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## Effect of milk replacer composition on growth and rumen development of neonatal Holstein calves

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EFFECT OF MILK REPLACER COMPOSITION ON GROWTH AND RUMEN  
DEVELOPMENT OF NEONATAL HOLSTEIN CALVES

A Thesis

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

in

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by

Anthony Joseph Bridges  
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## TABLE OF CONTENTS

ACKNOWLEDGMENTS .....	ii
LIST OF TABLES .....	iv
LIST OF FIGURES .....	v
ABSTRACT .....	vi
CHAPTER	
1    INTRODUCTION .....	1
2    REVIEW OF LITERATURE .....	4
Background .....	4
Protein and Fat .....	4
Milk Replacer Feeding Rate .....	9
Rumen Development .....	12
3    MATERIALS AND METHODS .....	15
Animals and Dietary Treatments .....	15
Sample Collection .....	17
Laboratory Methods .....	18
Calculations and Statistical Analysis .....	18
4    RESULTS AND DISCUSSION .....	20
Performance Data .....	20
Rumen Development Data .....	25
Blood Metabolites .....	26
5    SUMMARY AND CONCLUSIONS .....	35
Summary .....	35
Conclusions .....	35
REFERENCES .....	36
APPENDIX A. PLASMA IGF-1 ASSAY .....	40
APPENDIX B. $\beta$ -HYDROXYBUTYRATE COLORIMETRIC ASSAY .....	42
APPENDIX C. PLASMA UREA NITROGEN COLORIMETRIC ASSAY .....	43
APPENDIX D. ANALYSIS OF VOLATILE FATTY ACIDS IN RUMINAL FLUID .....	44
VITA .....	46

## LIST OF TABLES

1. Ingredients in 20.5% CP calf starter. ....	16
2. Guaranteed analysis for calf milk replacers containing 20% protein 20% fat (20/20), 25% protein 15% fat (25/15), and 28% protein 20% fat (28/20). ....	16
3. Least squares means for initial weights, average daily gain, weaning weights, and final weights for calves fed 20% protein 20% fat, 25% protein 15% fat, and 28% protein 20% fat MRs. ....	21
4. Least squares means for overall hip height, wither height, length, and fecal scores through 56 days of age of calves fed 20% protein 20% fat, 25% protein 15% fat, and 28% protein 20% fat MRs. ....	23
5. Least squares means for rumen pH and VFAs in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, and 28% protein 20% fat MRs. ....	29
6. Least squares means for blood hormones and metabolites in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat. ....	32

## LIST OF FIGURES

1. Least squares means of daily starter intakes in for calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲) MRs.....	21
2. Least squares means for body weights of calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲) MRs. ....	23
3. Least squares means for fecal scores of calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲) MRs. ....	24
4. Least squares means for rumen acetate in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat.....	29
5. Least squares mean for rumen propionate in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat.....	30
6. Least squares mean for rumen butyrate in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat.....	30
7. Least squares mean for total rumen VFA in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat.....	31
8. Least squares mean for rumen pH in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat.....	31
9. Least squares means for plasma IGF-1 concentrations of calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲)MRs. ....	32
10. Least squares means for plasma BHBA at weeks 2, 4, and 8.....	33
11. Least squares means for PUN concentrations of calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲)MRs. ....	34

## ABSTRACT

A study was conducted to determine effects of differing fat and protein levels in commercial milk replacers on the growth performance and rumen development of neonatal Holstein calves. Fifty-three calves (23 male; 30 female) were assigned to one of three dietary treatments which included 3 milk replacers (20% protein and 20% fat, 20/20; 25% protein and 15% fat, 25/15; 28% protein and 20% fat, 28/20) fed once per day at 10% of birth weight through weaning. Calves were offered a 20% CP calf starter and water free choice beginning on day 5. All calves were weaned on day 42. Body weights were measured at birth and weekly through weaning. Additionally, hip height, wither height, and body length were measured weekly. Feed intake and fecal scores were recorded twice daily. Beginning on day 7 and continuing weekly through weaning, blood samples were collected prior to morning feeding for analysis of IGF-I and on days 14, 28, 42, and 56 for PUN and  $\beta$ -hydroxybutyrate concentrations. On days 28 and 56 rumen fluid was collected for analysis of pH and VFA to evaluate possible differences in rumen development. Calves fed 28/20 milk replacer had greater ( $P < 0.05$ ) daily starter intake, body weights, PUN, and  $\beta$ -hydroxybutyrate concentrations when compared to calves fed 20/20. There was also a treatment by week interaction ( $P < 0.05$ ) for starter intake, with calves on 28/20 milk replacer consuming the greatest amounts after week 3 and maintaining the greater starter intake through week 8. A treatment over time effect was observed for fecal scores ( $P < 0.05$ ). No other differences were observed ( $P > 0.05$ ) in fecal scores among any treatments. Average wither heights were greater ( $P < 0.05$ ) in calves fed 28/20 milk replacer. There were no effects ( $P > 0.05$ ) of treatment on IGF-I concentrations, rumen pH, or concentrations of VFA. Acetate, as a percent of total VFA, tended ( $P < 0.10$ ) to be greater in calves fed 28/20 milk replacer. These data indicate that increasing the dietary protein content of

milk replacer without reducing fat content improved growth in young dairy calves without compromising health or rumen development.



# CHAPTER 1

## INTRODUCTION

The success of a dairy operation depends in part on a sound replacement program. Age at first calving is a major economic factor when raising dairy replacement heifers (Tozer and Heinrichs, 2001). Dairy heifers are an investment with no immediate returns from birth to first calving. They are especially expensive for the first 3 months of life. Dairy calf growers must find the most cost effective, efficient way for their heifers to achieve breeding weight without fattening in order to be more profitable.

Three phases are considered when feeding a young calf (NRC, 2001). The first phase, the liquid phase, occurs immediately postpartum. The newborn calf's digestive system is geared toward the utilization of Immunoglobulin-G through passive immunity until gut closure. During this time the calf relies on the dam's colostrum to provide all of the nutrients it requires. After gut closure the calf begins to rely on its own immune system.

The second phase to be considered in the feeding of a young calf is the transition phase. In this phase the calf is "transitioning" from a liquid diet to a diet of solid feed. At this time the calf is receiving both the liquid portion of its diet and a starter feed to prepare the calf for weaning. When the calf has become accustomed to eating dry feed and is consuming an adequate amount of starter the next phase begins.

The third and last phase of feeding the calf is the solid phase which begins at weaning. This is also the ruminant phase because the rumen of the young animal has developed enough that the calf no longer needs the nutrients supplied by the liquid portion of the diet. All nutrients needed by the growing calf will now be received from solid feed. Initially this feed is calf starter,

followed by a grower ration, and finally the animal will be introduced to a ration similar to that fed to the milking herd.

In the early 1950's milk replacer (**MR**) became available commercially because the price of milk was too high to feed whole milk to calves raised on the farm. Milk replacer is a form of liquid feed that is available to be incorporated into the diet of a young, growing animal. If used properly, MR is an excellent substitute for whole milk.

According to the National Animal Health Monitoring System 70.2% of dairy operations fed MR before weaning to dairy heifers in the U.S. in 2006 (USDA, 2007). There are regional differences in the usage of MRs, their nutrient specifications, and ingredients used in their formulation. They are often very economical and, in many situations, are more easily adapted to the needs of calf raising facilities than either whole or waste milk. However, farms with greater numbers of calves usually use more waste or whole milk to feed calves. Many of the management practices used in feeding MRs reflect accepted nutrition and management practices (Heinrichs et al. 1995).

Recently, researchers developed a MR that more closely resembles whole milk than traditional MR. Conventional MR typically is comprised of whey proteins and animal fat. Crude protein content is usually 20%, and the fat content typically ranges from 15 to 22% (DM basis). The newly developed MR contains 28% CP and 20% fat and is fed at a higher rate (Diaz et al. 2001). Calf performance on this program has resulted in greater average daily gain, skeletal growth, and lean muscle mass (Tikofsky et al., 2001; Blome et al., 2003). Although these calves consumed more MR, questions remain regarding starter consumption, rumen development, and weaning. Davis and Drackley (1998) suggested that replacement calves should be fed a restricted liquid diet to encourage dry feed intake, thereby allowing weaning to occur as early as possible.

It is well established that calves need fermentable carbohydrates and fiber, which are found in starter feed, to produce VFA for rumen epithelial development and establish microbial populations (Greenwood et al., 1997). A calf fed large amounts of liquid feed may eat less starter, thereby delaying rumen development and weaning.

Accelerated feeding programs require twice as much intake of MR with increased protein concentrations. Conventional practice on dairy farms is feeding 20% fat, 20% protein MR to calves twice daily. New research has been conducted with a MR containing fat and protein levels closer to those of whole milk (Diaz, 2001). It is recommended that these MRs be fed at twice the feeding rate of the conventional MRs to increase average daily gains and lean muscle growth (Tikofsky 2001, Blome 2003). The research conducted in this experiment was to study the effects of feeding the accelerated MR at conventional levels. The objective of this study was to determine the effects of differing fat and protein levels in commercial milk replacers on the growth and rumen development of neonatal Holstein calves.

## CHAPTER 2

### LITERATURE REVIEW

#### BACKGROUND

Early dairy farmers had only whole milk to feed their calves. Over the years technology has progressed and with it the feeding of calves has evolved. Milk replacers were formulated in the 1950's. The first MRs were gruels that led to poor calf performance unless supplemented with milk (Davis and Drackley, 1998). Milk replacers, which were less expensive than whole milk, allowed dairy farmers to sell whole milk that would otherwise have been fed to calves.

Technological developments and processing improvements since the mid-1980s have resulted in dramatic changes in the ingredients available for use in MR formulation (Davis and Drackley, 1998). Dried skim milk was used as the main component of MR until whey protein concentrate was found to be a more cost effective ingredient.

