fed MR containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and 27% CP: 20% fat with Calf Boost ®. Interaction of MR and week (P<0.05) SEM=10.156.
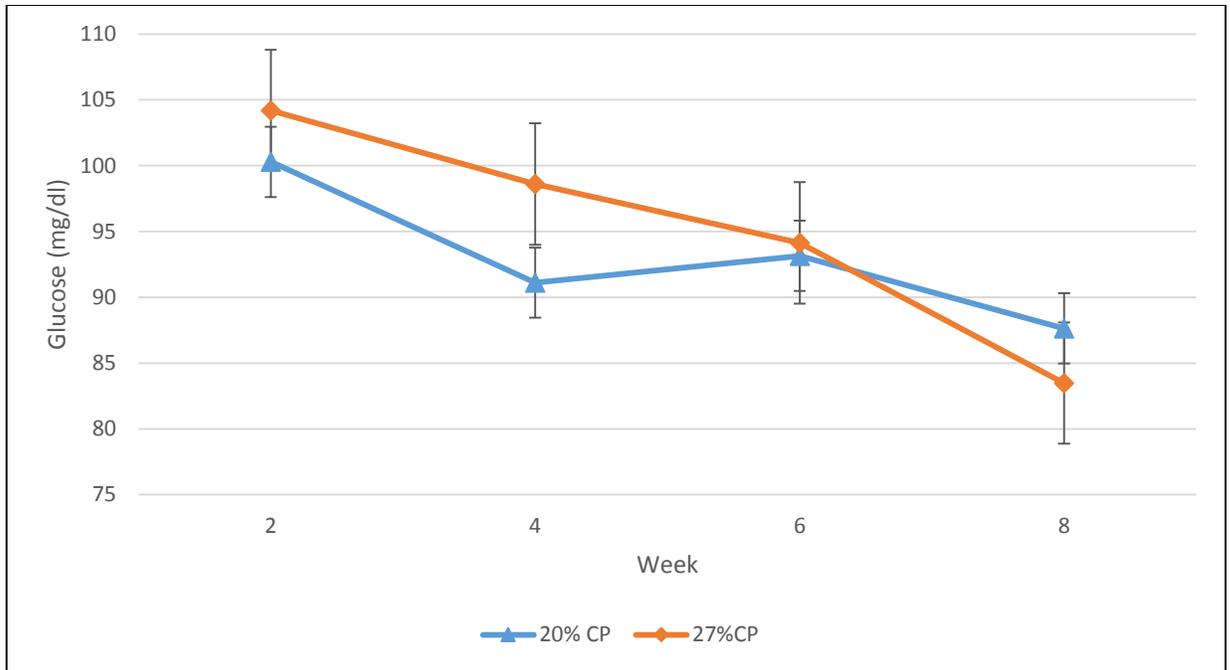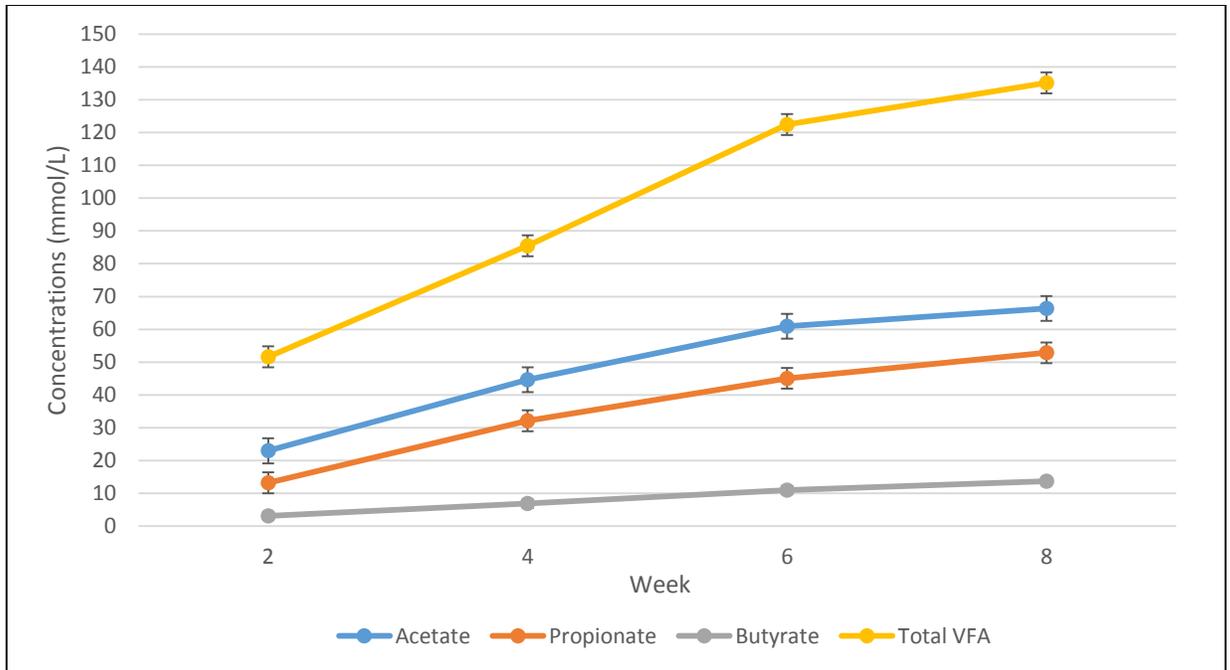
Figure 14. Least squares means of VFA concentrations. Main effect of week (P<0.001) SEM=3.8056. SEM=3.1918. SEM=1.2754. SEM=10.7732.

