

2016

Effects of Calf Starter Composition and Mixing Method on Growth and Rumen Health in Young Dairy Calves

Ruth Marisol Orellana Rivas

Louisiana State University and Agricultural and Mechanical College

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



Part of the [Animal Sciences Commons](#)

Recommended Citation

Orellana Rivas, Ruth Marisol, "Effects of Calf Starter Composition and Mixing Method on Growth and Rumen Health in Young Dairy Calves" (2016). *LSU Master's Theses*. 579.

https://repository.lsu.edu/gradschool_theses/579

This Thesis is brought to you for free and open access by the Graduate School at LSU Scholarly Repository. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Scholarly Repository. For more information, please contact gradetd@lsu.edu.

EFFECTS OF CALF STARTER COMPOSITION AND MIXING METHOD ON GROWTH
AND RUMEN HEALTH IN YOUNG 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

Ruth Marisol Orellana Rivas
B.S., Universidad Nacional de Agricultura, 2011
May 2016

ACKNOWLEDGEMENTS

First of all, I am grateful to God Almighty for all the blessings, opportunities and for giving me the strength and wisdom needed to complete this important step of my life.

I would like to express my gratitude to my advisor and mentor, Dr. Cathleen C. Williams, for helping me with patience, for her support, guidance, and love through this journey, without her help I would not be where I am. I would also like to thank to the members of my committee Dr. Bruce F. Jenny, and Dr. Vinicius R. Moreira for their guidance and support. Thanks to Dr. Kearney for helping me with statistics. Thanks to Dr. Christie Underwood for her help and technical support.

My sincere gratitude to the LSU AgCenter and Dr. William B. Richardson for the financial support which made possible my studies at LSU. I acknowledge with gratitude to Purina Animal Nutrition LLC. for the partial financial support to carry out my reserach. I would also like to thank to Westway Terminal Company, LLC. for the generous product donation.

I would like to thank all the farm and lab student workers Ashley Cooper, Alex Levin, Morgan Richard, Regan Gonzales, and Stephanie Hebert for their help with the handling of the calves, data collection, and sample analysis. Thanks to Ashley Dolejsiova for her teaching and help with my laboratory work and for being at my side as a friend. To my fellow graduate student Amanda Mathias I thank her for the help in data collection and classes. I would also like to extend my gratitude to my contemporary Steven Blair for his help with calves handling, data collection, laboratory work, and his support as a friend.

I would also like to thank my parents Nazario Orellana and Amanda Rivas for their love and support throughout these years of study. To my friends Patricia Reed, and Nell Ginn for

being like a family this time living in Baton Rouge, for their love, friendship, and support. Lastly but no less important to my best friend Anna Borjas who has been like a sister all these years and for supporting all my decision and helping me when I have needed her, for being with me in good and bad times.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT.....	ix
INTRODUCTION	1
REVIEW OF LITERATURE	4
Metabolic Changes during Calf Development	5
Volatile fatty acids.....	5
β -Hydroxybutyrate.....	5
Ruminal Ammonia	6
Ruminal pH.....	6
Plasma Urea Nitrogen.....	7
Glucose metabolism	7
Rumen Development and Normal Calf Growth.....	9
Physical Form of the Diet.....	13
Forage Inclusion in the Diet as a Source of Fiber	14
MATERIALS AND METHODS.....	16
Animal and Dietary Treatments	16
Sample Collection	18
Laboratory Procedures	19
Immunoglobulin G (IgG).....	19
Plasma Urea Nitrogen.....	19
β -Hydroxybutyrate.....	19
Plasma Glucose Concentration.....	19
Total Volatile Fatty Acids	19
Ammonia (NH ₃)	20
Statistical Analysis	20
RESULTS AND DISCUSSION.....	21
Feed Intake Data.....	21
Performance Data.....	22
Rumen Development Data	28
SUMMARY AND CONCLUSIONS	39
Summary	39
Conclusions.....	40
REFERENCES	41

APPENDIX A. BOVINE SERUM/PLASMA IgG TURBIDIMETRIC ASSAY	45
APPENDIX B. UREA NITROGEN (BUN) BERTHELOT/ COLORIMETRIC ASSAY	46
APPENDIX C. β -HYDROXYBUTYRATE COLORIMETRIC ASSAY	47
APPENDIX D. PLASMA GLUCOSE ASSAY	48
APPENDIX E. ANALYSIS OF VOLATILE FATTY ACIDS IN RUMINAL FLUID	49
APPENDIX F. PHENOL-HYPOCHLORITE ASSAY FOR AMMONIA	50
VITA.....	51

LIST OF TABLES

Table 1. Chemical analysis for treatment diets and feedstuff composition for farm mixed starter.....	17
Table 2. Least squares means for body weight, average daily gain (ADG) average daily starter intake, dry matter intake (DMI), feed efficiency, and fecal scores for calves fed farm mixed starter (FMS) or conventional commercial starter (CCS) through d 84 of age.	21
Table 3. Least squares means for wither height, hip height, hip width for calves fed farm mixed starter (FMS), and conventional commercial starter (CCS) through d 84 of age.	26
Table 4. Least squares means for rumen pH, acetate (mmol/L), propionate (mmol/L), butyrate (mmol/L), total VFA (mmol/L), molar % acetate, molar % propionate, molar % butyrate, NH ₃ (mg/dL), glucose, β-hydroxybutyrate (BHBA), plasma urea nitrogen (PUN) for calves fed farm mixed starter (FMS), and commercial calf starter (CCS) through d 84 of age.....	28

LIST OF FIGURES

Figure 1. Least squares means of average daily starter intake for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	22
Figure 2. Least squares means of body weight for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	23
Figure 3. Least squares means of ADG for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	25
Figure 4. Least squares means of feed efficiency for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	25
Figure 5. Least squares means of wither height for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	26
Figure 6. Least squares means of hip height for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	27
Figure 7. Least squares means of hip width for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	27
Figure 8. Least squares means of rumen pH for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	29
Figure 9. Least squares means of total volatile fatty acids (VFA) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	30
Figure 10. Least squares means of acetate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	30
Figure 11. Least squares means of propionate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	31
Figure 12. Least squares means of butyrate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	31
Figure 13. Least squares means of acetate (Molar %) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	33
Figure 14. Least squares means of propionate (molar %) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	33
Figure 15. Least squares means of butyrate (molar %) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	33

Figure 16. Least squares means of overall VFA percentages, for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	34
Figure 17. Least squares means of ammonia for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	36
Figure 18. Least squares means of plasma urea nitrogen (PUN) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	36
Figure 19. Least squares means of β -hydroxybutyrate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	37
Figure 20. Least squares means of plasma glucose for calves fed farm mixed starter (FMS) and commercial calf starter (CCS).	38

ABSTRACT

Twenty-four male Holstein calves (BW = 38.78 ± 7.26 Kg) were used to evaluate the effects of commercially available or farm mixed starter on animal performance, blood metabolites, and rumen health. Calves were randomly assigned to one of two treatments which included: commercial calf starter (minimum 20% CP; CCS) and starter of similar nutrient content but hand mixed on farm (FMS). Calves were offered treatments ad libitum on days 4 through 84. Milk replacer (MR) was offered beginning on day 4. On day 42, MR was reduced to once daily to decrease intake by 50%. On day 49 calves were weaned but remained in hutches for the remainder of the 84 d trial. Body weight (BW), hip height, withers height, hip width, and body length were recorded weekly, and grain and milk replacer intakes were measured twice daily. Rumen fluid and blood samples were collected every two weeks beginning on week 2 for analysis of ruminal pH, ammonia (NH₃), volatile fatty acids (VFA), plasma urea nitrogen (PUN), plasma glucose, and β -hydroxybutyrate (BHBA). There was a treatment by week interaction ($P < 0.05$) for BW, starter intake and PUN. Postweaning BW was greater ($P < 0.05$) for calves fed FMS. Calves fed FMS had higher ($P < 0.05$) overall ADG. However, there were no differences ($P > 0.05$) on ADG before weaning. There were no treatment effects ($P > 0.05$) on structural growth variables, feed efficiency, and fecal scores. Calves fed FMS had greater ($P < 0.05$) starter intake. There were no treatment effects ($P > 0.05$) on total VFA concentrations, VFA molar percentages, glucose, and BHBA. Ruminal pH was higher ($P < 0.05$) for calves fed FMS while ruminal NH₃ concentrations were greater ($P = 0.03$) for calves fed CCS. Calves fed CCS had greater ($P < 0.05$) PUN concentrations. These data suggest that farm mixed diets can equal commercial calf diets for general performance and rumen development when calf starter meets all the nutrient necessary for normal growth and is properly mixed.