Conventional MR feeding generally has been twice a day with fat and protein levels of 20% each or approximately so. Recent studies have shown that increasing the energy and protein content of MRs enhances the growth performance of neonatal calves (Diaz, 2001).

#### PROTEIN AND FAT

Increases in market prices for dried skim milk, coupled with the development of low-temperature ultrafiltration techniques for preparation of high-quality whey protein concentrates, have led to the almost complete replacement of dried skim milk with whey-derived products (Davis and Drackley, 1998). Milk replacer formulations generally are classified as all-milk protein or as alternative protein. Milk replacers consisting of all-milk proteins contain whey protein concentrate, dried whey, and delactosed whey as their protein sources. Many alternative protein sources are available, such as soy protein concentrate, soy protein isolates, animal

plasma, and modified wheat gluten. However, no alternative protein sources have been found that can replace milk proteins. At best, alternative protein sources are able to provide only a portion of the protein needed by the young calf (Davis and Drackley, 1998). The ability of these protein sources to supply an adequate amount and profile of amino acids for the growth of preruminant calves depends on the amino acid profile of the protein, the quality of the manufacturing process, and the calf's ability to digest the protein (NRC, 2001). For optimal growth, during the first 3 weeks of life, it is recommended that calves receive only MRs with all-milk protein sources included (NRC, 2001).

Levels of protein feeding have not been thoroughly researched until recently (Blome, 2003; Brown, 2005). Dairy calves are raised at as little cost as possible to keep them healthy until they are able to enter the milking herd. Research in the past was focused on how little protein calves would need for normal healthy growth instead of how much protein they are able to utilize optimally (Lassiter et al., 1963).

Lassiter et al. (1963) performed three trials to determine the correct protein level for MR. In trial 1, 32 calves were fed a MR ration consisting of protein levels from 15.2% to 30.9%. All calves were fed a 16.5% CP starter and hay starting on week 1. Calves on 30.9, 24.1, and 18.7% replacers performed similarly, while calves fed the 15.2% protein replacer grew at a slightly slower rate. In trial 2, 40 calves were assigned to 5 treatments, consisting of protein levels of 16.6, 19.6, 23.5, 26.2, and 29.7%. Results were similar to those in trial 1. Calves fed the lowest protein replacer grew at a slower rate compared to those fed at higher protein levels, especially from 2 to 49 days of age. Trial 3 was a metabolism trial performed with the replacers used in trial 1. Results of this experiment indicated that the optimum protein level in MRs is no more than 24 % and that the protein level could be as low as 19 % without affecting the growth of calves.

Diaz et al. (2001) conducted a study to determine the composition of growth of Holstein calves fed milk replacer from birth to 105 kilograms of body weight. Fifty-four calves were assigned at random to one of three treatments. All calves received milk replacer formulated to contain 30% CP and 20% fat. The MR was an all-milk protein formulation. Calves on treatment 1 were fed milk replacer on a dry matter basis of 1% of BW, treatment 2 at 3% of BW, and treatment 3 at 4% of BW. The calves assigned to treatment 1 and 2 were fed their MR reconstituted to 15% DM; treatment 3 calves received MR reconstituted to 18% DM. Calves were fed individually in buckets three times per day. This protocol was used so that protein or any other nutrient would not be a limiting factor to these calves. Six calves from each treatment were slaughtered at 65, 85, and 105 kg BW to study body composition. Treatment 3 calves had the lowest dry matter intake to reach the target slaughter weights, and by week 3 of life, calves assigned to treatment 3 were consuming greater quantities of MR than calves assigned to treatment 2. From this experiment Diaz et al. (2001) demonstrated that dairy calves were able to achieve feed efficiencies that were comparable to those of lambs and pigs.

Blome et al. (2003) studied 40 calves to test for differences in growth, nutrient utilization, and body composition in calves fed MRs containing different amounts of protein. After a 2 week standardization period eight calves were assigned to one of 4 treatments or to a baseline slaughter group. The baseline group was slaughtered on day 15, and the other calves were assigned to either a 16.1, 18.5, 22.9, or 25.8 % crude protein MR for treatments 1, 2, 3, and 4, respectively. MRs were fed at 12% of body weight for 6 weeks, and then all calves were slaughtered. This study found that as crude protein in the diet increased so did final body weight. Daily feeding was adjusted weekly according to body weight, so that the calves fed higher crude protein had a greater intake, as they grew more quickly. Wither height at the end of the study did

not differ significantly among treatments. Plasma urea nitrogen levels increased as crude protein level increased as well. Highest levels of plasma urea nitrogen were exhibited in calves fed the highest crude protein level, indicating that the calves on the 25.8% crude protein level did not utilize protein as efficiently as the calves on the other 3 treatments. Blome et al. (2003) determined that an increase in crude protein levels from 16 to 26% increased skeletal growth and lean gain.

Bartlett et al. (2006) conducted a study with 48 Holstein calves that were assigned to treatments in a  $2 \times 4$  factorial arrangement consisting of 2 feeding rates (1.25 or 1.75% of BW as DM daily, adjusted weekly) and 4 milk replacer CP concentrations (14, 18, 22, or 26% CP). It was determined from this study that increasing the feeding rate increased ADG, gain:feed, and empty-body gains. The percentage of fat in empty body weight gain and in the final empty body, and concentrations of IGF-I and insulin in plasma increased as well. Increasing the feeding rate decreased percentages of water and protein in the empty body and decreased PUN. Increasing dietary CP concentration increased ADG, body length, heart girth, and gains of water and protein but decreased fat deposition. Effects of dietary CP interacted with feeding rate for gains of lean tissue. As dietary CP increased, fat content in empty body gain decreased, and water and protein increased. These researchers found that efficiency of body weight gain can be markedly improved by increasing feeding rate and by increasing levels of protein in MR.

In 1973, Marshall and Smith performed a trial to determine the effect of milk fat levels on growth and efficiency of energy utilization for weight gain in dairy calves fed ad libitum. Milk was fed at 3, 6, and 9 % fat with each level fed ad libitum undiluted or diluted with 1.5 parts water. They found that calves on undiluted milk feeding programs had better growth performance than those on feeding programs in which milk was diluted. Weight gains were

greater for calves fed undiluted milk. Calves on the lowest level of undiluted milk (3% fat) had fewer digestive disturbances than calves on the diluted milk treatments.

To study effects of fat content of milk replacer and calf starter Kuehn et al. (1994) conducted an experiment with 120 Holstein calves at 3 different locations. Forty calves were used at each location. All calves were fed a high fat MR from day 5 to 13 at 10% of BW, at which time they began treatment protocols. Treatments consisted of 1) high fat MR (21.6%)/high fat starter (7.3%), 2) high fat MR (21.6%)/low fat starter (3.7%), 3) low fat MR (15.6%)/high fat starter (7.3%), and 4) low fat MR (15.6%)/low fat starter (3.7%). MR was fed at 8% of birth weight/d from day 14 to 35 and at 4% of birth weight/d from day 36 to 42. Calves were weaned on day 43, and starter was fed until day 56. High fat milk replacer depressed dry matter intake before and after weaning, and high fat starter depressed dry matter intake after weaning. Before weaning, calves gained more body weight when fed low fat MR. Calves fed low fat starter gained more body weight after weaning. On day 56 body weights were greatest for calves fed low fat MR and starter and lowest for those fed high fat MR and starter. Calves fed a MR with 15.6% fat and a starter without added fat performed better than calves fed a MR with 21.6% fat or starter with added fat.

Tikofsky et al. (2001) utilized 32 Holstein bull calves to investigate effects of fat levels in MR. Eight calves were assigned to 1 of 3 treatments in a randomized block design by age and weight. The fourth calf of every block was selected for a baseline slaughter group. All treatments were isonitrogenous and isocaloric. Fat levels were 14.8, 21.6, and 30.6 % of DM for treatments 1, 2, and 3, respectively. Baseline calves were slaughtered on the day the rest of the calves started treatment. Calves started treatment at 2 to 6 days of age and were grown to 85 kg of live body weight. These researchers determined that when fat was substituted for carbohydrate as an



energy source, dietary fat was used as a fuel source for body fat deposition, and not for additional protein retention. It was concluded that addition of fat to MR diets does not promote lean tissue gain.

A study conducted by Brown et al. (2005) to determine if increased energy and protein intake from 2 to 14 wk of age would affect mammary development in heifer calves. Holstein heifer calves were assigned to 1 of 4 treatments. Treatments were 2 different levels of protein and energy intake in the first period (2-8 weeks) and 2 different protein and energy intakes in the second period (8-14 weeks). The levels of protein and energy intake were moderate and high in the first period and low and high in the second period. The calves on the moderate diet in the first period were fed MR at 21.3% CP, 21.3% fat, fed at 1.1% of body weight on a dry matter basis and a 16.5% CP grain mix so that they would gain 400 grams/d. The calves on the low diet in the second period were fed only the starter at 16.5% CP. The calves on the high diet in period 1 were fed MR at 30.3% CP, 15.9% fat, fed at 2.0% of body weight on a dry matter basis and a 21.3% CP grain mix available ad libitum. The calves on the high diet in period 2 received only the starter at 21.3%CP. Eleven calves were slaughtered at 8 weeks of age and 41 calves were slaughtered at 14 weeks of age. Mammary tissue was collected and analyzed. These researchers concluded from this study that increasing protein and energy intake in Holstein heifer calves from 2 to 8 wk of age can increase the rate of development of mammary tissue.

### **MILK REPLACER FEEDING RATE**

Huber et al. (1984) conducted a study to determine the effects of feeding different amounts of milk on performance, health, and absorption capability of neonatal Holstein calves. Sixteen calves (male n = 8, female n = 8) were assigned to one of two treatments. Treatment 1 calves were fed 4.1 kg of milk replacer from day 3 to weaning on day 48. Treatment 2 calves

were fed 4.1 kg of milk replacer increased to 7.0 kg over time during the first 2 weeks of the study. Then, calves on treatment 2 were fed 7.6 kg of milk replacer through day 42 when they were gradually weaned to day 48. Commercial starter, alfalfa hay, and water were available at all times. The results of this study indicated that higher feeding rates resulted in greater weight gain but decreased starter intake.