Figure 15. Least squares means of fecal scores for calves fed MR containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and 27% CP: 20% fat with Calf Boost ®. SEM=0.161.

Table 2. Least squares means for rumen VFA, and pH in calves fed varying milk replacers with or without Calf Boost®.

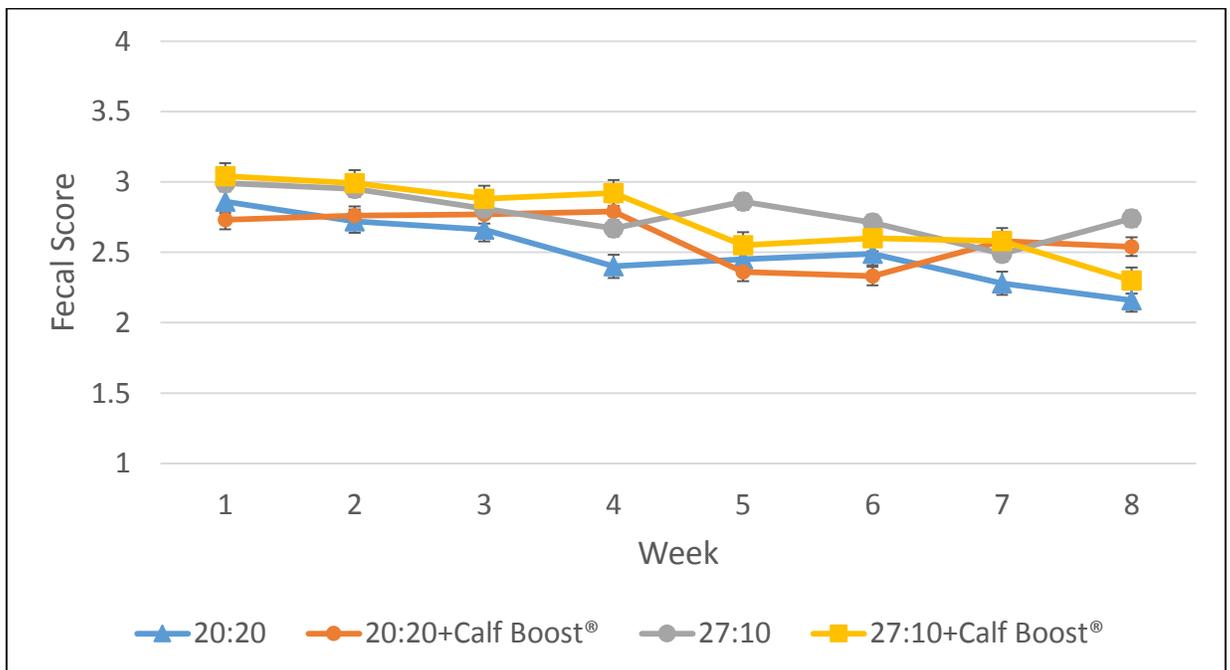| Item	Treatments[1]				SE[2]	P Value
	20:20	20:20+CB	27:10	27:10+CB		
Acetate, mmol/L	51.71	43.32	50.32	49.73	6.79	0.6984
Propionate, mmol/L	38.07	31.34	36.15	37.84	4.29	0.6434
Butyrate, mmol	9.38	7.71	8.39	9.22	1.71	0.8864
Total, mmol/L	103.3	104.15	101.03	86.168	10.77	0.6785
Rumen pH	5.81	5.81	5.65	5.58	0.08	0.3829

[1] 27:10= 27% CP: 10% fat; 27:10+CB= 27% CP: 10% fat with the addition of Calf Boost ®; 20:20= 20% CP: 20% fat; 20:20+CB= 20% CP: 20% fat with the addition of Calf Boost®.
[2]Standard error of the means

Heat Stress

The instances of heat stress observed in this study were mostly observed in the first 4 weeks and may exaggerate this effect further in the small sample size, due to the lack of replacer feeding during weeks 6 through 8. Effect of the interactions of stress and week on AM MR intake are presented in Figure 16 and show a lack of MR consumption during week six of the trial for heat stressed calves. This observance is misleading in its significant presentation, as it is a result of the absence of heat stress during week 6 and not the absence of MR. This does, however, further substantiate the previous assumption that heat stress was lacking during the later weeks of the trail period and had an ill effect on the analysis of these data.

Least squares means for both AM and PM water intake are presented in Figure 17 and illustrate a significant difference (P<0.05) in water intake between AM and PM measurement periods. In this case, these can be understood as daylight and night time hours, with AM being the daylight hours between 6am and 5pm and PM being the night

time hours between 5pm and 6am. When related in this way the difference is expected, with water being consumed more during the warmer and drier daylight hours than in the cooler and moist night time hours. As expected, water intake increased as calves aged (P < 0.05). Calves consumed more water after week 6, when the weaning process was taking place. These observations are consistent with findings by Thibeau et al. (2011).

Least squares means for calf rectal temperatures and respiration rates are presented in Figures 18 and 19 respectively. Ante meridiem rectal temperatures were found to be consistently lower than those measured during PM hours. Respiration rates were found to be higher during the PM hours taken after long exposer to the heat of the day. Respiration rates were not elevated after week 5 of this study, and temperatures taken during the AM periods followed the same trend. However, PM temperatures were not closer than 1ºC to normal rectal temperature of 38.6ºC in calves throughout the study.