INTRODUCTION

Nutrition of young dairy calves is the most important factor for calf health and profitability in dairy operations. One of the goals in dairy production is to raise healthy replacement heifers with the required body weight and proper skeletal structure for breeding at an acceptable time. An appropriate feeding program must be used in order to reach this goal while keeping the cost to a reasonable level. Liquid feeding represents a major expense in young calves' diets, mainly because of the high price of milk or milk replacer and the labor required to feed the animals (Drackley, 2008). When the calf is weaned the cost decreases greatly. For this reason, an early weaning program is recommended in the dairy industry. In order to have a successful and economical weaning process the rumen must be fully developed at the time milk is no longer offered to the calf.

As early research shows solid feed is the major diet component promoting rumen development in calves (Warner et al., 1956). Also, dry feed intake is most cost effective. In contrast to adult ruminants, calves have unique dietary requirements mostly because their nonfunctional rumen and digestive system are similar to that found in monogastrics (Becker et al., 1951). It is relatively simple to meet the nutrient requirement of the calf with a milk-based diet. However, this type of diet will not provide any rumen development mostly because milk bypasses the rumen, therefore there will not be substrate available to undergo the different processes needed for rumen development (Temate et al., 1961; Church, 1988). Large amounts of milk offered to the calf will delay dry feed consumption (Weary et al., 2007) making the rate of weight gain less economically efficient. For this reason, encouraging solid feed intake at an early age will guarantee a successful weaning process.

The development of the rumen will provide to the calf's digestive system the changes necessary for the transition from monogastric to functional ruminant. This transition is fundamental for the survival of the calf after weaning. Several physiological and metabolic changes will occur during this transition. From the physiological standpoint the animal will increase its rumen capacity, develop a functional microbial population, and efficient absorptive structures will grow in the rumen wall, giving to the animal the ability to utilize the solid feed nutrients (Brownlee, 1956; Warner et al., 1956; Flatt et al., 1958; Sander et al., 1959; Tamate et al., 1961; Jami et al., 2013; McCann et al., 2014). Additionally in combination with these physiologic changes the animal will experience a number of metabolic adaptations such as an increase in ruminal fermentation end-products (VFA), noticeable presence of plasma β -hydroxybutyrate, variation in ruminal pH, increase in urea recycling rate, and decrease in blood glucose concentrations (Hibbs et al., 1956; Murdock and Wallenius, 1980; Quigley et al., 1991; Beharka et al. 1998; Hayashi et al., 2006).

Digestibility and further utilization of dry feed rely greatly on rumen fermentation which is promoted by age and rumen development. In order to increase fermentation rates, a dense and stable microbial population must be established in the rumen. Volatile fatty acids (VFA), particularly butyrate and propionate, resulting from microbial fermentation will have a great impact on ruminal epithelial development; this consequently will improve the absorption ability of the rumen wall (Sander et al., 1959; Tamate et al., 1961). Highly fermentable grain based diets are fed to calves to encourage rumen development. These types of feeds will provide the substrate and environment required by ruminal microbes to proliferate and begin ruminal fermentation influencing overall growth and rumen development.

Considerable disagreement exists when referring to dairy calf diets. This is not only at the research level but also on the farm. The feed components, form of diet, mixing methods, and the inclusion of forage, are among the topics of debate. Calf starters are high-quality concentrates containing highly digestible energy (Van Soest, 1994). For this reason, calf starter should be a great source of substrate for microbial fermentation. However, when fed incorrectly it can lead to problems such as lower pH, papillae keratinization, and bloating.

It is challenging for the farmer to decide if the best option will be to purchase a pre-made starter or find the right ingredients to mix it at the farm. Particle size influences rumen development greatly. Ground diets are not recommended because these can have a negative impact on rumen capacity and the overall ruminal environment. Therefore, the processing method to mix feedstuffs should be a critical factor to consider. The method used can alter the overall form of the diet, in particularly starter particle size. Since little information is available on this issue, the objective of this experiment was evaluate performance, blood metabolites, and rumen development of Holstein calves fed commercially prepared or farm mixed calf starter.

REVIEW OF LITERATURE

The newborn calf has a rudimentary stomach, and during the first weeks of life is considered a monogastric animal. The gastrointestinal tract of a young calf has all the structures of an adult ruminant including the reticulum, rumen, omasum, and abomasum compartments. However, these are not completely developed and are nonfunctional with the exception of the abomasum. The abomasum is the main stomach compartment at early age. In adult ruminants the rumen is the largest compartment followed by the abomasum. However, at birth the abomasum is the largest stomach compartment. The rumen increases in size at higher rate compared to the abomasum. In the first week of age the abomasum comprehend the 49% of the total bovine stomach tissue, while the reticulum-rumen is the 38%. By week 8 the rumen has increased in size being the 60% of the total stomach tissue while the abomasum is the 27% (Church, 1988).

In bovine embryos a primitive digestive tract similar to that found in other mammalians can be identified at the end of the fourth week after conception (Warner, 1958). Differentiation of the rumen is apparent in 28 mm embryos, and in 77 mm embryos the rumen formation and its caudal position is definitive (Warner, 1958). At birth the abomasum is the largest organ, mainly because it is the functional stomach compartment at that time (Becker et. al. 1951). Diet is the primary factor affecting development of the forestomach (reticulum, rumen and omasum) in newborn calves (Preston, 1963). It has been demonstrated that feeding the calf dry feed has a great impact on rumen development (Warner et al., 1956).

A series of metabolic changes in the young calf ultimately cause the development of the rumen. Diet plays an important role in this process. The neonatal calf must receive the correct diet based not only in its nutritional requirement but also in what is required for rumen

development and transition to a functional ruminant. Some of the metabolic indicators of rumen development are explained in the following pages.

Metabolic Changes during Calf Development

Volatile fatty acids

Volatile fatty acids are short chain fatty acids which are fermentation end-products of the rumen microflora. There are several VFA produced in the rumen; however, acetate, propionate and butyrate are found in high concentrations; with acetate being the highest. Concentrations of VFA in an adult ruminant vary depending on the diet. In general, with high forage diets the molar proportions of acetate: propionate: butyrate average 65:25:10, while with high concentrate diets, such as in dairy producing cow diets, the molar proportions average 50:40:10 (Church, 1988).

Volatile fatty acids are essentially important in rumen development, Sander et al. (1959) and Tamate et al. (1962) found that rumen papillae developed greatly when VFA were present or dry feed was being fed. They also found that the major VFA developing papillae are butyrate and propionate, with butyrate being the major stimulator of rumen papillary growth.

β -Hydroxybutyrate

The end-products of microbial fermentation are VFA, and absorption of these products occurs through the rumen wall to the bloodstream. Absorption rate of VFA is affected by ruminal pH and carbon chain length of each acid, therefore VFA are absorbed as follows: butyric > propionic > acetic (Curch, 1988). Butyric acid is oxidized to β -hydroxybutyrate, in the rumen epithelium, and used as an energy source.

B-Hydroxybutyrate is an indicator of rumen development, owing to the fact that VFA are produced by microbial fermentation and absorbed through the rumen epithelium. Quigley et al.

(1991) determined that concentrations of BHBA are closely related to dry feed consumption.

Murdock and Wallenius (1980) also observed that butyrate concentrations in the rumen increased with age and calf starter consumption.

Ruminal Ammonia

Ammonia is the nitrogen source required by most ruminal bacteria and is the principal end-product of microbial protein degradation (Bryant and Robinson, 1962). Rumen NH_3 is high in newborn calves but tends to decrease with age (Godfrey, 1961). With the initiation of microbial fermentation utilization of NH_3 by microbes begins. The rumen epithelium begins to absorb ammonia, and this leads to lower concentrations in the rumen (Anderson et al., 1987).

Beharka et al. (1998) found an interaction between treatment and age for ammonia concentration in a study where unground or ground starter was being fed to calves. Calves fed ground feed had higher ruminal ammonia at week 2 and lower ammonia at week 6 compared to those animals fed the unground diet. Ruminal pH

Ruminal pH

Rumen pH in mature ruminants ranges from 5.8 to 6.8 (Van Soest, 1994). According to Beharka et al. (1998), pH in neonatal calves can be influenced by age and form of diet. Hibbs et al. (1956) observed lower ruminal pH in calves fed grain diets in an experiment where the effect of roughage to grain ratio on rumen development was being studied. Beharka et al. (1998) fed a ground diet and an unground diets to neonatal calves to determine the effect on microbial and rumen development. Lower pH was recorded in calves fed the ground diet.

Early microbial activity in calves is favored by grain based diet offered at early age. This will allow the production of fermentation end-products, such as VFA. Acetate, propionate and

butyrate will lower ruminal pH, affecting rumen environment and therefore altering microbial population.

Plasma Urea Nitrogen

Protein or non-protein nitrogen (NPN) is fermented in the rumen and converted to ammonia, which is either utilized by microbes or absorbed through the rumen wall into the bloodstream and then converted to urea by the liver. After ammonia is converted to urea this can be excreted in urine or recycled to the rumen, via saliva or diffusion through the rumen wall, where it is converted to ammonia and can be utilized by rumen microbes. Hayashi et al. (2006) demonstrated that urea recycling rate and loss increases with age and reticulorumen development in calves. Recycled urea can serve as an indicator of rumen development. An increase in recycled urea and a decrease of ammonia should be observed as rumen develops (Davis and Drackley, 1998). After weaning nitrogen concentration should keep increasing as microbial activity increases and anatomical rumen development takes place.