Jasper and Weary (2002) studied the effects of ad libitum milk intake with 28 female Holstein calves. Calves were assigned to treatments consisting of waste milk either 1) conventionally fed at 10% of BW at equal amounts twice a day or 2) free choice milk from a nipple installed in the pen wall. All calves were gradually weaned from day 37 to 42. Calves were allowed free access to a barley-based starter, chopped fescue hay, and water throughout the experiment. Calves fed ad libitum consumed 89% more milk but only ate 16% as much starter and 17% as much hay as the limit-fed calves. However, shortly after weaning, treatment differences were negligible. They concluded that calves can be fed ad libitum with no detrimental effects on grain intake after weaning.

Cowles et al. (2006) performed a study on 34 Holstein heifer calves. Calves were assigned to 1 of 4 treatments in a 2 x 2 factorial arrangement. Calves were assigned to either a conventional (20% protein/ 20% fat) or intensified (28% protein/ 20% fat) MR feeding regimen with or without the addition of 1 g of lactoferrin. Conventional MR was fed at 562 g daily, and intensified MR was fed at  $0.2 \text{ Mcal/kg BW}^{0.75}$  for d 2 to 9 and at  $0.27 \text{ Mcal/kg BW}^{0.75}$  from d 10 to 42. Calves had free access to a pelleted starter and fresh water throughout the experiment and were studied for 14 d postweaning. Cowles et al. concluded that addition of lactoferrin had no beneficial effect on calves; however, calves fed the intensified feeding regimen had significantly

higher gains preweaning. The calves fed conventionally had higher gains during weaning. Intensified MR feeding appears to depress starter intake.

Hill et al. (2006) conducted an experiment to study the effects of different feeding rates of high protein MRs on calf performance. In trial 1 calves were fed a 26% CP, 17% fat MR at either .681 kg/d increased to .794 kg/d by day 8 or increased to .908 kg/d by day 15. Calves in trial 2 were fed at either .681 kg/d throughout the trial or increased to .908 kg/d by day 15. Calves on trial 3 were fed .681 kg/d of 26% protein, 17% fat MR increased to 1.135 kg/d by day 22, 28% protein, 20% fat MR increased to 1.135 kg/d, or 20% CP, 20% fat MR at .454 kg/d. Calves on all trials were fed MR from day 3 to 4 until weaning by day 42. Calves also had access to an 18% crude protein calf starter and water at all times. Trial 1 resulted in starter intake from 0 to 56 days declining as MR feeding rate increased. Trial 2 resulted in calves fed .681 kg/d having slower ADG from day 0 to 21 and day 0 to 42 but faster ADG from day 42 to day 56 compared with calves fed .908 kg/d. Overall ADG from day 0 to 56 was not different. Starter intake among treatments was greater for calves fed .681 kg/d than for calves fed .908 kg/d from day 0 to 42, day 42 to 56, and day 0 to 56. Efficiency of calves fed .681 kg/d was not as high from day 0 to 21 and day 0 to 42 but these calves were more efficient from day 42 to 56 than calves fed .908 kg/d. Efficiency from day 0 to 56 was not significantly different. Results from trial 3 indicated that both sets of calves fed at the highest rates, 1.135 kg/d grew the fastest and had the greatest efficiency from day 0 to 42 but grew the slowest and had the lowest efficiency for the rest of the trial. Results of all of these trials indicated that calves fed 26% CP, 17% fat MR did not reduce starter intake and increased the ADG compared with calves fed the 20% CP, 20% fat MR at .454kg/d. Hill et al. (2006) suggested that .681 kg/d was the maximum amount of a high CP MR that could be fed without having a weaning and post-weaning decrease in calf performance.

## **RUMEN DEVELOPMENT**

Both the rate of intestinal development and the process of transitioning calves from their neonatal reliance on nutrients supplied from milk to nutrients supplied from grain are of substantial economic importance to the producer. Improvements to the calf nutritional regime can decrease mortality and disease susceptibility, increase postweaning rate of gain, ultimately enhancing the rate of herd genetic improvement (Baldwin et al., 2004).

Warner et al. (1956) studied 36 Holstein bulls and 10 Holstein heifers. Calves were fed diets of mostly milk, grain, hay, or a mixture of grain and hay. Calves were slaughtered at birth, 4, 7, 10, 13, and 16 weeks of age in order to study the development of the stomach. From 4 weeks of age an increase in stomach development could be seen in calves receiving dry feed as opposed to those fed milk alone. Papillae development on the rumen wall as well as volume and tissue development of the fore-stomachs was observed.

Lengemann and Allen (1959) studied 24 male dairy calves on limited and liberal milk feeding regimens. Calves were assigned to one of four groups. Control calves were fed milk at 10% of body weight and a 14% CP calf starter free choice to 3 lbs. per day. Two groups were fed milk at 10% of body weight for the first 3 weeks. Then milk was gradually reduced until calves were completely weaned at week 7. These calves were fed a 25% CP starter with a limit of 3 lbs. per day. One group of these calves was fed aureomycin in their calf starter. The other group received no feed additives. The fourth group of dairy calves was fed milk only at 12% body weight until week 9 of the study. Then calves were gradually weaned to week 11. Lengemann and Allen (1959) found that calves fed milk liberally showed slow rumen development and did not attain the total bacterial numbers seen in adult cattle until 9 weeks and never approached the values for the non-aureomycin or aureomycin calves. They also noted that for the milk only

calves butyric acid was significantly lower on weeks 7 and 8, and propionic acid levels were significantly lower for weeks 3 through 7. These levels were measured as a percent of the total rumen acids. Lengemann and Allen (1959) concluded that the major factor affecting rumen function development was the diet.

Stobo et al. (1966) studied 30 Ayrshire bull calves from 21 days of age until 83 days of age. From 3 weeks of age calves were fed 7.5% crude protein hay ad libitum with a 20% crude protein concentrate diet of 0.45, 0.91, 1.36, 1.81, or 2.27 kgs per day. Six calves were slaughtered at 3 weeks of age before consuming dry feed. The remaining calves were slaughtered at 84 days of age in order to determine digestive tract development. Calves that were fed more concentrate consumed less hay. The calves on treatments 4 and 5 exhibited extensive ruminal development when compared to calves on the other treatments. Stobo et al. determined that calves consuming diets with increased protein content had more favorable conditions for rumen papillary development.

Hamada et al. (1976) pooled data on kids from many different feeding trials which used different types of solid feed. Kids from 37 days of age were slaughtered and relationships between age, food intake, weight gain, and final body weight were studied, as well as weights of stomach compartments, heart, liver, kidneys, and spleen were analyzed. Also Hamada et al. determined from these data that growth of fore-stomachs, especially rumen mucosa, relative to body weight was stimulated only in feeding periods of solid feed.

Kertz et al. (1979) pooled research data on 277 calves from 0 to 28 days of age on an early weaning program over 3 years. The first day of life calves nursed their dams, then they were moved to the calf starting unit where they were fed colostrum for 2 more days. From 4 days of age to 4 weeks of age calves were fed milk replacer. Calves were fed milk replacers of 22%

protein with a variety of fat levels (8%, 10%, 12%, and 15%) with a variety of different ingredient variables, namely soy protein concentrate, fat level, milk protein, soy flour, fish flour, fish protein concentrate, and fish protein hydrolysate. All calves were fed a 16% crude protein and 6 to 7% crude fiber starter ration. Calves fed milk replacers which contained protein of poorer quality consumed less calf starter and gained less body weight.

Rumen development is mainly affected by calf starter intake. The effect milk replacer has on rumen development is indirect through its effect on calf starter consumption. Calves fed milk replacer according to an accelerated feeding program consume at least twice as much milk replacer as calves fed conventionally. Williams et al. (2007) observed a dramatic decrease in starter intake in calves fed in a typical accelerated feeding program when compared to calves fed in a conventional feeding program. The accelerated feeding program consisted of milk replacer containing 28% protein and 20% fat at a feeding rate of 0.57 kg powder during days 2 through 9, 1.13 kg during days 10 through 41, and 0.57 kg from day 42 through weaning on day 49. The conventional feeding program consisted of feeding 0.45 kg of 20% protein, 20% fat milk replacer from day 2 through 41 followed by 0.23 kg from day 42 through weaning on day 49. While these researchers reported an increase in growth with the accelerated program, they observed no effect of feeding program on rumen pH or volatile fatty acids. The effect of nutrient composition of milk replacers used in accelerated feeding programs, when fed at conventional feeding rates, on starter intake and rumen development remains to be determined.

## CHAPTER 3

### MATERIALS AND METHODS

#### ANIMALS AND DIETARY TREATMENTS

Fifty-three newborn Holstein calves (male n = 23, female n = 30) were utilized in a randomized block design to assess the effects of three different milk replacers containing different fat and protein levels on growth and rumen development of neonatal Holstein calves. All calves in this study were born and raised at the LSU Dairy Science Research and Teaching Farm in Baton Rouge, LA. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center.

Calves were removed from their dams, weighed, and placed in individual calf hutches according to standard operating procedures of the LSU Dairy Science Research and Teaching Farm. Calves were vaccinated with bovine rotavirus-coronavirus vaccine (Calf Guard) as soon as they were placed in a hutch, and their navels were disinfected with a 7% iodine solution. Calves were housed in individual hutches throughout the experiment. Hutches, which measured 2.5 m<sup>2</sup>, were connected to wire enclosures measuring 2.3 m<sup>2</sup>. All hutches were located on a bed of scallop rock approximately 8 cm thick. Hutches were spaced in intervals of approximately 1.5 m in up to 5 rows of up to 6 hutches each. During the first 48 h after birth calves were bottle fed at least 4 quarts of colostrum from their dams twice a day. On day 3 of life calves were trained to drink from a bucket.