Average daily temperatures, relative humidity, and calculated temperature humidity index ratings are presented in Table 3. Temperatures were originally measured and averaged on the Fahrenheit scale and later converted to Celsius for THI calculations. Table 3 presents data from all 27 weeks of the trial, and clearly shows that heat stress caused by ambient temperatures and relative humidity resulted in calculated THI ratings of 72 and above were isolated to weeks 1, 3, and 5 of this trial. These data substantiate the absence of data previously presented with observations lacking heat stress during weeks 6 through 8 of this trial.
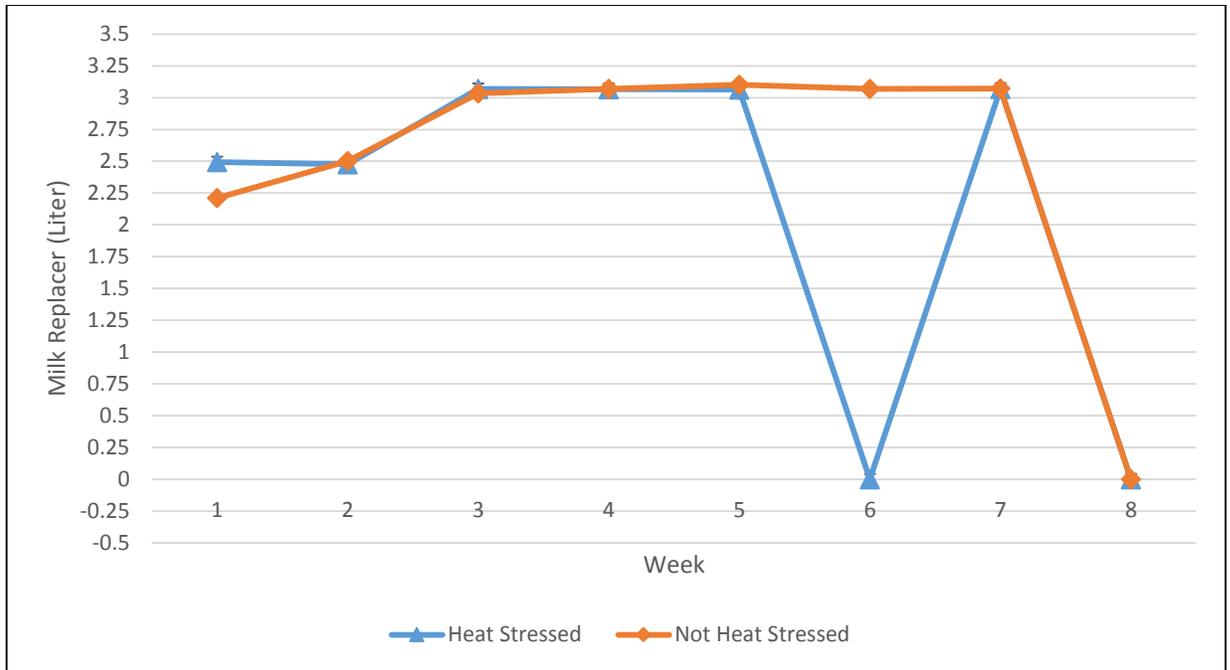
Figure 16. Least squares means of AM milk replacer intake. Interactions between heat stress and week on milk replacer intake throughout an 8 week trial period. (P<0.05) SEM=0.04386.