Plasma urea nitrogen is an indicator of the amount of protein being degraded within the rumen. When protein is deficient in the diet, ruminal ammonia concentrations are relatively low and the proportion of urea recycled back to the rumen increases. Therefore concentrations of PUN should decrease as amount and quality of protein decreases in the diet being fed. Increased production of urea in the liver is due to an increase in dietary nitrogen intake, and increased levels of PUN will raise urinary excretion rate (Van Soest, 1994).

Glucose metabolism

At birth and in the first weeks after birth glucose levels in calves are similar to the levels found in non-ruminant animals. Glucose is the primary source of energy in neonatal calves, however this condition changes with age and the shift from monogastric-like digestion to

ruminant digestion. Plasma glucose concentrations decrease with age reaching normal levels of an adult ruminant at 3 months of age (Quigley et al., 1991).

Decrease in blood glucose may be associated with rumen development, however Reid (1953), in a study with lambs, concluded that the decline in glucose concentrations in lambs and calves are due to other factors than rumen development. Decreased blood glucose concentrations with advanced age is the result of reduced milk intake as well as loss of glucose from erythrocytes (Reid, 1953).

The majority of non-structural carbohydrates are fermented in the rumen, with little escape to the small intestine for digestion and absorption. This results in small amounts of glucose coming from the gastrointestinal tract of the ruminant causing lower blood glucose concentrations. Glucose required at tissue level is supplied by gluconeogenesis in adult ruminant animals. A diverse number of gluconeogenic pathways can synthesize glucose from propionate, amino acids, lactate, and glycerol.

As mentioned before, neonatal calves function as monogastric animals, however after the first weeks of age several changes in glucose metabolism take place. These changes include a decrease in the glucose pool, decrease in glucose utilization, decreased insulin response, and lower rate of glucose absorption from the small intestine. Also, with rumen development different enzymatic changes occur due to the decrease in glucose oxidation by glycolytic pathways, and the increase in gluconeogenesis activity in the liver. Key enzymes in glycolysis and the pentose phosphate pathway are higher in newborn calves than in mature ruminants. Also, important enzymes in gluconeogenesis and the incorporation of pyruvate and propionate into glycogen increase with age (Church, 1988).

Rumen Development and Normal Calf Growth

For a successful weaning process and calf performance after weaning, adequate development of the rumen is required. Different factors such as rudimentary reticulo-rumen, existence of the esophageal groove, the functional abomasum, and the enzymatic processes taking place in the digestive system oblige the young calf to function as monogastric. Several anatomical and enzymatic changes have to take place in order to make the transition from a monogastric to a ruminant animal. An efficient dietary program is needed to achieve this transition without affecting animal performance. Knowledge of the process of rumen development is needed to understand the different changes occurring in the young calf as it grows.

Some important factors must be considered when referring to rumen development. Microorganisms growing in the rumen of calves have a great impact on rumen development and can be influenced by diet and management (McCann et al., 2014). In a comparison made by Jami et al. (2013) between 1-day old and 3 day old calves along with matures ruminants, only few bacteria genera were shared between the newborn calf and the rest of the animals, suggesting that the ruminal bacteria population changes rapidly with age. With aging and development of the rumen a decrease in aerobic and facultative bacteria occurs while anaerobic bacteria population increases (Jami et al., 2013). This change in bacteria genera is enhanced by dry feed intake, because substrate for fermentation is available.

Water is considered one of the most important nutrients and water availability is no less important for young calves. A liquid environment is needed for ruminal fermentation and microorganism survival in the rumen. It has to be considered that milk is not a source of water for the calf, since it bypasses the reticulo-rumen compartments through the esophageal groove

(Church, 1988). Water entering the rumen comes from water being offered to the animal. Therefore for optimal rumen development water must be supplied at early age. Some advantages of offering water are promotion of dry feed intake, increased body weight gain, and reduced scours.

At birth little rumen musculature activity is noticeable. The consumption of dry feed is probably the most influential factor for development of rumen musculature and capacity (Brownlee, 1956; Warner et al., 1956). Warner et al. (1956) found that calves offered hay or grain had a greater increase in reticulo-rumen capacity compared to only milk fed calves, with hay being the major promoter of increased rumen capacity. Poor quality dry feed or inert physical objects can increase rumen capacity and musculature (Flatt et al., 1958; Temate et al., 1962). However, feeding the animal hay or inert objects instead of highly fermentable carbohydrates can lead to poor papillae development.

Ruminal motility is an important factor to consider in normal rumen function. Normal ruminal contractions are important, so feed can enter the rumen and the fermented material can leave it. Asai (1973) studied rumen motility in calves fed different diets. This study shows that when calves are fed milk only, there are no or little contractions in the reticulo-rumen. In calves fed grain diets or when an inert object (sponge) + VFA (acetate, propionate and butyrate) were inserted in the rumen, rumen motility was established by week three after birth (Asai, 1973). Therefore, if a coarse grain diet is fed to the calf from the first week of age, acceptable rumen motility will be established by week two or three, initiating the overall rumen development that will take place in the next weeks.

Another but not less important criterion to consider in rumen development is the ability of the rumen epithelium to absorb fermentation end-products. Volatile fatty acids diffuse through

the rumen wall into the blood stream. The rumen absorptive structures are known as papillae, these are “finger-like” protuberances that greatly increase the rumen surface area enhancing fermentation end-products absorption. Again, these rumen structures develop with age and in great extent due to dry feed diets being fed to the calf. The presence of fermentation end-products, VFA, are the stimulus for fully development of rumen papillae (Warner et al., 1956; Sander et al., 1959; Tamate et al., 1962).

Rumen papillae are present in neonatal calves. Papillae length in calves from 1 to 3 days of age is around 1 - 2.6 mm (Tamate et al., 1962). Tamate et al. (1962) studied the effect of various diets on the anatomical development of the calf’s stomach. This study used three groups of calves, the first group was fed milk only, the second was fed milk + hay + grain, and calves in group three received various substances or inert objects in addition to milk (intraruminal substances included sponges + acetate and propionate, sponges + butyrate, NaCl, KCl, NaHCO₃, KHCO₃, whole milk and carbonated water). With regard to papillae growth, this research showed that animals fed grain/hay had significantly greater papillary growth with age than those fed other type of diets.

Papillae in calves offered high concentrate diets are longer, wider, and less dense than those from only milk fed animals (Harrison et al., 1960; Tamate et al., 1962; Stobo et al., 1966; Zitnan et al., 1998). Several studies have demonstrated that the primary stimuli of rumen papillae are VFA, especially butyric acid and propionic acid; with butyrate being the VFA which has the greater effect in rumen epithelium development (Sander et al., 1959; Tamate et al., 1962).

Feedstuffs and Rumen Development

The importance and impact of diet in rumen development has been previously discussed. The rumen will be functional and similar to that found in adult animals sooner if a proper diet is

fed to the young calf. Few microbial genera are present in the rumen at birth (Jami et al., 2013); however the influence of certain feeds offered to the young ruminant these microorganisms will grow and new anaerobic bacteria will establish in the rumen. These bacteria will produce VFA which will impact rumen development. Liquid and solid feeds have great impact on rumen development.

The neonatal calf depends on a liquid diet consisting of milk or milk replacer to supply nutrients needed for growth. Milk bypasses the rumen through the esophageal groove and passes to the abomasum. Milk replacer components such as lactose, fat, and protein are initially digested in the abomasum and digestion is completed in the intestine by different enzymes (Drackley, 2008). Milk replacer digestion in the gastrointestinal tract provides the calf enough fatty acids, glucose and amino acids for maintenance, body function, and growth (Khan et al., 2011). Therefore, feeding poor quality and/or low amounts of milk replacer can lead to decreased growth rate and reduced metabolic development.

It is generally recommended to offer dry feed to young calves as soon as possible. Calf starter or complete calf starters are adjusted to fulfill the qualities needed for metabolism and rumen development in the young calf. Calves receiving solid feeds undergo different physical and metabolic changes at earlier age than if they were only fed a liquid diet. Solid feeds will provide the substrate and environment necessary for microbial proliferation. When the fermentation process starts, VFA are released and play an important role in rumen development (Warner et al., 1956; Sanders et al., 1959; Tamate et al., 1961; Stobo et al., 1966). Solid feeds can consist of highly fermentable concentrates and/or forages. Both types of feeds influence rumen development; however, early research demonstrated that high grain diets have the greatest impact in rumen development (Warner et al., 1956; Stobo et al., 1966).