Beginning on the third day calves received commercial MR powder at 10% of birth weight, reconstituted to 15% solids every day at 0700 hours for 6 weeks. Calves were assigned to treatments randomly by sex and birth date. Calves on treatment 1 received a 20% protein, 20% fat (Land O' Lakes, 20/20) MR. Treatment 2 calves received a 25% protein 15% fat (Land

Table 1. Ingredients in 20.5% crude protein calf starter on a dry matter basis.

<b>Ingredients</b>	<b>% as Fed</b>
Cracked Corn	43.7
McNess Calf Premix B <sup>1</sup>	1.5
Pro-Lak	2.5
Sweet Stuff	7.5
Vitamin E-20,000 IU/lb.	0.075
Bovatec Premix <sup>2</sup>	0.6
Calcium Carbonate	0.35
Dried Distiller Grain	5.0
Soybean Meal 48	15.0
Cargill Pellet Milk +	2.5
Oats, crimped	16.275
Molasses	5.0

<sup>1</sup>McNess Calf Premix B: 17.80% Ca, 4.5% P, 1.0% Mg, 0.60% K, 7.80% Na, 12.20% Cl, 8.00% S, 44 ppm Co, 875 ppm Cu, 2530 ppm Fe, 57 ppm I, 2860 ppm Mn, 19.5 ppm Se, 2879 ppm Zn, 211 KIU/lb Vitamin A, 21.6 KIU/lb Vitamin D, 380 KIU/lb Vitamin E.

<sup>2</sup>Bovatec Premix consists of 9.0 lb of Soybean Meal 48 % plus 1.0 lb of Bovatec 68 hand mixed

Table 2. Guaranteed analysis for calf milk replacers containing 20% protein 20% fat (20/20), 25% protein 15% fat (25/15), and 28% protein 20% fat (28/20) on an as-fed basis.

	<b>20/20</b>	<b>25/15</b>	<b>28/20</b>
Crude Protein, min. %	20	25	28
Crude Fat, min. %	20	15	20
Crude Fiber, max. %	.15	.15	.15
Calcium, min. %	.50	.75	.50
Calcium, max. %	1.00	1.25	1.00
Phosphorus, min. %	.60	.70	.60
Vitamin A, min. IU/lb.	35,000	23,100	35,000
Vitamin D <sub>3</sub> , min. IU/lb.	7,500	5,000	7,500
Vitamin E, min. IU/lb.	150	100	150



O' Lakes, 25/15) MR. Calves on treatment 3 received a 28% protein 20% fat (Cow's Match, Land O' Lakes, 28/20) MR. All MRs (Table 2) were manufactured by Land O' Lakes Corporation and were medicated with decoquinate at 45.4 grams/ton. On day 42 calves were abruptly weaned.

On day 3 a calf starter formulated to contain 20.5% crude protein (Table 1.) was made available to all calves. Through day 56 calves were offered fresh feed twice a day at 0630 and 1700 hours. Orts were collected at the beginning of every feeding, weighed, and fresh starter was returned to the calf. Calves received water ad libitum throughout the study.

### **SAMPLE COLLECTION**

Weekly, from 24 hrs. to 8 weeks of age at 0600 hours, before milk replacer feeding, calves were weighed and skeletal measurements (hip height, wither height, and body length) were taken. Average daily gain and stature calculations were derived from these measurements. Fecal scores were recorded twice daily at feeding times on a 1-4 scale, 1 being the most solid and 4 being the most fluid, as previously described by Larson (1977).

Blood samples were collected weekly before the morning feeding of milk replacer until 8 weeks of age from all calves for measurement of blood metabolite concentrations. Plasma and serum samples were collected via jugular venipuncture into 10 mL evacuated tubes (Kendall Monoject; Kendall Medical, St. Louis, MO). The plasma samples were taken in tubes containing sodium heparin as an anticoagulant. Serum samples were taken in tubes with no additives. All samples were centrifuged at  $1,876 \times g$  for 15 minutes or until separation occurred. Plasma and serum were removed, and samples were stored at  $-20\text{ }^{\circ}\text{C}$  until analysis for plasma urea nitrogen (PUN), insulin-like growth factor-1 (IGF-1), and  $\beta$ -hydroxybutyrate ( $\beta$ -HBA).

Rumen fluid was collected before the morning feeding of milk replacer from all calves at weeks 4 and 8 via esophageal stomach tube. Samples were taken in graduated tubes and pH was measured immediately after sample collection. After pH measurement 20% (vol/vol) phosphoric acid was added to rumen fluid samples as a preservative, and samples were stored at -20 °C until analysis of volatile fatty acid levels.

### **LABORATORY METHODS**

Plasma samples from each week were analyzed for IGF-1 using the radioimmunoassay protocol from Sticker et al. (1995) modified for cattle. PUN levels were measured from weeks 2, 4, 6, and 8 using commercial spectrophotometric kits, (Urea Nitrogen (BUN) Berthelot/Colorimetric; Pointe Scientific, Inc., Canton, MI).  $\beta$ -HBA levels were analyzed from plasma collected at weeks 2, 4, and 8 measured with commercial spectrophotometric kits ( $\beta$ -Hydroxybutyrate Liquicolor® Procedure No. 2440; STANBIO Laboratory, Boerne, Texas). A 4 ml sample of rumen 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 rumen fluid and meta-phosphoric acid was then centrifuged at  $30,000 \times g$  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 .53 mm and a film thickness of 1.2  $\mu$ m (Alltech Associates, Inc.; Deerfield, IL). The reagent preparation procedure and temperature gradient program for VFA analysis was adapted from Grigsby et al. (1992) and Bateman et al. (2002), respectively.

### **CALCULATIONS AND STATISTICAL ANALYSIS**

Variables measured daily were reduced to weekly means prior to analysis. All dependent variables were analyzed using the mixed procedure of SAS (Littell et al., 1998). For all variables

except ADG, weight at birth, weight at the end of the trial and milk replacer intake the model included treatment, sex, week, and their two- and three-ways interactions as fixed effects. Weekly averages were analyzed as repeated measures using a first-order auto regressive covariance structure for all variables except body weight and starter intake. For body weight and starter intake a first-order auto regressive with non homogeneous variance covariance structure was used because as time progressed body weight and starter intake increased along with its variance. Calf within sex by treatment was the subject of the repeated statement. The covariance structure was selected by choosing the best fitting model according to the Akaike Information Criterion. For body weight, hip height, wither height, and body length response variables measured at birth were included into the model as a covariate. The natural log of IGF-1,  $\beta$ -hydroxybutyrate, and plasma urea nitrogen was taken to correct either a non-homogeneity variance problem and/or a problem of residuals being not normally distributed.

Milk replacer intake, ADG, weight at birth, and weight at trial end were analyzed including in the model treatment, sex and their interaction as fixed effects. Two contrasts statements were built to test: 1) 20% protein 20% fat vs. 25% protein 15% fat and 2) 20% protein 20% fat vs. 28% protein 20% fat. Values reported are least squares means. Significance was declared at  $P < 0.05$ , and a trend was reported if  $0.05 < P < 0.10$ .

## CHAPTER 4

### RESULTS AND DISCUSSION

#### PERFORMANCE DATA

As expected, starter intake increased with age of the calves (Figure 1.) ( $P < .05$ ). Milk replacer consumption did not differ significantly among treatments. Calves fed 28/20 consumed more starter daily overall ( $P = 0.03$ ) (Figure 1). When comparing calves fed 20/20 versus calves fed 28/20, the calves fed 28/20 consumed more starter per day ( $P = 0.02$ ). However, when comparing calves fed 20/20 versus calves fed 25/15 there was no difference in starter consumption ( $P > 0.10$ ). A significant treatment by week interaction was observed ( $P = 0.04$ ). Starter consumption was not significantly different in week 1 between calves fed 20/20 and calves fed 28/20. Calves fed 25/15 consumed less starter in week 1 than calves on other treatments. Beginning in week 2 calves fed 28/20 consumed more starter weekly than all other calves on experiment. Hill et al. (2006) and Jasper and Weary (2002) indicated that 28/20 fed at 1.135 kg/d and milk fed ad libitum, respectively, decreased starter intake preweaning. The current trial suggests that feeding 28/20 at 10% of birth weight encourages starter intake when compared to feeding 20/20 in once a day calf feeding systems.

Average daily gain, initial body weights, weaning weights, and weights at the end of the trial are presented in Table 3. There was a tendency for birth weight to be significantly different among treatments ( $P = 0.10$ ). Therefore, body weight at birth was used as a covariate for the remainder of the analyses. Overall average daily gains (ADG) for calves fed 28/20 were greater than those for all other calves on this experiment ( $P = 0.03$ ). When comparing calves fed 20/20 to calves fed 28/20, calves fed 28/20 had significantly higher average daily gains ( $P = 0.02$ ).