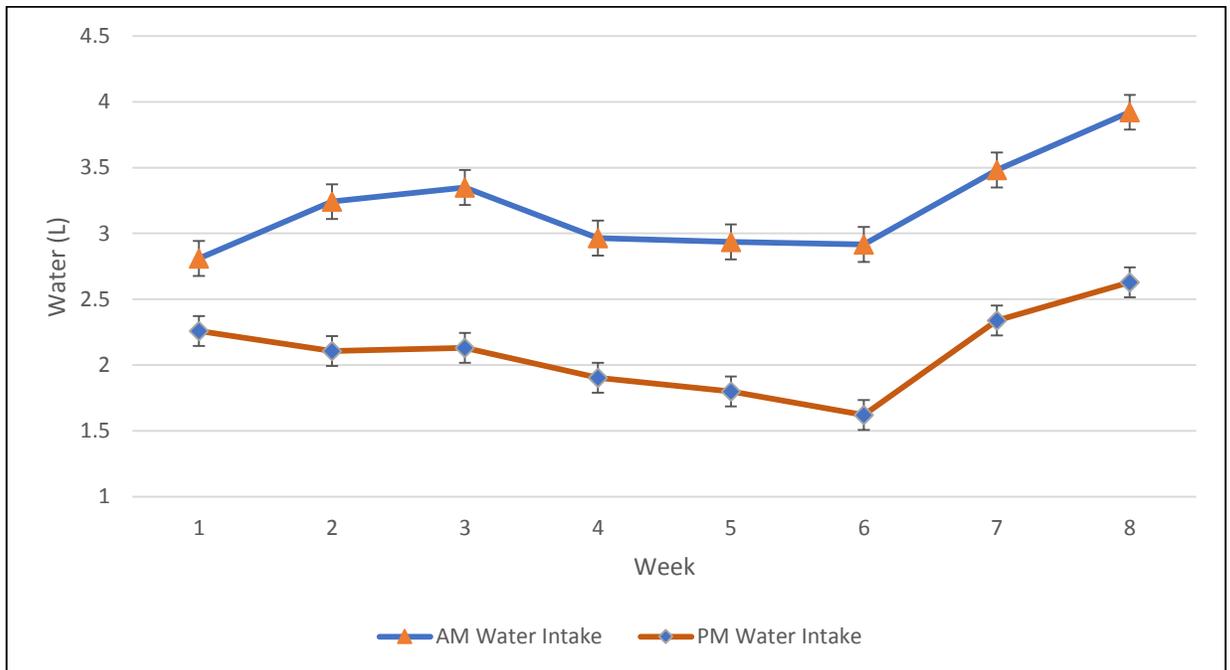
Figure 17. Least squares means of weekly water intake during both AM and PM offerings. Main effect of week. (P<0.05) Main effect of time of day. SEM=0.1827.
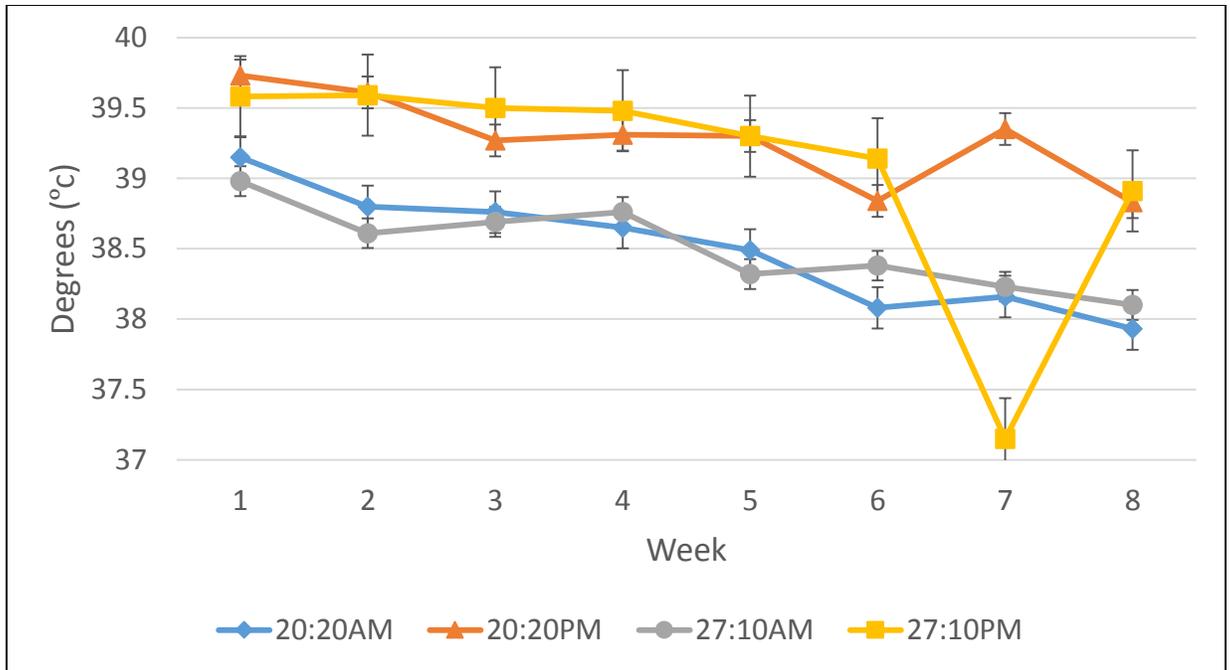
Figure 18. Least squares means of bi-weekly rectal temperatures for calves fed MR containing 20% CP: 20% fat, 20% CP: 20% fat with Calf Boost ®, 27% CP: 10% fat, and 27% CP: 20% fat with Calf Boost ®. Interaction of MR and week (P=<0.05) AM SEM=0.0.1287. PM SEM=0.777.
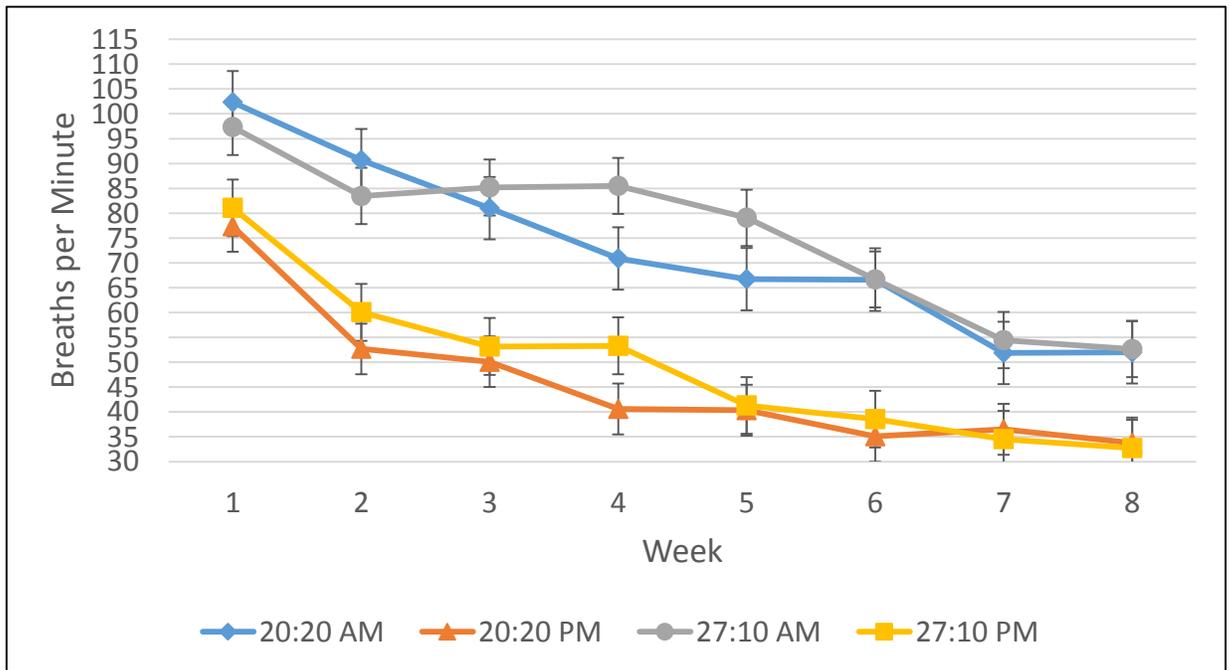
Figure 19. Least squares means of bi-weekly respiration rates for calves fed MR containing 27% CP: 10% fat, 27% CP: 10% fat with Calf Boost ®, 20% CP: 20% fat, and 20% CP: 20% fat. Main effect of time of day (P<0.01) AM SEM=6.448. PM SEM=4.071.