Physical Form of the Diet

Feed particle size has an important impact on rumen development. Ruminant environment, VFA, and papillae shape and function are influenced by the form of the diet being fed to the calf. The environmental changes in the rumen caused by different feed particle size lead to changes in rumen microbes, which consequently will have an effect on VFA concentrations in the rumen. Animals fed small particle size feeds will have a decrease in the acetate to propionate ratio (Kerley et al., 1985). However, some researchers found no differences in VFA molar concentrations and acetate to propionate ratio when feeding ground or unground diet (Beharka et al., 1998). Scientists who observed differences in VFA concentration also observed differences in pH and microbial population. For this reason the changes in VFA concentrations is attributed mostly to environmental alteration of the rumen when feeding small particle size.

Ruminal pH is affected by particle size, as calves fed ground feed have lower ruminal pH compared to calves fed coarsely unground feed (Beharka et al., 1998). This same tendency is observed in adult animals when cows are fed finely ground total mixed rations. Pelleted feeds, when roughage is incorporated as form of pellet, will induce lower ruminal pH (Bull et al., 1965). Calves fed ground diet have increased number of amylolytic bacteria and fewer cellulolytic bacteria than calves fed an unground diet, likely due to the lower pH caused by the ground diet (Beharka et al., 1998)

Digestive flow in the gastrointestinal tract is also affected by particle size. As particle size decreases rate of passage increases (Kerley et al., 1985). Decreased particle size will reduce retention time in the rumen and will result in decreased nutrient utilization by microbes and the animal. The reduction in digestibility due to decreased time for ruminal fermentation will lead to an increased feed intake (Hodgson, 1971). Beharka et al. (1998) did not observe differences in

liquid flow rates until week 10 when calves fed the ground diet had faster rate of passage than the calves fed an unground diet. These researchers attributed lack of differences in the first weeks of age to low ruminal fill.

Forage Inclusion in the Diet as a Source of Fiber

There is much controversy about the addition of forage to the diet of young dairy calves. Fermentation end-products are needed for papillae growth and overall rumen development, therefore it is recommended to offer high grain diets with no inclusion of forage during the first weeks of age to encourage VFA production, particularly butyrate and propionate, to promote rapid rumen development and the transition from monogastric to ruminant. The inclusion of forage should begin around week 8, when the rumen microbial population is somewhat established and the rumen epithelia is capable of absorbing the fermentation end-products. At this time the animal will be capable of digesting fiber and utilizing the nutrients from forage that was not possible at early age.

Several researchers have found that addition of forage to the calf diets will negatively affect dry matter intake, feed efficiency, average daily gain, and rumen development (Brownlee, 1954; Hibbs et al. 1956; Murdock and Wallenius, 1980; Hill et al., 2008). However, several other scientists have reported positive effects when forage is added to the diet of young calves as a source of fiber or just as an additional component of the diet. These authors observed higher dry matter intake, greater average daily gain, increased final body weight, and greater feed efficiency (Coverdale et al., 2004; Khan et al., 2011; Castells et al., 2012; Beiranvand et al., 2014). In addition to these findings Suarez-Mena et al. (2016) reported no differences in ruminal fermentation and rumen development among treatments when calves received different straw particle size in starter diets. Suarez et al. (2007) found that the addition of roughage to the diet

did not affect growth but positively affected the microscopic appearance of the rumen epithelium.

Quality of the fiber added to the diet is essential for palatability and digestibility. The use of different types of roughage including grass, hay, legume hay or straw are common fiber sources utilized in young dairy calf diets. Murdock and Wallenius (1979) studied the addition of different types of fiber in the diet. Dietary treatments were complete calf starters containing alfalfa hay, cottonseed hull, or alfalfa-beet pulp. Calves fed the cottonseed hull ration had increased feed intake, greater body weight gain, and higher feed acceptability. Also, they observed an increased incidence of bloating in calves fed the diet containing alfalfa hay.

Castells et al. (2012) conducted a series of three experiments to evaluate the effect of different forage sources on performance and feeding behavior. All the experiments had a control treatment which consisted of feeding the animal with calf starter only. The rest of the treatments contained calf starter plus one of the following components: chopped alfalfa, ryegrass hay, chopped oat hay, chopped barley straw, corn silage, or triticale silage. Animals fed chopped oat hay, triticale silage, and chopped barley straw treatments had greater calf starter intake and average daily gain compared to the control. While the animals on the chopped alfalfa hay and chopped oat hay diets had greater forage intake than those fed the other treatment diets. However, chopped alfalfa hay treatment was not different in starter intake when compared to the control. The inclusion of forage in these experiments also had a positive effect in average daily gain, with oat hay, triticale silage and barley straw treatments having the greatest effect on average daily gain.

MATERIALS AND METHODS

This study was conducted at the LSU Dairy Science Research and Teaching Farm in Baton Rouge, during fall 2014 and winter 2015. All procedures for this experiment were approved by the Louisiana State University Agricultural Center Institutional Animal Care and Use Committee.

Animal and Dietary Treatments

Twenty-four Holstein male calves ($BW = 38.78 \pm 7.26$ Kg) were used in a 12 week study to determine the effects of feeding starter from a commercial mill or farm mixed with similar nutritional characteristics on animal performance, blood metabolites, and rumen health. Calves were separated from the dam at birth, navel was dipped in a 2% iodine solution to prevent umbilical infections, and body weight was recorded. Calves were vaccinated for Rotavirus and Coronavirus (Calf Guard, Pfizer Animal Health, Lenexa, KS) prior to receiving colostrum. Calves received 4 to 6 liters of colostrum in the first 24 hours after birth which was followed by transition milk on day 2.

On day 3 calves were transported from the LSU AgCenter Southeast Research Station to the experiment location. When calves arrived to the farm they were given 4 liters of electrolytes (LAND O LAKES® Electrolytes, Arden Hills, MN) and housed in individual calf hutches measuring 2.5m^2 with a 2.8m^2 wire enclosure on rock bedding. Calves were randomly assigned to one of two treatments in a completely randomize design: 1) commercial calf starter (CCS), and 2) farm mixed calf starter (FMS) with equal nutrient content. Calf starter and milk replacer chemical analysis, and farm mixed starter ingredients are presented in Table 1.

Beginning day 4 calves were offered milk replacer (MR) (Purina® Herd Maker® Protein Blend, Shoreview, MN) and were trained to drink from buckets. Milk replacer was mixed at 15%

solid. Calves were fed 2 liters twice daily during weeks 1 through 6 to provide 2.28 kg of dry matter per day. Morning and afternoon feedings were scheduled at 7 a.m. and 4 p.m. Beginning day 43 milk replacer feeding was reduced to 1 time per day to all treatment groups to decrease milk replacer intake by 50%. On day 49 calves were weaned and kept in hutches until day 84.

Table 1. Chemical analysis for treatment diets and feedstuff composition for farm mixed starter.

Chemical Analysis	¹ Treatment	
	² FMS	³ CCS
Dry Matter, (%)	85.76	92.55
TDN, (%DM)	79.33	77.78
Crude Protein, (%DM)	23.07	23.27
Neutral Detergent Fiber, (%DM)	19.48	21.08
Acid Detergent Fiber, (%DM)	8.02	8.71
Ash, (%DM)	6.28	6.84
Ingredients, % As Fed		
Cracked Corn	49.00	-
Soy Bean Meal	30.43	-
Chopped Alfalfa Hay	7.00	-
Rolled Oats	6.00	-
Molasses	5.00	-
Vitamins/Minerals mix	2.57	-

¹FMS = farm mixed calf starter, CCS = commercial calf starter.

²Farm mixed calf starter contained calcium 1.22%, phosphorus 0.41%, magnesium 0.23%, potassium 1.39%, sulfur 0.25%, sodium 0.29%.

³Commercial calf starter contained decoquinatate 33.06 mg/kg, calcium (min) 0.70%, calcium (max) 1.20%, phosphorus (min) 0.45%, selenium (min) 0.30 ppm, Zinc (min) 2,750 ppm, vitamin A (min) 6,500 IU/lb.

On day 4 calves were offered calf starter (commercial calf starter or farm mixed starter, depending on treatment assignment) *ad libitum* throughout day 84. Beginning calf starter feeding, calves received 113 g of starter AM and PM. Feed refusal was recorded before each new

feeding. Starter was increased in 113 g increments when calves refused less than 30 g of feed per feeding. Water was offered free choice daily from the beginning of the trial.

Sample Collection

Fecal scores were recorded twice daily using a scoring scale based on fecal fluidity ranged from 1-4 (1=firm, 2=soft, 3=runny, and 4=watery) adapted from Larson et al., (1977). Intakes of milk replacer, and calf starter refusal were recorded twice daily. Body weight, hip height, wither height, hip width, and body length were weekly measured from week 1 through 12.