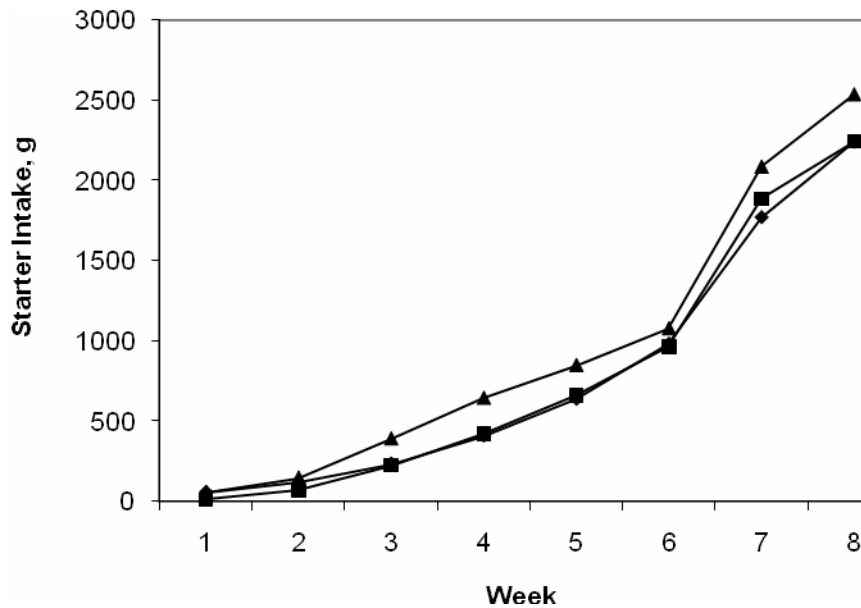


Figure 1. Least squares means of daily starter intakes for calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲) MRs. Main effect of treatment ( $P < 0.05$ ) and time ( $P < 0.05$ ). Interaction of treatment and time ( $P < 0.05$ ). Probability of orthogonal contrasts: 28/20 vs. 20/20 ( $P < 0.05$ ). SEM = 0.26.

Table 3. Least squares means for initial weights, weaning weights, final weights, and average daily gain for calves fed 20% protein 20% fat (20/20), 25% protein 15% fat (25/15), and 28% protein 20% (28/20) fat MRs.

	Treatment				P-Value		
	20/20	25/15	28/20	SEM <sup>1</sup>	Trt	20/20 vs. 25/15	20/20 vs. 28/20
Birth Weight	41.34	38.50	37.60	3.26	0.09	0.10	0.03
Weaning Weight, kg	58.80	59.89	63.47	2.45	0.01	0.48	0.004
Final Weight, kg	69.81	71.40	76.80	3.33	0.004	0.45	0.002
ADG, kg	0.57	0.58	0.67	0.07	0.03	0.84	0.02

SEM<sup>1</sup> = Standard Error of the Mean

However, no significant difference in average daily gain was observed between calves fed 20/20 and calves fed 25/15. Similarly, weaning weights were greater ( $P < 0.01$ ) in calves fed 28/20 compared to those fed 20/20 MR. This increased growth continued after weaning, as body weight at the end of the eighth week were greater in calves consuming 28/20 milk when compared to the 20/20 MR. No differences were observed ( $P > 0.10$ ) in weaning weights or end of trial weights for mean body weight in calves fed 25/15 MR compared to those fed 20/20.

Mean weekly body weights are presented in Figure 2. No significant differences in body weight were observed between calves fed 25/15 and 20/20 ( $P > 0.10$ ). From week 2 until the final weight at week 8, including weaning, calves fed 28/20 weighed more than calves fed 20/20 ( $P < 0.05$ ), which continued until the end of the trial. These results are similar to those from experiments by Quigley et al. (2006) and Brown et al. (2005), both of which used 28% CP, 20% fat milk replacers. However, these researchers fed these higher protein milk replacers at higher feeding rates.

Least squares means for wither height, hip height, body length, and fecal scores are presented in Table 4. A tendency was observed for calves fed 28/20 to have greater overall wither heights than all other calves on this experiment ( $P = 0.08$ ). Significantly greater wither heights were observed in calves fed 28/20 when compared to calves fed 20/20 ( $P = 0.04$ ). However, when calves fed 20/20 were compared with calves fed 25/15 no significant differences were observed ( $P > 0.10$ ). These results conflict with those of Blome et al. (2003) who observed no difference in wither heights between calves fed MR containing 16.1, 18.5, 22.9, or 25.8% CP. However, Brown et al. (2005) reported similar findings in an experiment with calves fed either a 30.3 % CP, 16% fat MR with a 21.3% CP starter or a 21.3% CP, 21.3 % fat MR with a 16.5% starter.

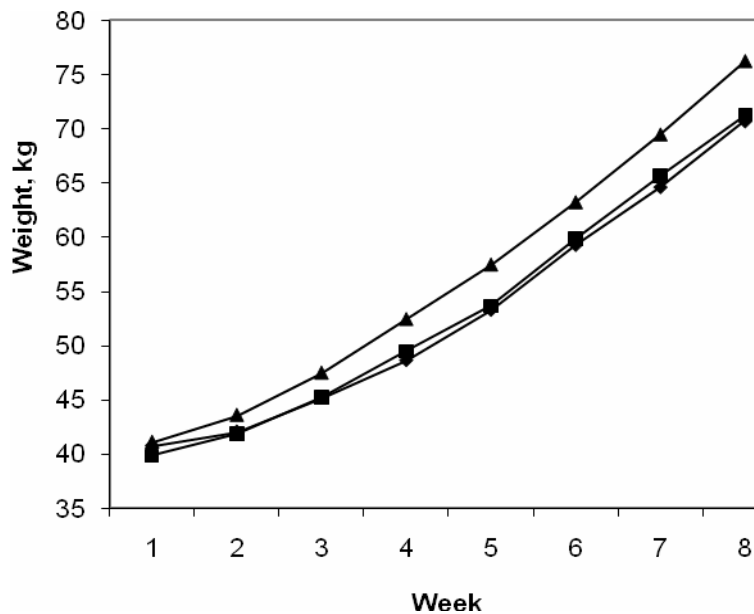


Figure 2. Least squares means for body weights of calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲) MRs. Main effect of treatment ( $P < .05$ ) and time ( $P < .05$ ). Interaction of treatment and time ( $P < .05$ ). Probability of orthogonal contrasts: 28/20 vs. 20/20 ( $P < .05$ ). SEM = 3.15.

Table 4. Least squares means for overall hip height, wither height, length, and fecal scores through 56 days of age of calves fed 20% protein 20% fat (20/20), 25% protein 15% fat (25/15), and 28% protein 20% fat (28/20) MRs.

	Treatment				Trt	P Value	
	20/20	25/15	28/20	SEM <sup>1</sup>		20/20 vs. 25/15	20/20 vs. 28/20
Hip (cm)	84.89	84.89	85.46	0.42	0.44	0.99	0.30
Wither (cm)	79.96	80.13	80.93	0.35	0.08	0.71	0.04
Length (cm)	64.48	64.19	64.95	0.96	0.30	0.58	0.37
Fecal Score <sup>2</sup>	2.57	2.55	2.64	0.02	0.08	0.58	0.10

<sup>1</sup>SEM = Standard Error of the Mean

<sup>2</sup>Fecal Score scale: 1=normal; 2=soft; 3=runny; 4=watery

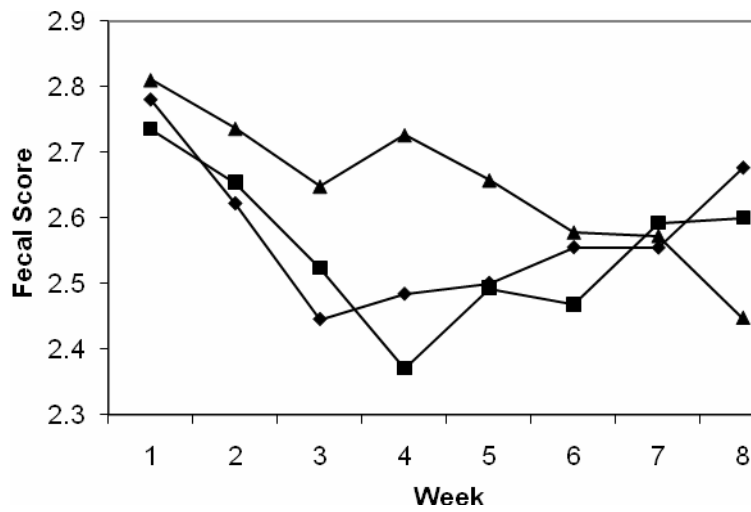


Figure 3. Least squares means for fecal scores of calves fed 20% protein 20% fat (♦), 25% protein 15% fat (■), and 28% protein 20% fat (▲) MRs. Main effect of time ( $P < .05$ ). Treatment by time interaction ( $P < 0.05$ ) SEM = 0.06.



No differences ( $P > 0.10$ ) were observed among treatments in hip height or body length. Bartlett et al. (2006) reported a significant increase in body length with an increase in crude protein concentration of the diet. However, the calves on that project were fed according to daily body weight, as opposed to the calves on this project, which were fed according to birth weight.

The greater wither height observed in 28/20 calves suggests that the higher protein milk replacer increased structural growth. However, the fact that treatments had an effect on wither height and not on hip height or body length suggests variation in measurement.

Fecal scores for the 8 week trial are presented in Figure 3. As calves aged, fecal scores consistently decreased ( $P < 0.05$ ). Average fecal scores throughout the study for 20/20, 25/15, and 28/20 were 2.58, 2.55, and 2.65, respectively. A treatment by time interaction was observed ( $P < 0.05$ ). At week 4 calves fed 25/15 had the lowest fecal scores, and calves fed 28/20 had the highest. Then, at week 8, calves consuming 28/20 had the lowest fecal scores, while calves consuming 20/20 had the highest. Calves fed 28/20 tended to have higher fecal scores than all other calves on this study ( $P = 0.09$ ). This would suggest that the higher protein content of the 28/20 MR increased the softness or fluidity of the calf feces. Data from every treatment group are much higher than those reported by Quigley et al. (2006), on a similar experiment with calves fed a 20/20 MR or a 28/17 MR.