Table 3. Highs and lows of ambient temperature and relative humidity data by project week and the calculated THI.

| Project Week	Avg. Weekly High Temp (°F)	Avg. Weekly High Temp (°C)	Avg. Weekly Humidity for High Temps (%)	Avg. Weekly High THI	Avg. Weekly Low Temp (°F)	Avg. Weekly Low Temp (°C)	Avg. Weekly Humidity for Low Temps (%)	Avg. Weekly Low THI
1	81	27	72	73	75	24	87	72
2	80	27	72	71	75	24	86	71
3	84	29	68	73	77	25	84	73
4	83	28	66	71	75	24	85	71
5	83	28	68	73	76	25	85	72
6	79	26	73	70	73	23	86	70
7	79	26	72	71	73	23	87	70
8	73	23	68	63	67	20	85	63
9	76	25	73	68	70	21	89	67
10	67	19	69	58	59	15	86	55
11	69	21	72	61	62	17	84	58
12	65	18	67	55	59	15	82	55
13	59	15	58	47	50	10	72	43
14	68	20	66	58	61	16	76	57
15	48	9	75	41	47	8	80	42
16	65	18	70	56	54	12	89	51
17	45	7	81	40	43	6	90	40
18	55	13	67	46	45	7	82	41
19	55	13	68	47	49	10	82	45
20	49	10	74	43	45	7	86	42
21	44	6	58	33	34	1	69	27
22	57	14	55	43	49	10	80	44
23	51	11	45	34	43	6	74	36
24	40	4	70	33	34	1	78	29
25	52	11	82	48	45	7	91	43
26	48	9	79	42	41	5	92	39
27	66	19	71	57	55	13	87	52

CHAPTER V
CONCLUSION

The data from this study show the benefits of increased CP and decreased fat concentrations in MR (20:20 vs 27:10) along with increased feeding rates. Calves fed 27:10 CP:fat MR were observed to be 12kg heavier and larger than calves fed 20:20 CP:fat MR at weaning and 10kg heavier at the end of the 56 day trial. It was observed that calves fed 27% CP were significantly heavier from week 2 until the end of the trial. The differences did not disappear or converge after weaning, meaning that development was positively affected by increased feeding rates and MR CP composition.

Amplicalf® calf starter was fed ad libitum throughout the study and no decrease in growth occurred during or after weaning onto this product. The use of this pre-made textured starter is also credited with the immediate increase in starter consumption. In previous studies done here at LSU, calf starter intake was shown to be extremely low and negligible for the first week of each study, however calves offered Amplicalf in this study were observed to begin eating starter immediately. The use of this starter left no gaps in intake in this study and may also be partly responsible for the exceptional growth of these calves.

Multivitamin and mineral supplementation did not to promote increased growth, rumen development, or intake during this study. This supplementation was also ineffective at alleviating any ill effects brought on by heat stress. This may have been due, in part, to the lack of heat stress in this study, and the lack of significant effects on calf performance. Also, the high quality MR fed could is likely to have dampened any positive effect of the supplements that may have otherwise been observed. The

PalaMountains Calf Boost® product may serve better in a program that requires

supplementation than in carefully formulated diets such as these. Also, the use in beef

cattle or group feeders may yield slightly more significant results than dairy animals who

are pushed through development much faster and with higher quality feed.

In conclusion the use of "full potential" feeding programs and increased CP

concentrations may be used to greatly increase calf growth, regardless of whether heat

stress is present. The use of quality starter has shown to provide adequate, if not

exceptional, growth performance yields in neonatal Holstein calves. Multivitamin and

mineral supplementation was shown to be unnecessary in the conditions under which this

study was conducted.

REFERENCES

Anderson, K. L., T. G. Nagaraja, and J. L. Morrill. 1987. Ruminal metabolic development in calves weaned conventionally or early. J. Dairy Sci. 70:1000-1005.

Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. Farnham Royal, Slough, England: Commonwealth Agricultural Bureaux.

Baldwin, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant J. Dairy Sci. 87: E55-65E.

Bartlett, K.S., F.K. McKieth, M.J. VanderHaar, G.E. Dahl, and J.K. Drackley. 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. J. Anim. Sci. 84: 1454-1467.

Beede, D. K., and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62543.

Beharka, A. A., T. G. Nagaraja, J. L. Morrill, G. A. Kennedy, and R. D. Klemm. 1998. Effects of form of the diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. J. Dairy Sci. 81:1946-1955.

Blome, R. M., J. K. Drackley, F. K. McKeith, M. F. Hutjens, G. C. McCoy. 2003. Growth, nutrient utilization, and body composition of dairy calves fed milk replacers containing different amounts of protein. J. Anim. Sci. 81:1641-1655.