Blood was collected 24 hours after birth via jugular venipuncture into 10 mL evacuated tubes with no anticoagulant. This sample was centrifuged for 15 minutes, serum was collected, protected from UV light, and frozen at -20°C until analysis to estimate IgG concentrations. All calves with the exception of two passed the IgG test. Blood was collected via jugular venipuncture tubes at weeks 2, 4, 6, 8, 10, 12. One 10mL tube with no anticoagulant, one 10ml tube with sodium heparin, and one 5mL tube containing potassium oxalate and sodium fluoride were used. All samples were centrifuged for 15 minutes at 600 x g, plasma and serum were collected, protected from UV light, and stored at -20°C until analysis. These samples were used later for plasma urea nitrogen (PUN), plasma glucose, and β -hydroxybutyrate (BHBA) analysis.

Ruminal fluid was collected by stomach tube on weeks 2, 4, 6, 8, 10, 12 between 10:00 to 11:00 am for volatile fatty acids (VFA), ammonia, and pH analysis. A SHUCO-VAC Vacuum pump by Allied Healthcare Products, INC. (St. Louis, MO) and a 1/4" non-toxic esophageal PVC tube were used to collect ruminal fluid from calves. pH was recorded using a Thermo Scientific Orion 2 Star pH benchtop by Fisher Scientific (Waltham, MA) meter, 1 mL of phosphoric acid (20% W/V) was added prior to freezing (-20°C).

Laboratory Procedures

Immunoglobulin G (IgG)

Serum IgG concentrations were measured using a immunoturbidimetric method (MBC QTII® Bovine Serum IgG; Midland BioProducts Corporation®, Boone IA) (Appendix A)

Plasma Urea Nitrogen

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

β-Hydroxybutyrate

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

Plasma Glucose Concentration

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

Total Volatile Fatty Acids

A 4 mL Sample of ruminal fluid was mixed with 1 mL of 25% (wt/vol) metaphosphoric acid containing 2 g/L of 2-ethylbutyric acid, which was used as internal standard for VFA quantification. Concentration 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 E).

Ammonia (NH₃)

Ruminal fluid samples were thawed at room temperature and clarified by centrifuging at 30,000 x g for 20 minutes. The clarified supernatant were then decanted and analyzed for NH₃ using a modified phenol-hypochlorite method adapted from Broderick and Kang (1980) (Appendix F).

Statistical Analysis

The mixed procedure of SAS® (Version 9.4, SAS Inst. Inc., Cary, NC) was used to analyze the data as a repeated measures analysis of variance. Response variables included BW, ADG, hip height, wither height, hip width, body length, feed intake, PUN, Glucose, BHBA, NH₃, and VFA. Response variables measured daily were reduced to weekly prior to analysis. Fixed effects in the model included treatment, week, and treatment by week interaction. The random effect was calf within treatment. When overall differences were detected, post hoc analyses were conducted with pairwise t test comparisons of least-squares means. Weeks 1-7 and weeks 8-12 were considered as preweaning and postweaning periods. Values reported are least squares means. Significance was declared at $P < 0.05$ and tendencies were declared at $P < 0.10$.

RESULTS AND DISCUSSION

Feed Intake Data

Least squares means for pre and postweaning starter intake are presented in Table 2 and Figure 1. Starter intake and dry matter intake (DMI) increased with age of the calves ($P < 0.001$).

Table 2. Least squares means for body weight, average daily gain (ADG) average daily starter intake, dry matter intake (DMI), feed efficiency, and fecal scores for calves fed farm mixed starter (FMS) or conventional commercial starter (CCS) through d 84 of age.

Item	Treatment			P-Value
	FMS	CCS	SEM ¹	
Body weight, kg				
Preweaning, d 0-49	51.54	49.28	1.23	0.206
^A Postweaning, d 50-84	94.83	86.46	2.69	0.038
^B Entire Trial d 0-84	68.19	63.58	2.04	< 0.001
ADG, kg/d				
Preweaning, d 0-49	0.60	0.53	0.03	0.159
^A Postweaning, d 50-84	1.22	1.05	0.05	0.025
^A Entire Trial d 0-84	0.86	0.75	0.03	0.022
Starter Intake, g/d				
Preweaning, d 0-49	466.66	419.53	50.975	0.511
^A Postweaning, d 50-84	3187.34	2796.32	131.52	0.0428
^B Entire Trial d 0-84	1600.28	1409.86	117.33	0.0046
TDMI ² , g/d				
Preweaning, d 0-49	19.45	18.06	0.81	0.239
Postweaning, d 50-84	3.54	3.38	0.32	0.734
Entire Trial d 0-84	10.17	9.50	0.48	0.330
Feed Efficiency ³				
Preweaning, d 0-49	1.69	1.73	0.12	0.830
Postweaning, d 50-84	0.48	0.43	0.02	0.136
Entire Trial d 0-84	1.19	1.19	0.07	0.979
Fecal Score ⁴				
Entire Trial d 0-84	2.49	2.38	0.06	0.160

¹SEM = Standard error of mean.

²TDMI = total DMI (milk replacer and starter feed).

³Kilograms of BW gain/kg of TDMI.

⁴Fecal Score = 1 = normal, 2 = soft, 3 = runny, and 4 = watery.

^AMain effect of treatment ($P < 0.05$).

^BTreatment by week interaction ($P < 0.05$).

There was a tendency for calves fed FMS to have greater starter intake over the entire study ($P = 0.080$) than those fed CCS. Starter intake was greater ($P < 0.05$) in the postweaning period for calves fed FMS. However, no difference was observed in the preweaning period. There was a treatment by week interaction ($P < 0.001$), with calves fed FMS consumed more starter during weeks 11 and 12. These results can be compared with Coverdale et al. (2004), who observed that animals fed coarse calf starter with 7.5% Bromegrass and 15% grass hay had greater starter intake than calves offered a commercial coarse starter and ground starter. In addition, some research suggests that the addition of alfalfa hay to the diet will increase DMI (Beiranvand et al., 2014). In contrast, Castells et al. (2012) found no treatment effect on feed intake when conventional calf starter versus calf starter + alfalfa hay diets were compared.

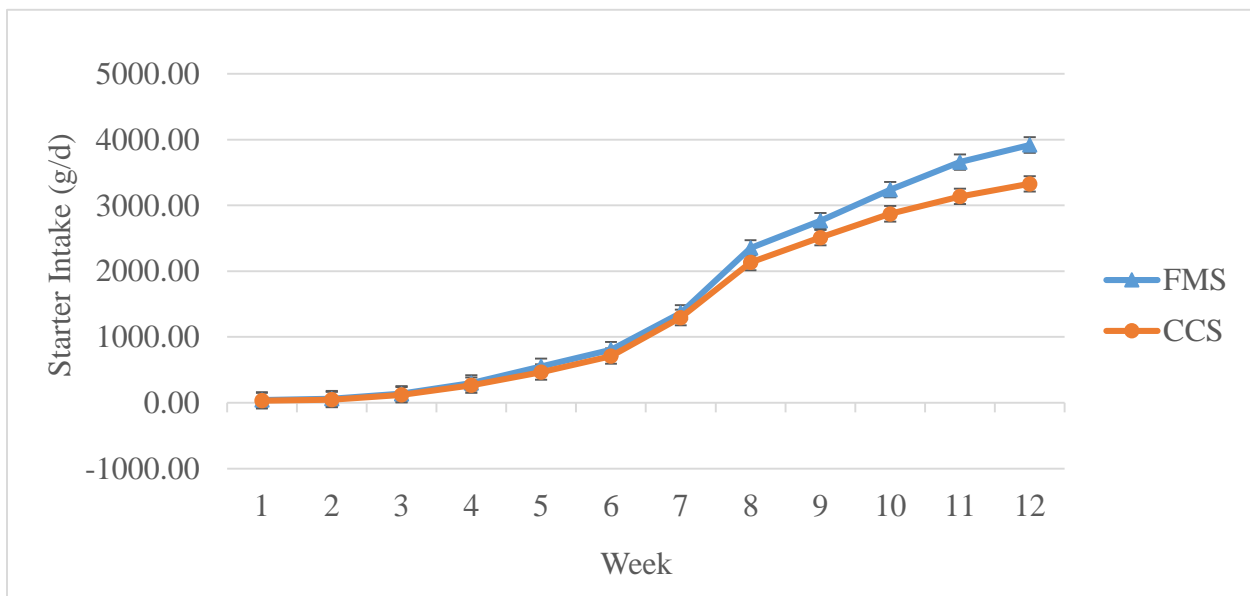


Figure 1. Least squares means of average daily starter intake for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Interaction of treatment by week ($P < 0.001$). SEM 843.16.

Performance Data

Least squares means for body weight (BW) are presented in Table 2 and Figure 2. No treatment effect on body weight was observed in the preweaning period ($P < 0.05$). However,

there was a treatment effect ($P < 0.05$) in the postweaning weeks; calves fed the FMS had increased body weight after weaning. Additionally, calves fed the FMS diet tended to have a greater body weight over the entire trial ($P = 0.07$). There was treatment by week interaction ($P < 0.001$), as well as a main effect of week on body weight ($P < 0.001$). Coverdale et al. (2004) observed similar outcomes when hay was added to the diet, and intake limited until weaning. However in a second experiment where the starter was offered *ad libitum*, no treatment effect on overall BW was observed, but animals under the starter + hay diet had a tendency for higher BW in the postweaning period (Coverdale et al., 2004).