### **RUMEN DEVELOPMENT DATA**

Data for rumen pH and concentrations of acetate, butyrate, and propionate are presented in Table 5. No effects ( $P > 0.10$ ) were observed among treatments for overall means for each VFA and total VFA. Least squares means for acetate, propionate, butyrate, and total VFA are presented in Figures 4, 5, 6, and 7, respectively. Calves fed 20/20 were observed to have the highest total VFA concentration at week 4 and the lowest total VFA concentration in week 8

when compared to all other calves on the experiment ( $P < 0.05$ ). However, no effect of treatment, sex, or time was observed for total VFA concentrations. Acetate concentrations were not significantly affected by any treatment or time ( $P > 0.10$ ). A main effect of time ( $P < 0.05$ ) was observed on concentrations of propionate and butyrate. Propionate levels increased over time ( $P = 0.02$ ). Calves fed 20/20 were observed to have the highest propionate concentration at week 4 and the lowest propionate concentration in week 8 when compared to all other calves on the experiment ( $P = 0.03$ ). Butyrate concentrations decreased over time ( $P = 0.04$ ). Coverdale et al. (2004) reported similar levels of volatile fatty acids in a study conducted with calves fed a 20/20 MR along with a coarse commercial starter. Williams et al. (2007) observed that treatment had no effect on rumen concentrations of acetate, propionate and butyrate in calves fed an accelerated MR (28% protein, 20% fat) versus a conventional MR (20% protein, 20% fat). These data compare favorably with those of the current study in relation to propionate and butyrate; however, in relation to acetate, the data are conflicting.

Overall there were no differences among treatments for rumen pH throughout the 8 week trial (Table 5). Least squares means for rumen pH over time are presented in Figure 8. A treatment by time interaction ( $P < 0.05$ ) was observed for rumen pH. Calves consuming 20/20 had the highest pH at week 4 and the lowest pH at week 8. Calves fed 28/20 had the lowest pH at week 4 and the highest pH at week 8. A main effect of time ( $P < 0.05$ ) was observed on all treatments. These results agree with those of Laborde (2008) and Beharka et al. (1998), who reported an increase in rumen pH with age in young calves.

### **BLOOD METABOLITES**

Least squares means for plasma concentrations of IGF-1,  $\beta$ -hydroxybutyrate, and urea nitrogen are presented in Table 6 and in Figures 9, 10, and 11, respectively. Plasma

concentrations of IGF-1 were not affected by treatment ( $P > 0.10$ ). As expected a significant effect of time was observed ( $P < 0.01$ ) with IGF-I concentrations increasing as the calves aged. These data conflict with the findings of Quigley et al. (2006) which showed that IGF-1 levels increased with increased consumption of dietary crude protein. However, in this study neonatal calves were fed either a fixed amount of conventional (20% protein, 20% fat) MR or a variable amount of accelerated (28% protein, 17% fat) MR. Varying the amount of MR fed may have affected the IGF-I levels. In a similar study Williams et al. (2007) observed no change in IGF-I levels when comparing an accelerated feeding program to conventional feeding methods.

Overall  $\beta$ -HBA levels were greatest for calves fed 28/20 ( $P < 0.01$ ). A tendency was observed for calves fed 25/15 to have greater concentrations of  $\beta$ -HBA than calves fed 20/20 ( $P > 0.10$ ).  $\beta$ -HBA concentrations increased ( $P < 0.01$ ) over time for all treatments (Figure 10). These data indicate that calves fed MR with the highest protein level were metabolizing more butyric acid, which is indicative of more progressed rumen development. These data compare favorably with Khan et al. (2007), which also showed an increase in  $\beta$ -HBA over time, especially in calves consuming more calf starter. Others have agreed with this increase in  $\beta$ -HBA with age (Laborde, 2008; Coverdale et al., 2004; Quigley et al., 1991).

A significant effect of time was observed on PUN concentrations ( $P < 0.01$ ). Overall calves fed 20/20 had lower PUN concentrations than all other calves on the experiment ( $P < 0.01$ ). Concentrations of PUN were greater for calves fed 28/20 ( $P < 0.01$ ) and 25/15 ( $P < 0.01$ ) than calves fed 20/20 (Table 6). These data compare favorably with the findings of Blome et al. (2003) which found that PUN levels were increased in calves fed higher crude protein MR when compared to calves fed lower crude protein MR. However, the values for PUN in the current

study were comparable than those reported by Blome (2003), Terosky et al. (1997), and Diaz et al. (2001).

Table 5. Least squares means for rumen pH and VFAs in calves fed MRs containing 20% protein 20% fat (20/20), 25% protein 15% fat (25/15), and 28% protein 20% fat (28/20) MRs.

	Treatment			SEM <sup>1</sup>	Trt	P Value	
	20/20	25/15	28/20			20/20 vs. 25/15	20/20 vs. 28/20
pH	6.32	6.42	6.43	0.7854	0.52	0.35	0.30
Acetate, mM	30.94	33.84	33.09	6.46	0.54	0.36	0.59
Butyrate, mM	5.08	4.84	5.04	0.44	0.92	0.71	0.94
Propionate, mM	24.90	26.74	24.57	6.19	0.67	0.46	0.90
Total VFA, mM	64.66	69.33	67.26	13.45	0.69	0.40	0.65

<sup>1</sup>SEM = Standard Error of the Mean

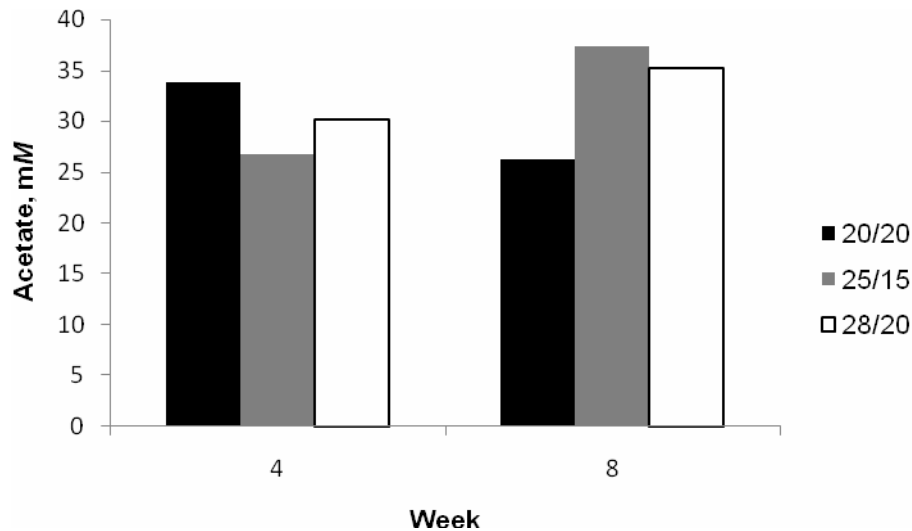


Figure 4. Least squares means for acetate in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat. Main effect of treatment ( $P < 0.05$ ). Main effect of time ( $P < 0.05$ ). Probability of orthogonal contrasts: 25/15 vs. 20/20 ( $P < 0.05$ ), 28/20 vs. 20/20 ( $P < 0.05$ ). SEM = 0.01

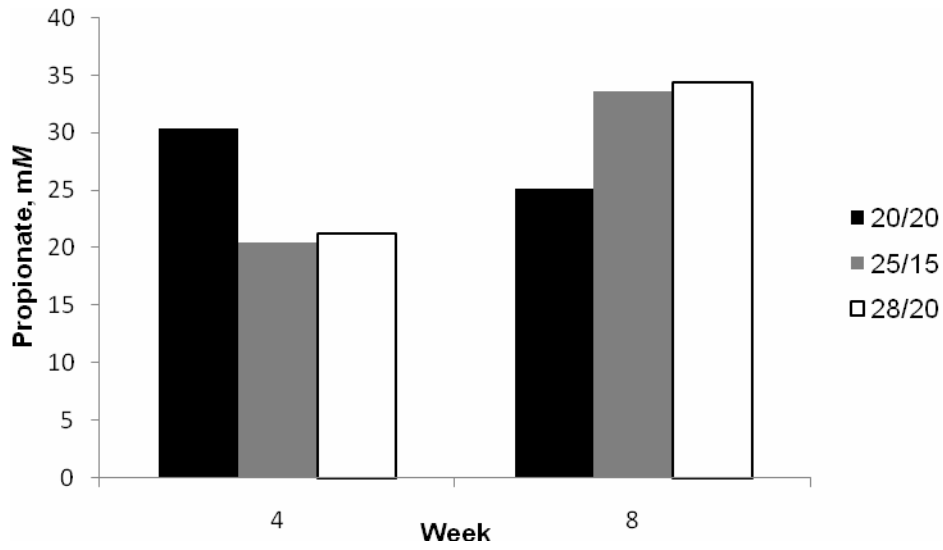


Figure 5. Least squares mean for propionate in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat. Main effect of time ( $P < .05$ ). SEM = 0.04

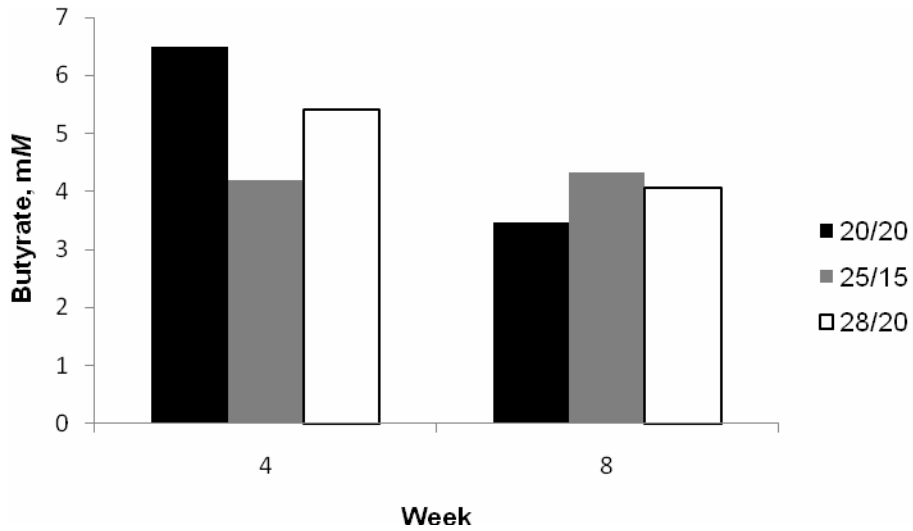


Figure 6. Least squares mean for butyrate in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat. Main effect of time ( $P < .05$ ). SEM = 0.03