Bridges, A. J. 2009. Effect of Milk Replacer Composition on Growth and Rumen Development of Neonatal Holstein Calves. MS Thesis. Louisiana State University, Baton Rouge.

Brown, E. G., M. J. VandeHaar, K. M. Daniels, J.S. Liesman, L. T. Chapin, D. H. Keisler, and M. S. Weber Nielsen. 2005. Effect of increasing energy and protein intake on body growth and carcass composition of heifer calves. J. Dairy Sci. 88: 585-594.

Brown, R. E., C. L. Davis, J. R. Staubus, and W. O. Nelson. 1960. Production and absorption of volatile fatty acids in the perfused rumen. J. Dairy Sci. 43:1788-1797.

Collier, R.J., D.K. Beede, W.W. Thatcher, L. A. Israel, C.J. Wilcox. 1982a. Influences of environment and its modification on dairy animal health and production. J. Dairy Sci. 65: 2213-2227.

Combs, G.F., Jr. 1992. The Vitamins: Fundamental Aspects in Nutrition and Health. San Diego, CA: Academic Press, Inc.

Cowles, K.E., R.A. White, N.L. Whitehouse, and P.S. Erickson. 2006. Growth characteristics of calves fed an intensified milk replacer regimen with additional lactoferrin. J. Dairy Sci. 89: 4835-4845.

Davis, C. L., and J. K. Drackley. 1998. *The Development, Nutrition, and Management of the Young Calf*. Iowa State University Press, Ames, IA.

Diaz, M.C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. J. Dairy Sci. 84: 830-842.

Geiger, A.J., S.H. Ward, C.C. Williams, B.J. Rude, C.J. Cabrera, K.N. Kalestch, and B.E. Voelz. 2014. Short communication: Effects of increasing protein and energy in the milk replacer with or without direct-fed microbial supplementation on growth and performance of pre-weaned Holstein calves. J. Dairy Sci. 97:7212-7219.

Guyton, A. C. and J. E. Hall. 1996. Pages 927-983 in Textbook of Medical Physiology. 9thed.W. B. Sanders company, Philadelphia, Pennsylvania.

Hayashi, H., M. Kawai, I. Nonaka, F. Terada, K. Katoh, and Y. Obara. 2006. Development changes in the kinetics of glucose and urea in Holstein calves. J. Dairy Sci. 89:1654-1661.

Hibbs, J. W., H. R. Conrad, W. D. Pounden, and Norma Frank. 1956. A high roughage system for raising calves based on early development of rumen function. VI. Influence of hay to grain ratio on calf performance, rumen development, and certain blood changes. J. Dairy Sci 39:171-179.

Hsu, W. H. and M. H. Crump. 1989. The Endocrine Pancreas. Pages 186 in Veterinary Endocrinology and Reproduction. 4th ed. L.E. Mc Donald and M. H. Pineda, ed., L.E. and Febiger, Phildelphia, PA.

Jaster, E.H., G.C. McCoy, N. Spanski, and T. Tomkins. 1992. Effects of extra energy as fat or milk replacer solids in diets of young dairy calves on growth during cold weather. J. Dairy Sci. 75:2524-2531.

Jergens, M. H. 1997. "Nutrients and Digestive System." Animal Feeding and Nutrition. Kendall/Hunt Publishing Company. pp 11.

Kadzere, C.T., M.R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: a review. Livestock Prod. Sci. 77: 59-91.

Kellems, R., & Church, D. (2002). *Livestock feeds and feeding* (5th ed.). Upper Saddle River, N.J.: Prentice Hall.

Kertz, A.F., L.F. Reutzel, and J.H. Mahoney. 1984. Ad libitum water intake by neonatal calves and its relationship to calf starter intake, weight gain, feces score, and season. J. Dairy Sci 67:2964-2969.

Kuehn, C. S., D. E. Otterby, J. G. Linn, W. G. Olson, H. Chester-Jones, G. D. Marx, and J. A. Barmore. 1994. The effect of dietary energy concentration on calf performance. J. Dairy Sci. 77: 2621-2629.

Larson, L. L., F. G. Owen, J. L. Albright, R. D. Appleman, R. C. Lamb, and L. D. Muller. 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. 60:989-991.

Lassiter, C. A., L. D. Brown, R. M. Grimes, and C. W. Duncan. 1963. Effect of protein level in milk replacers on growth and protein metabolism of dairy calves. J. Dairy Sci. 46: 538-543.

Lyford, S. J. and J. T. Huber. 1993. Digestion, Metabolism and Nutrient Needs in Preruminants. Pages 401-420 in The Ruminant Animal Digestive Physiology and Nutrition. D.C. Church (Ed) Waveland Press, Inc., Prospect Heights, Illinois.

Mader, T., S. Davis, J. Gaughan, and T. Brown-Brand. 2004. Wind speed and solar radiation adjustments for the temperature humidity index. In *16th Conference on biometeorology and Aerobiology.* Vancouver, British Columbia, Canada.

Marshall, S. P. and K. Smith. 1973. Effect of milk fat levels on growth and efficiency of energy utilization for weight gain in dairy calves fed ad libitum. J. Anim. Sci. 37: 833-836.

Martin, W. G., H. A. Ramsey, G. Matrone, and G. H. Wise. 1959. Responses of young calves to a diet containing salts of volatile fatty acids. J. Dairy Sci .42:1377-1386.

Murdock, F. R. and R.W. Wallenius. 1980. Fiber sources for complete calf starter rations. J. Dairy Sci. 63:1869-1873.