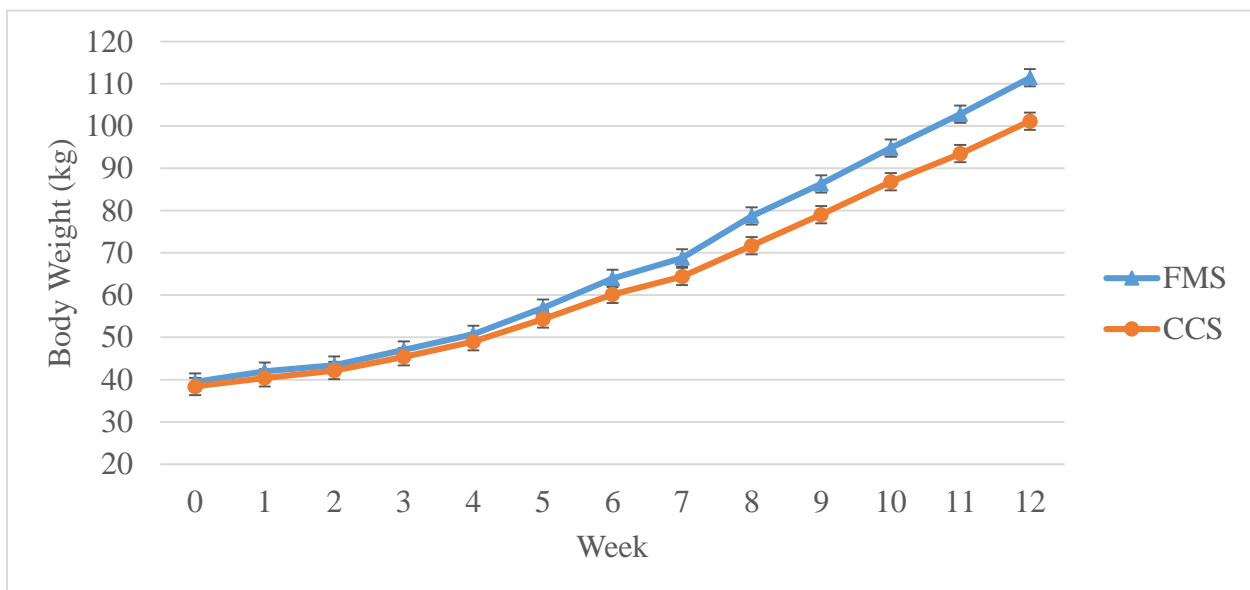


Figure 2. Least squares means of body weight for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Interaction of treatment by week ($P < 0.001$). SEM 2.04.

Least squares means for average daily gain (ADG) are presented in Table 2 and Figure 3. Similar to the pattern observed in BW and starter intake, treatment effects were observed ($P < 0.05$) on overall ADG and post-weaning ADG. However, there was no treatment effect ($P > 0.05$) on preweaning ADG. Animals fed FMS had higher ADG over the entire study but no differences were found during the pre-weaning period. Comparable to these results Castell et al (2012) observed that calves fed calf starter with the addition of alfalfa hay had similar

preweaning ADG than calves fed only calf starter; but, greater ADG over the 10 week trial. Contrary to this, Hill et al. (2008) reported that ADG was repressed on calves from 30 to 60d old when fiber in the form of 5 to 10% cotton seed hulls or 2.5 to 5% chopped hay was added to the calf starter.

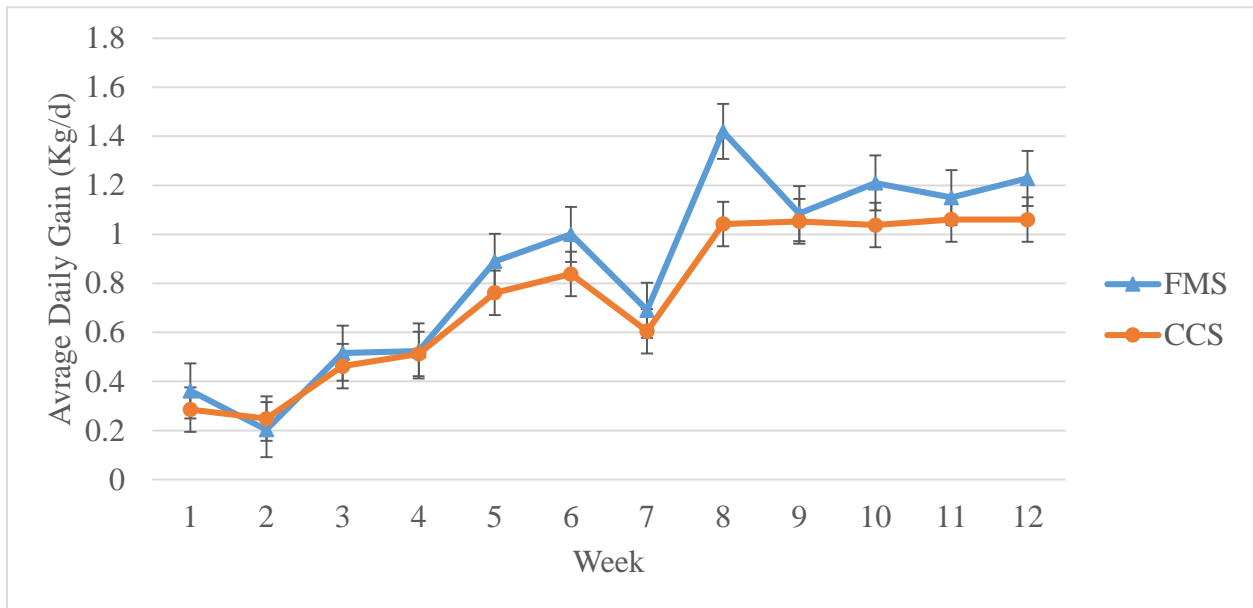


Figure 3. Least squares means of ADG for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of treatment ($P < 0.05$). SEM 0.031. Main effect of week ($P < 0.001$). SEM 0.062.

Least squares means for feed efficiency are presented in Table 2. Despite the tendency for increased feed intake, as well as the tendency for higher body weight in calves fed FMS, no differences ($P < 0.05$) on feed efficiency were observed. This agrees with Castells et al. (2012) who reported no differences in feed efficiency when distinct forages were added to the diet. Coverdale et al. (2004) had similar results when comparing commercial calf starter versus coarse + hay starter diets. Least squares means for weekly feed efficiency are presented in Figure 4, which shows the main effect ($P < 0.001$) of week. Feed efficiency decreased with age.

Least squares means for fecal scores are presented in Table 2. There was no treatment effect ($P = 0.16$) on fecal score throughout the trial. Fecal scores were within the range of healthy

calves throughout the entire trial. Electrolytes were fed only at the beginning of the trial when calves arrived at the farm, thus further electrolyte administration was not needed. Coverdale et al. (2004) observed no treatment effects on fecal scores when evaluating coarse starter with 7.5% of bromegrass hay, coarse starter with 15% grass hay, commercial coarse starter, and ground starter.