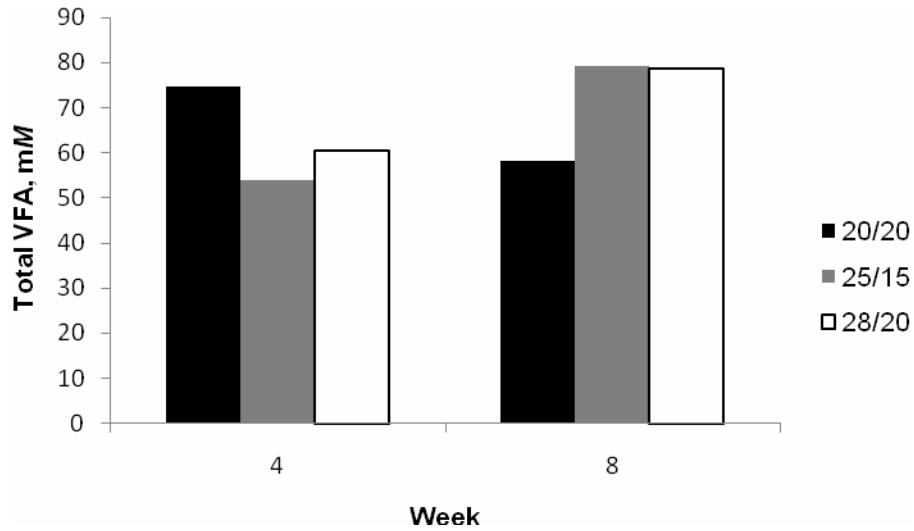


Figure 7. Least squares mean for total VFA in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat. SEM = 18.14

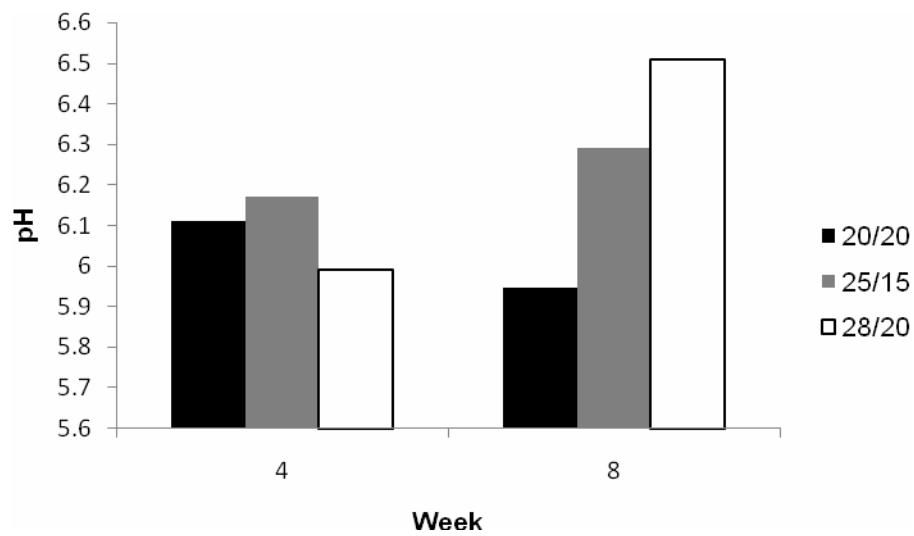


Figure 8. Least squares mean for rumen pH in calves fed MRs containing 20% protein 20% fat, 25% protein 15% fat, or 28% protein 20% fat. Main effect of time ( $P < .05$ ). SEM = 0.12

Table 6. Least squares means for blood hormones and metabolites in calves fed MRs containing 20% protein 20% fat (20/20), 25% protein 15% fat (25/15), or 28% protein 20% fat (28/20).

	Treatment				P Value	
	20/20	25/15	28/10	Trt	20/20 vs. 25/15	20/20 vs. 28/20
IGF-1, mg/dL	105.05	96.07	129.21	0.19	0.59	0.21
PUN, mg/dL	6.05	8.58	8.76	<0.01	<0.01	<0.01
$\beta$ -HBA, mM	0.14	0.16	0.19	<0.01	0.06	<0.01

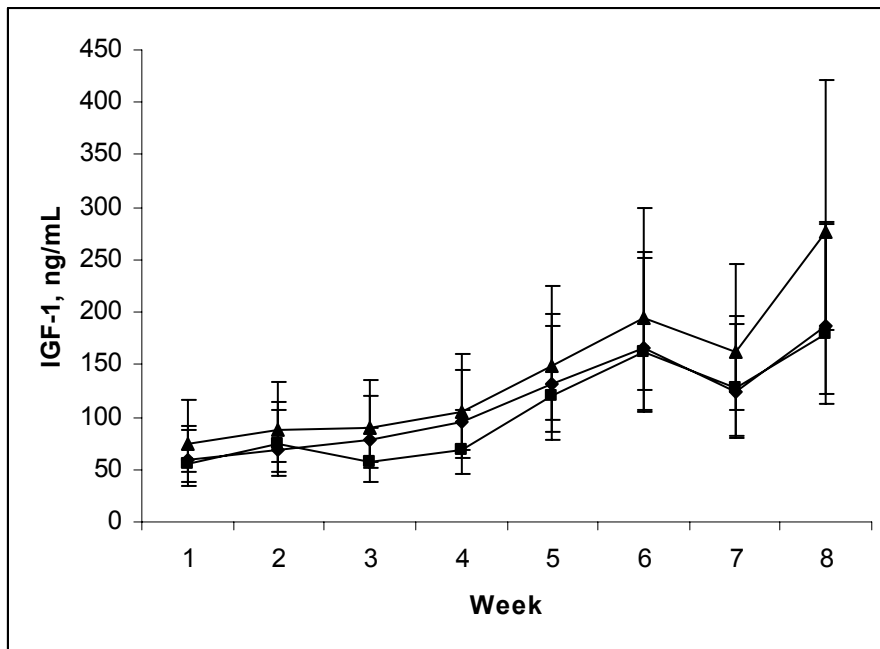


Figure 9. Least squares means for plasma IGF-1 concentrations of calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲) milk replacers. Main effect of time ( $P < 0.01$ ). Error bars denote confidence limits of back-transformed values at a Confidence Interval of 95%.



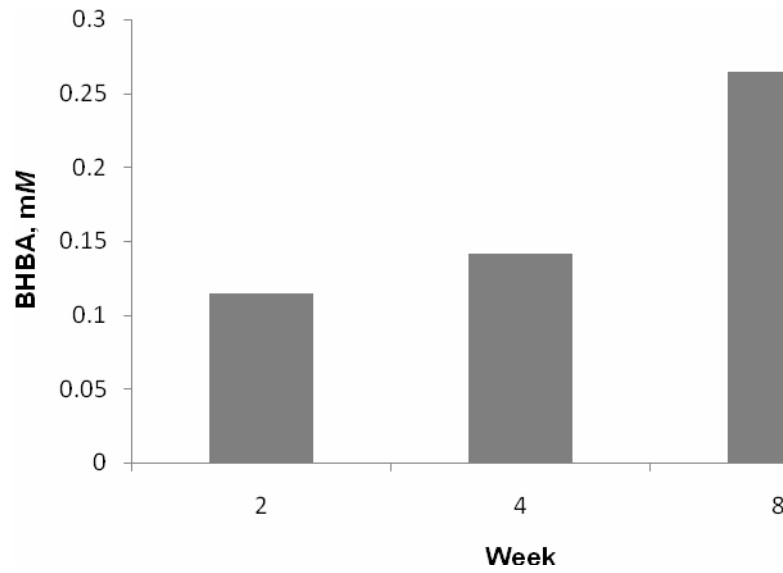


Figure 10. Least squares means for plasma  $\beta$ -HBA at weeks 2, 4, and 8. Main effect of time ( $P < .01$ ). (Confidence Interval 95.00%: week 2, 0.10 to 0.13; week 4, 0.13 to 0.16; week 8 0.24 to 0.29)

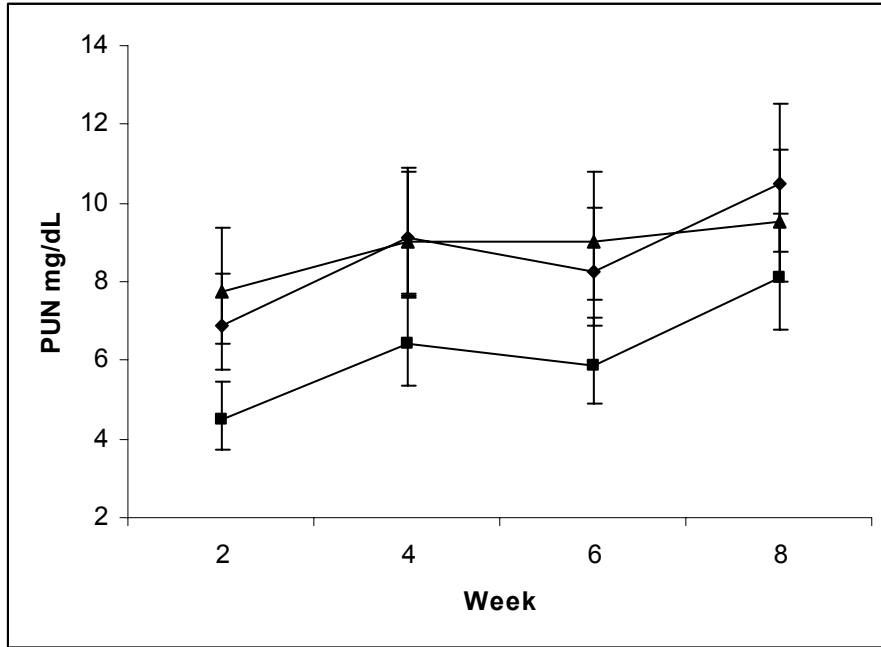


Figure 11. Least squares means for PUN concentrations of calves fed 20% protein 20% fat (◆), 25% protein 15% fat (■), and 28% protein 20% fat (▲)MRs. Main effect of time ( $P < 0.01$ ). Probabilities of orthogonal contrasts: 28/20 vs 20/20 ( $P < 0.01$ ), 25/15 vs. 20/20 ( $P < 0.01$ ). Error bars denote confidence limits of back-transformed values at a Confidence Interval of 95%.