National Animal Health Monitering System. 2001. National Dairy and Heifer Evaluation Project.

NAHMS (National Animal Health Monitoring System). Highlights of Dairy 2007 Part II: Changes in the U.S. dairy cattle industry, 1991–2007.

National Research Council. 2001. Nutritional requirements of dairy cattle. National Academy Press, Washington, DC.

Obara, Y. and K. Shimbayashi. 1980. The appearance of recycled urea in the digestive tract of goats during the final third of a once daily feeding of a low-protein ration. Br. J. Nutr. 44:295-305.

Preston, T.R. 1963. The nutrition of the early weaned calf. *World Rev.Nutr.Diet.* 4:117-139.

Quigley, J. D., L. A. Caldwell, G. D. Sinks, and R. N. Heitmann.1991. Changes in blood glucose, nonesterified fatty acids, and ketones in response to weaning and feed intake in young calves. J.Dairy Sci. 74:250-257.

Quigley, J. D. III. and J. K. Bernard. 1992. Effects of nutrient source and time of feeding changes in blood metabolites in young calves. J. Anim. Sci. 70:1543-1549.

Raeth-Knight, M., H. Chester-Jones, S. Hayes, J. Linn, and H.G. Jung. 2007. Effects of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. J. Dairy Sci. 90: 1802-1809.

Randall, E. M., and K.P. Swannack. 1975. Once a day feeding for calves. Expl. Husb. 28:44-52.

Ravagnolo, O., Misztal, I. 2000. Genetic component of heat stress in dairy cattle, parameter estimation. J. Dairy Sci. 83:2126–2130.

Reddy, P.G., J.L. Morrill, R.A. Frey, M.B. Morrill, H.C. Minocha, S.J. Galitzer, and A. D. Dayton. 1985. Effects of supplemental vitamin E on the performance and metabolic profiles of dairy calves. J. Dairy Sci, 68:2259-2266.

Reddy, P.G., J.L. Morrill, H.C. Minocha, M.B. Morrill, S.J. Galitzer, A. D. Dayton, and R.A. Frey. 1986. Effects of supplemental vitamin E on immune system of calves. J. Dairy Sci, 69:164-171.

Roseler, D. K., J. D. Ferguson, C. J. Sniffen, and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525-534.

SAS/STAT User's Guide: Statistics, Version 6, 4th Edition. 1989. SAS Inst., Inc., Cary, NC.

Sanchez, W. K., McGuire, M. A., & Beede, D. K. (1994). Macromineral nutrition by heat stress interactions in dairy cattle: Review and original research. Journal of Dairy Science, 77(7), 2051-2079

Sander, E.G.,R.G.Warner,H.N.Harrison,and J.K.Loosli. 1959. The stimalatory effect of sodium butyrate and sodium propionate on the development of ruminal mucosa in the young calf. J. Dairy Sci.421600.

Sockett, D.C., C.E. Sorenson, N.K. Betzold, J.T. Meronek, and T.J. Earleywine. 2011. Impact of three times versus twice a day milk replacer feeding on calf performance, likelihood to reach lactation and future milk production in a commercial dairy herd. J. Dairy Sci. 94:264-274.

Stanley, C. C., C. C. Williams, B. F. Jenny, J. M. Fernandez, H. G. Bateman II, W. A. Nipper, J. C. Lovejoy, D. T. Gantt, G. E. Goodier. 2002. Effects of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves. J. Dairy Sci. 85:2335-2343.

Stelwagen, K., and D. G. Grieve. 1992. Effect of plane of nutrition between 6 and 16 months of age on body composition, plasma hormone concentrations and lst lactation milk production in Holstein heifers. Can. J. Anim. Sci. 72:337–346.

Stobo, I. J. F., J.H. B. Roy, and Helen J. Gaston. 1966. The effect of diets containing different proportions of concentrates to hay on rumen development. Br. J. Nutr. 20:171.

St. Pierre, N.R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86: E52-E77.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408-420.

Thibeau, S. S., C. C. Williams, B. F. Jenny, and A. H. Dolejsiova. 2011. Effects of shade on heat stress reduction in Holstein dairy calves. J. Dairy Sci. 94(Suppl):215.

Thomas, M. 2014. Effects of feeding milk replacer once, twice, or three times daily on growth and performance in neonatal Holstein calves. MS Thesis. Louisiana State University, Baton Rouge.

Tikofsky, J. N., M. E. Van Amburgh, and D. A. Ross. 2001. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. J. Anim Sci. 79: 2260-2267.

Tomkins, T., and E.H. Jaster. 1991. Preruminant calf nutrition. *Vet. Clin. N. Am. Food Anim. Prac.* 7:557-576.

Van Amburgh, M.E., and F. Soberon. 2013. "Early life nutrition and management and the impact on lifetime productivity of calves." Four-State Dairy Nutrition & Management Conference (M. E. Van Amburgh, 2013)pp.36-43.

Van Soest, P. J. 1994. "Function of the Ruminant Forestomach." Nutritional Ecology of the Ruminant. Cornell University: Cornell University Press. pp 230-252.

Warner, E.D. 1958.The Organogenesis and Early Histogenesis of the bovine stomach. *Am. J. Anat.*102:33-53.

Warner, R.G., and W. P. Flatt 1965. Anatomical development of the ruminant stomach. In: *Physiology of the Digestion in the Ruminant,* edited by. R.W. Dougherty, R.S. Allen, W. Burroughs, N.L. Jacobson, and A.D. McGilliard, 24-38. Washington, D.C.:Butterworths.