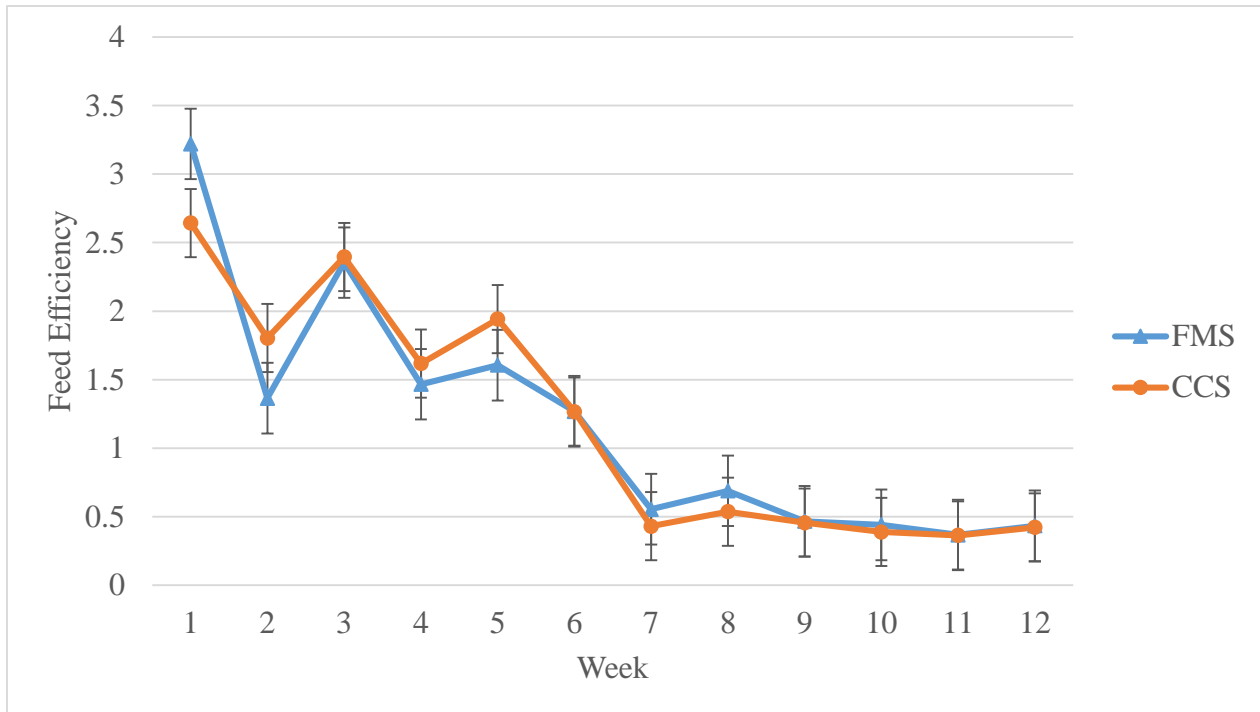


Figure 4. Least squares means of feed efficiency for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 0.25.

Least squares means for structural growth are presented in Table 3 and Figures 5, 6, and 7. All structural growth variables increased with age ($P < 0.001$). There were no treatment effects ($P > 0.05$) on body length, wither height, hip height, and hip width. These findings are consistent with those described by Beiranvand et al. (2014), who observed no effect on skeletal growth when 5 to 10% alfalfa hay was added to the calf starter. In contrast, Hill et al. (2008) reported a linear decline in hip width as chopped hay increased in the diet

Table 3. Least squares means for wither height, hip height, hip width for calves fed farm mixed starter (FMS), and conventional commercial starter (CCS) through d 84 of age.

Item	Treatment			P-Value
	FMS	CCS	SEM ¹	
Wither Height, cm				
Prewaning, d 0-49	80.83	80.24	0.74	0.598
Postweaning, d 50-84	90.79	90.11	0.79	0.564
Entire Trial d 0-84	84.97	84.35	0.79	0.581
Hip Height, cm				
Prewaning, d 0-49	85.38	84.78	0.87	0.624
Postweaning, d 50-84	95.54	94.40	0.92	0.391
Entire Trial d 0-84	89.60	88.79		0.511
Hip Width, cm				
Prewaning, d 0-49	18.33	18.22	0.25	0.776
Postweaning, d 50-84	23.16	22.56	0.29	0.162
Entire Trial d 0-84	20.34	20.03	0.26	0.407
Body Length, cm				
Prewaning, d 0-49	67.48	67.32	0.59	0.850
Postweaning, d 50-84	79.85	78.88	0.74	0.368
Entire Trial d 0-84	72.63	72.14	0.60	0.569

¹SEM = Standard error of mean.

^AMain effect of treatment (P < 0.05).

^BTreatment by week interaction (P < 0.05).

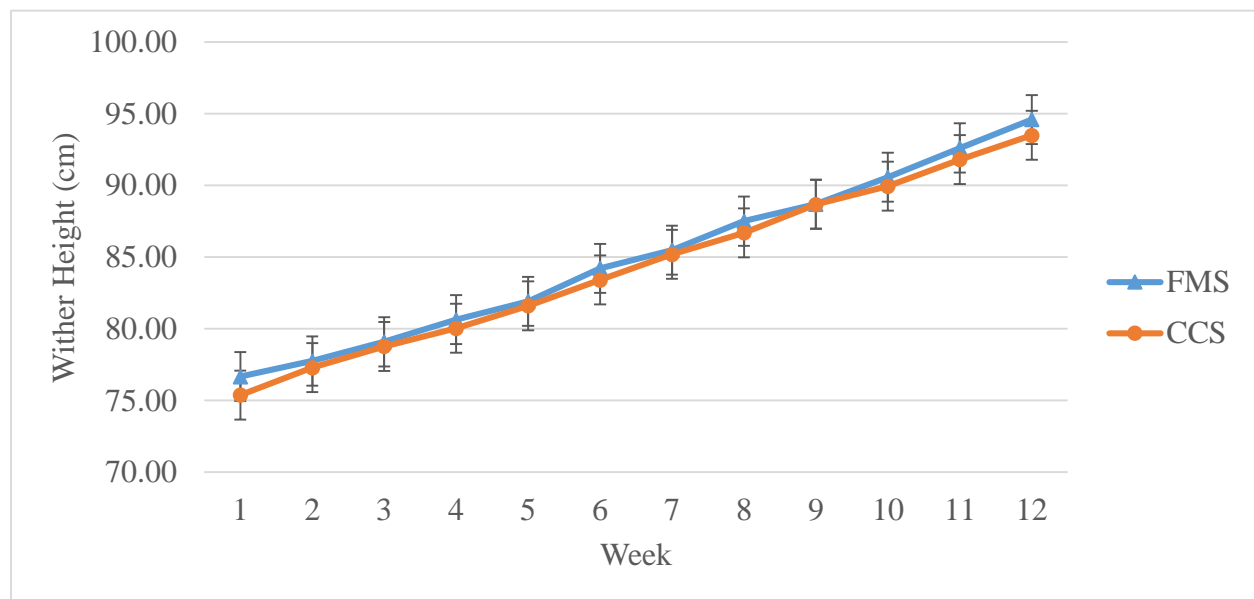


Figure 5. Least squares means of wither height for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week (P < 0.001). SEM 0.843.

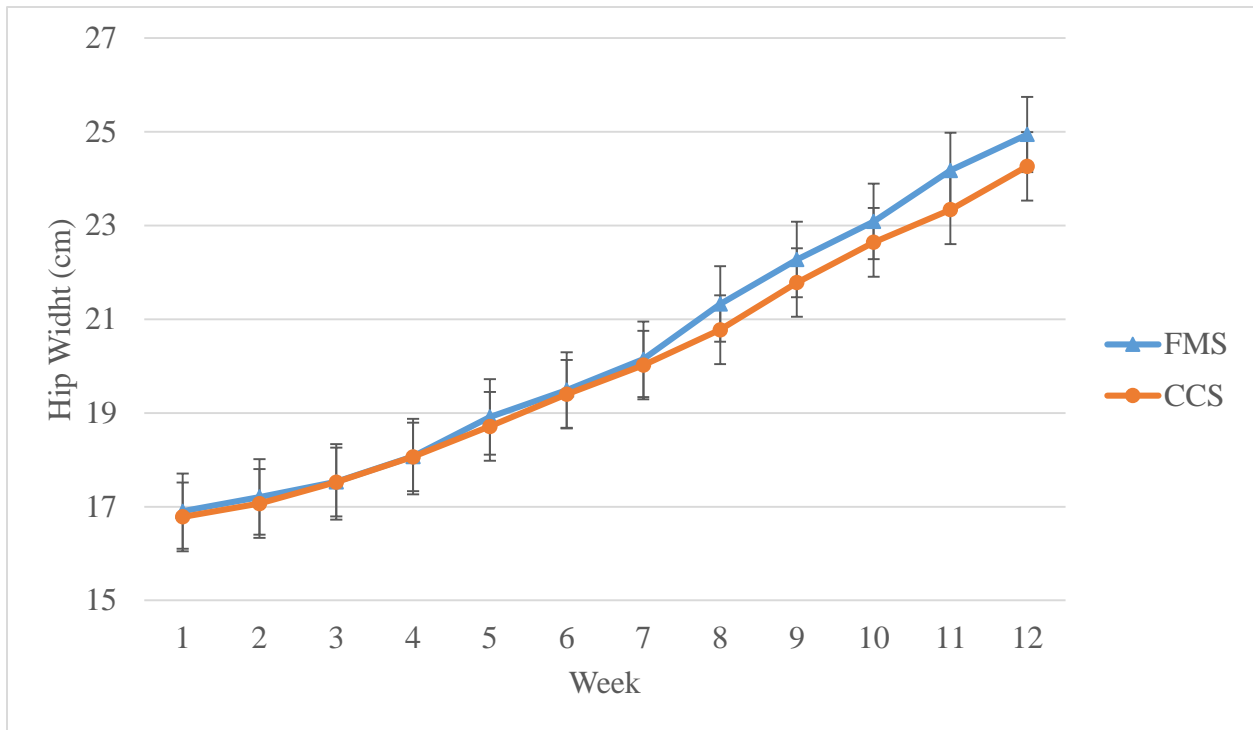


Figure 6. Least squares means of hip height for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 0.84.