## **CHAPTER 5**

### **SUMMARY AND CONCLUSIONS**

#### **SUMMARY**

The objective of this study was to examine the effects of feeding milk replacers of different protein and fat concentrations on calf growth and development as compared to the industry standard (20/20). The MR with 25% protein 15% fat only differed from the 20% protein 20% fat MR in affecting levels of plasma urea nitrogen.

MR containing 28% protein and 20% fat encouraged starter intake, encouraged body weight gain, and caused an increase in wither height when compared to a 20% protein and 20% fat MR. Levels of the individual volatile fatty acids acetate, butyrate, and propionate were not affected, nor were total levels of volatile fatty acids. IGF-1 levels were not affected by treatment; however, PUN levels were significantly greater for calves fed 28/20 as were  $\beta$ -HBA levels.

Findings of starter intake, body weight, and skeletal measurements along with volatile fatty acid measurements indicate that 28/20 MR encourages lean gain, without negatively changing the environment in the rumen or negatively affecting calf health. A significant increase in  $\beta$ -HBA concentrations in the blood suggests this MR may have affected starter intake which in turn affected rumen development. PUN levels indicate that with an increase of protein in the neonatal calf's liquid diet more protein is not utilized and is passed through the animal.

#### **CONCLUSIONS**

A 28/20 MR fed at conventional feeding rates increases growth and calf performance without any deleterious effects on growth or rumen function when compared to a 20/20 MR fed at conventional feeding rates.

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## APPENDIX A: PLASMA IGF-1 ASSAY

### **Day 1 – Sample Preparation:**

- 1) Create a protocol for the sample extraction in Excel:
  1. The samples are in single. Put sample number (1,2,3...end) in column A, put sample ID in column B
  2. Samples 1-3 are to be LE2, Low Bovine, and Blank
  3. Follow with your samples
  4. End with LE2, Low Bovine, and Blank (there will be a total of N+6 tubes)
- 2) Label tubes for sample extraction (1, 2, ... N+6)
- 3) Add 300 $\mu$ L of glycine-HCl (pH =3.2) mixture to all tubes.
- 4) Pipette 200  $\mu$ L of each sample INTO the glycine-HCl mixture in the appropriate tube (do NOT dribble down the side of the tube).
- 5) When all sample are pipetted, vortex the tubes gently (but thoroughly mix) for 5 seconds.
- 6) Cover, label, and place in fridge for 24 hours.

### **Day 2 – Sample Neutralization and Assay Set-up:**

- 7) To each sample extraction tube, add 2.6 mL of PBS-EDTA.
- 8) Vortex thoroughly (several repetitions of 3-5 seconds each).
- 9) Create an assay protocol per normal (2TC, 3 NSB, 3BC, 8 STD), sample 1 in duplicate, sample 2, in duplicate,... sample N +6 in duplicate, 3 NSB, 3 BC, 8 STD, 2 TC).
- 10) Label assay tubes (total number will be  $2(N+6) + 32 = 2 N + 52$ ).
- 11) *Sample-standard Phase:* Pull the TC, NSB, BC, and STD tubes and set aside. Into all sample tubes, pipette 150  $\mu$ L of IGF-1 Assay buffer (PBSG:PBS-EDTA 1:100). Then into appropriate duplicate tubes, pipette 50  $\mu$ L of the sample. Pipette directly down into the buffer.
- 12) Pipette 200  $\mu$ L of IGF-1 Assay Buffer into all NSB and BC tubes. For the STD, first pipette the appropriate amount of IGF-1 assay buffer to make the difference between the 8 standard volumes and 200  $\mu$ L (that is 198, 196, 192, 185, 175, 150, 100, and 0  $\mu$ L), Then pipette the appropriate amount of standard (50ng/mL) into the tubes (2, 4,8,15, 25, 50, 100, 200  $\mu$ L, respectively). Pipette standard directly into the buffer.
- 13) *Antibody Phase:* Put all tubes except TC and NSB back into the racks in numerical order. To all other tubes, add 200  $\mu$ L of IGF-1 Antiserum (1:60,000). To the NSB tubes, add 200  $\mu$ L of IGF-1 NSB solution (PBSG:PBS-EDTA:protamine sulfate 100mL:100mL:0.2mg/mL).
- 14) Place the NSB and TC tubes in racks and vortex all. Cover, label, and incubate 24-48 hr in fridge (48 is ideal but 24 is okay if in a hurry).

### **Day 4 (or 3) – Hot Hormone Addition:**

- 15) Remove all racks from fridge. Add 200  $\mu$ L of <sup>125</sup>I-IGF-1 solution to all tubes (this solution should be between 40,000 and 60,000cpm/200  $\mu$ L)
- 16) Cover and vortex. Incubate in fridge 24-48 hours (48 is ideal...).



**Day 6 (or 5 or 4) – Second Antibody Precipitation:**

17) Remove racks from fridge. To all tubes except TC, add 200  $\mu$ L of 4X NRS (1:105). Follow this with 200  $\mu$ L of 4X pARGG (1:4.5). Vortex all tubes and incubate in fridge over night (24 hrs is ideal; however precipitation likely is complete in 12-18 hrs.)

**Day 7 – Pour Off:**

18) Load all tubes except TC into centrifuge carriers. Spin at 3,000 rpm for 30 minutes. Immediately when centrifuge stops, remove, decant and drain and blot on paper towels. Add 1 mL cold PBS to all tubes, and repeat centrifuge-decant-drain-blot.

## APPENDIX B. $\beta$ -HYDROXYBUTYRATE COLORIMETRIC ASSAY

(REF:  $\beta$ -Hydroxybutyrate Liquicolor® Procedure No. 2440; STANBIO Laboratory, 1261 North Main Street, Boerne, Texas 78006)

### **Reagents:**

- 1) Enzyme (R1) (Cat. No. 2441)
- 2) Catalyst (R2) (Cat. No. 2442)
- 3) Standard, 1mmol/L (Cat. No. 2443)

### **Procedure:**

- 1) Incubate the needed amount of Reagent A (Enzyme) at 25°C for 3 minutes.
  - 2) To two cuvettes, add 1075  $\mu$ L of Reagent A (Cuvettes 1 and 2).
  - 3) To cuvette 1, add 30  $\mu$ L of sample to be tested and immediately measure the OD at 505nm (To).
  - 4) To the same cuvette 1, add 0.18 mL of Reagent B (Catalyst) and measure the final OD at 505nm (Tf) at 10 minutes.
  - 5) To cuvette 2, add 30  $\mu$ L of Hydroxybutyrate Standard and immediately measure OD at 505nm (To, std).
  - 6) To the same cuvette 2, add 0.18 mL of Reagent B and measure the final OD at 505nm (Tf, std) at 10 minutes.
- 1) Subtract To from Tf to obtain OD (10 min) for both serum and standard.

### **Calculation**

$$\beta\text{-Hydroxybutyrate (mM)} = \frac{\text{OD (10 min) Sample}}{\text{OD (10 min) Std}} \times 1\text{mM} \times \text{dilution of serum}$$

## APPENDIX C: PLASMA UREA NITROGEN COLORIMETRIC ASSAY

(REF: Urea Nitrogen (BUN) (Berthelot/Colorimetric) Assay; Pointe Scientific, Inc., 5449 Research Drive, Canton, MI 48188

### **Procedure:**

- 1) Turn spectrophotometer on to warm up (~30 min.). Label cuvettes UNKNOWN, CONTROL, STANDARD, and BLANK in duplicate.
- 2) Transfer 0.5mL of COLOR RGT to vials labeled: UNKNOWN, CONTROL, STANDARD, BLANK.
- 3) Add 0.010mL (10 $\mu$ L) of sample in to corresponding vial.
- 4) Add 0.5 mL of ENZYME RGT to all vials, mix gently, and incubate for ten minutes at room temperature (26°C)
- 5) Add 2.0mL of BASE RGT, mix and incubate at room temperature (26°C) for ten minutes.
- 6) Set the absorbance reading to 0.00 against BLANK.
- 7) Set wavelength at 630nm.
- 8) Read the STANDARD cuvettes and enter the standard value.
- 9) Read and record the absorbances of all the cuvettes.

### **Calculation:**

$$\text{Sample Urea Nitrogen} = \frac{\text{Abs of Sample}}{\text{Abs Standard}} \times \text{C Standard mg/dL} = \text{C sample}$$

## 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) metaphosphoric acid (fluka #79615) acid solution containing 2 g/L of 2-ethyl butyric acid (216.5  $\mu$ L 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<sub>2</sub>O. Store in refrigerator when not in use.

MW	Acid	Volume ( $\mu$ L)	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 step 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 provides a 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<sub>2</sub>/ air flame.
- 6) Helium is used as the carrier gas with a splitless injection at a flow of 60 mL/min.

## VITA

Anthony Joseph Bridges was born August, 1983, in Minden, Louisiana to Mr. Robin and Mrs. Gail Gorrell Bridges. Upon graduation from Mt. Olive Christian School, Anthony enrolled at Louisiana State University with a concentration in dairy science production. In May 2005 he received his Bachelor of Science degree in dairy science. After receiving his bachelor's degree, Anthony began his graduate work in dairy science with a concentration in nutritional physiology. He will receive the degree of Master of Science in August, 2009. Anthony is currently employed at Calf Source, LLC, located in De Pere, Wisconsin.