Whitelaw, F.G., T.R. Preston, and R.D. Ndumbe. 1961. The nutrition of the early-weaned calf. 1. The effect on nitrogen retention of diets containing different levels of groundnut meal. *Anim.Prod.* 3:121-126.

Williams, C.C., D.T. Gantt, C.F. Hutchison, C.C. Stanley, and M.A. Froetschel. 2007. Effects of early intensified nutrition on growth and metabolism of neonatal dairy calves. J. Dairy Sci. 90 (Suppl 1): 112.

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

Calculation

β-Hydroxybutyrate (mM) = <u>OD Sample - Blank </u> x Standard Concentration

OD Std - Blank

APPENDIX B.
UREA NITROGEN (BUN) BERTHELOT/ COLORIMETRIC ASSAY

Reagents:

1) Enzyme Reagent (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 (10μl) 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 minutes at room temperature 2-26ºC).
4) Add 2.0 mL of BASE RGT, mix gently and incubate at 37ºC for 5 min. (Alternative: React to 10 minutes at room temperature 2-26ºC).
5) Set the wave length 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 concentrations of Standard (25mg/dl_). Read unknown concentration directly

Calculation:

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

$\underline{A\ (U)}$ x C (S) mg/dl =C (U) mg/dl

A (S)

APPENDIX C.
PLASMA GLUCOSE ASSAY

(REF: Glucose Oxidase Reagent Set®; Pointe Scientific, 5449 Research Drive, Canton, Michigan 48188)

Reagents:

1) Glucose Oxidase >15u/ml, Peroxidase (horseradish) 1.2u/ml,

2) 4-Aminoantipyrine 0.38mM, Phosphate Buffer, pH 7.5± 0.1,

3) Sodium p-hydroxybenzoate 10 mM

4) Non-reactive stabilizers and fillers

5) Sodium Azide 0.1%

Procedure:

1) Pipette 1.0ml of working regent to all tubes and place in a 37ºC heating bath for at least five minutes.

2) Add 0.01ml (10µl) of sample to respective tubes. Mix ad incubate at 37ºC for exactly five minutes.

3) After incubation, zero spectrophotometer with the reagent blank. Read and record absorbances of all tubes at 500nm (500-520nm).

4) To determine results see calculations.

Calculations:

A= Absorbance

A(Patient) x Concentration of = Glucose (mg/dl)

A(Standard)   Standard(mg/dl)

APPENDIX D.
ANALYSIS OF VOLATILE FATTY ACIDS IN RUMINAL FLUID

Based on preparation procedures described in Grigsby et al., 1992. J. Anim. Sci. 70:1941-1949, and temperature gradient program described in Bateman et al., 2002. Prof. Anim. Sci. 18:363-367.

Reagents

1) 25% (wt/vol) metaphophoric acid (fluka #79615) acid solution containing 2 g/L of 2-ethyl butyric acid (216.5 µL 2-EB to 100 mL m-phos acid solution; Aldrich #10, 995-9).

2) VFA standard

   a) Add the following volumes of acids to a 100-mL volumetric flask and fill volume with dH$_2$O. Store in refrigerator when not in use.

MW	Acid	Volume (µL)	Conc (g/L)	Conc (mM)
60.06	Acetic	330	3.46	57.62
74.08	Propionic	400	3.97	53.59
88.10	Isobutyric	30	0.29	3.29
88.10	Butyric	160	1.53	17.37
102.13	Isovaleric	40	0.375	3.67
102.13	n-Valeric	50	0.471	4.61

Sample and Standard Preparation

1) Centrifuge strained ruminal fluid at 30,000 x g for 20 min (this step may be skipped).
2) Mix 4 mL of rumen fluid supernatant with 1 mL of m-phosphoric acid solution containing 2-EB.
3) Allow to stand in ice bath for 30 min (this stepped may be skipped).
4) Centrifuge at 30,000 x g for 20 min.
5) Remove the supernatant for GC analysis.
6) To insure that standard is prepared in the same manner as the samples, treat the mixed sample from step A-2 above as a sample.

Remember to correct the dilution factor from the m-phos solution when calculating the final VFA concentrations (4mL fluid mixed with 1 mL acid providea correction factor of 1.25).

For use on Shimadzu GC, samples should be in 2 mL autosampler vials. The optimal vials that we have used are ordered from Cole-Parmer. They are Target autosampler vials (#A98810-00). These are a screw cap vial so you also need caps, and the septa color is important. The autosampler recognizes white as the color of the septa (#A98801-23).

Temperature Gradient Program

1) The column temperature at the beginning of the program is 115°C and is held there for 0.1 min.
2) It is then increased at a rate of 10°C/min to 150°C and held there for 0.1 min.
3) It is then further increased at a rate of 11°C/min to 170°C and held there for 1 min.
4) The injector of the chromatograph is held at 250°C and the detector is held at 275°C.
5) Peak detection is by a flame ionization that uses a H_2/ air flame.
6) Helium is used as the carrier gas with a splitless injection at a flow of 60 mL/min.

VITA

Steven Blair is the son of Steve and Sarah Blair of Marrero, Louisiana. He graduated from Archbishop Shaw High School in May of 2008, and began his bachelor's degree in August of 2008 at Southeastern Louisiana University, transferring to Louisiana State University in August of 2009. It was here that he discovered his passion for research in the field of animal science. In May of 2012, he earned his Bachelor of Science degree in Animal, Dairy, and Poultry Science with a concentration in Sciences and Technologies. After graduating, Steven began his graduate studies in August of 2012 in reproductive physiology and transferred to dairy nutrition and management. He will receive his master's degree in August, 2015.