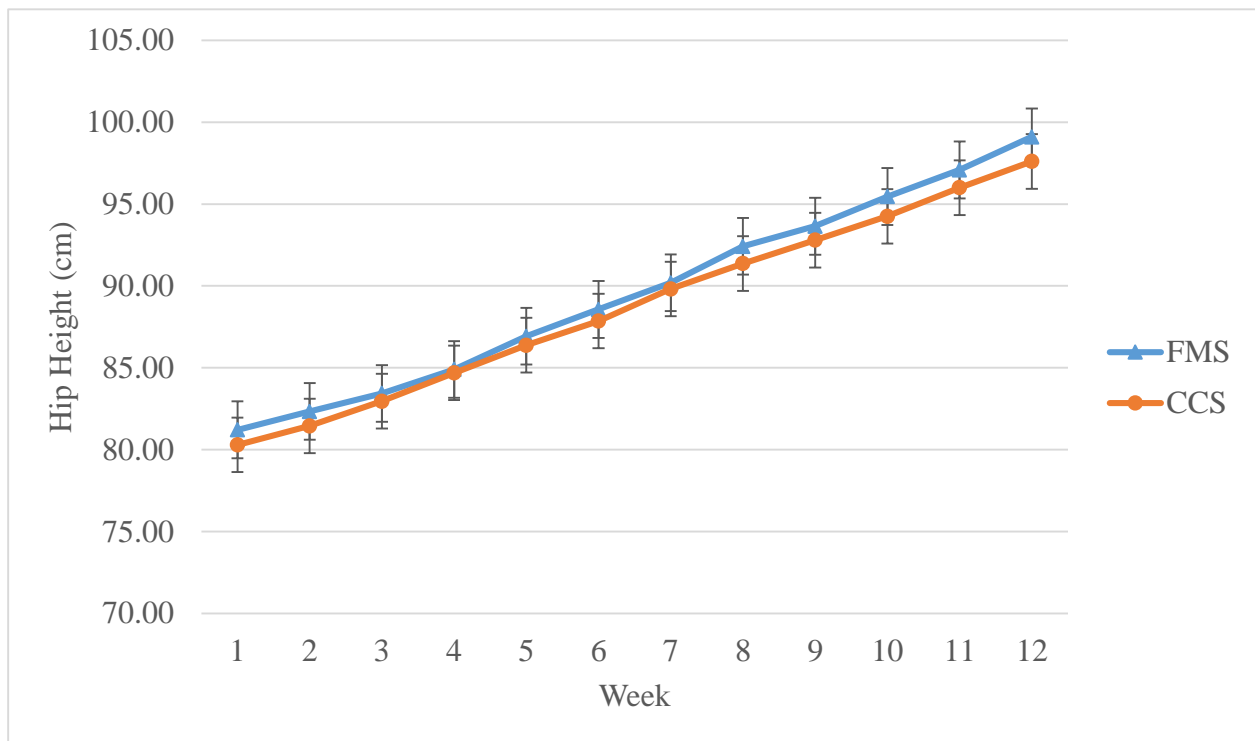


Figure 7. Least squares means of hip width for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 0.20.

Rumen Development Data

Least squares means of rumen pH are presented in Table 4 and Figure 8. Rumen pH decreased with age ($P < 0.001$), with the exception of week 12 where rumen pH increased in calves fed FMS. This can be compared to the findings of Beharka et al. (1998) who also observed a decrease in pH during the first weeks of age and an increase in pH by week 10 when ground or unground diets containing 25% alfalfa hay and 75% grain mix were fed to calves. The lower pH throughout the study may have been influenced by the large content of highly fermentable carbohydrates in the diets.

Table 4. Least squares means for rumen pH, acetate (mmol/L), propionate (mmol/L), butyrate (mmol/L), total VFA (mmol/L), molar % acetate, molar % propionate, molar % butyrate, NH₃ (mg/dL), glucose, β -hydroxybutyrate (BHBA), plasma urea nitrogen (PUN) for calves fed farm mixed starter (FMS), and commercial calf starter (CCS) through d 84 of age.

Item	Treatment			
	FMS	CCS	¹ SEM	P-Value
^A Rumen pH	5.90	5.68	0.07	0.031
Acetate, mmol/L	43.03	38.71	4.23	0.478
Propionate, mmol/L	37.24	31.92	3.49	0.293
Butyrate, mmol/L	8.69	9.85	0.84	0.337
² Total VFA, mmol/L	92.38	83.78	8.22	0.467
Acetate, molar %	49.61	49.17	0.99	0.751
Propionate, molar %	37.13	35.70	1.03	0.337
Butyrate, molar %	9.24	9.88	0.82	0.584
^A NH ₃ , mg/dL	8.80	14.82	1.30	0.003
Glucose mg/dL	102.04	103.15	2.76	0.779
BHBA Mmol/L	0.14	0.15	0.01	0.189
^B PUN mg/dL	13.64	14.30	1.22	0.042

¹SEM = Standard error of mean.

²Total volatile fatty acids

^AMain effect of treatment ($P < 0.05$).

^BTreatment by week interaction ($P < 0.05$).

A treatment effect ($P = 0.031$) was observed over the entire trial. Calves fed FMS had higher ruminal pH than calves fed CCS. Rumen pH in calves fed FMS may have been affected by the

inclusion of forage in the diet. Other authors have had similar results in previous studies when forage was added to the diet (Khan et al., 2011; Beiranvand et al., 2014).

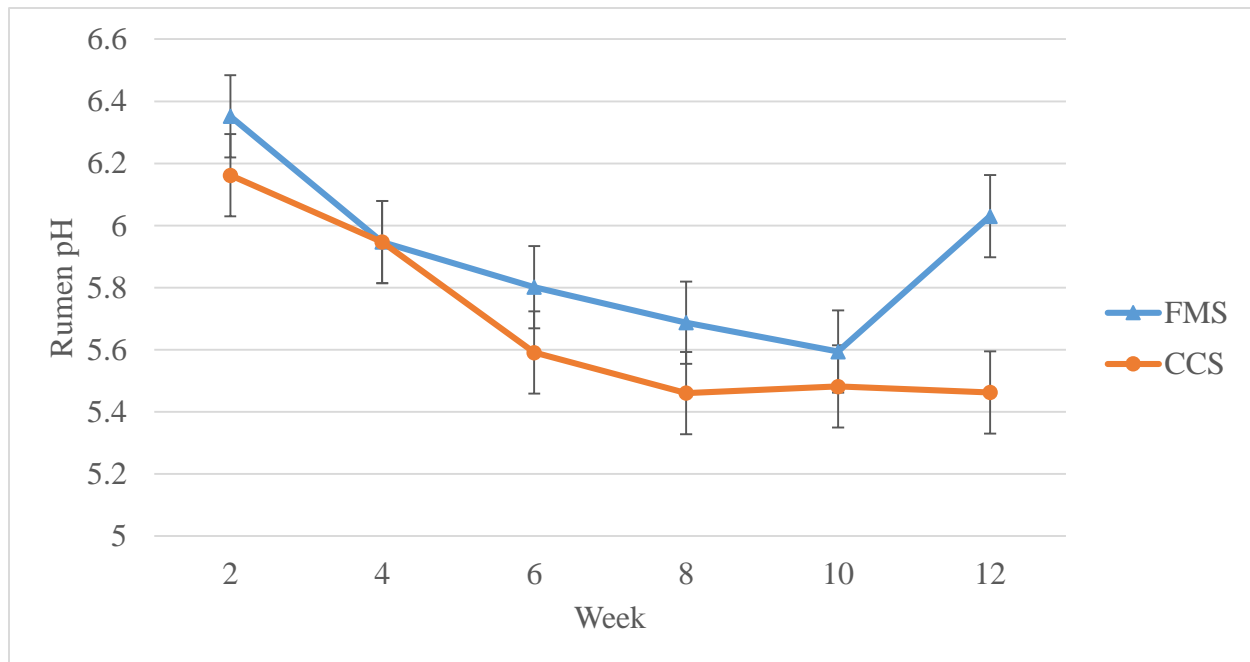


Figure 8. Least squares means of rumen pH for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.05$). SEM 0.07.

Least squares means for rumen concentrations of total VFA, acetate, propionate, and butyrate are presented in Table 4 and Figures 9, 10, 11, and 12. There were no treatment effects ($P > 0.05$) on acetate, propionate, butyrate, and total VFA. A significant effect of time was observed for acetate ($P < 0.001$), propionate ($P < 0.001$), butyrate ($P < 0.001$), and total VFA ($P < 0.001$). These effects indicate an increase in total VFA over time, demonstrating rumen fermentation. The increasing VFA with age follows the same pattern that other researchers observed (Stobo et al. 1966; Beharka et al. 1998; Coverdale et al., 2004). The greatest concentrations of VFA were observed in weeks 8 and 10, after which VFA concentrations declined during week 12. This may explain the low ruminal pH observed during the same weeks and its increase in week 12 for FMS fed calves. Butyric acid was the only VFA which increased

in week 12, and this was most observed in calves fed CCS. Rumen pH did not increase during week 12 in the calves fed CCS.

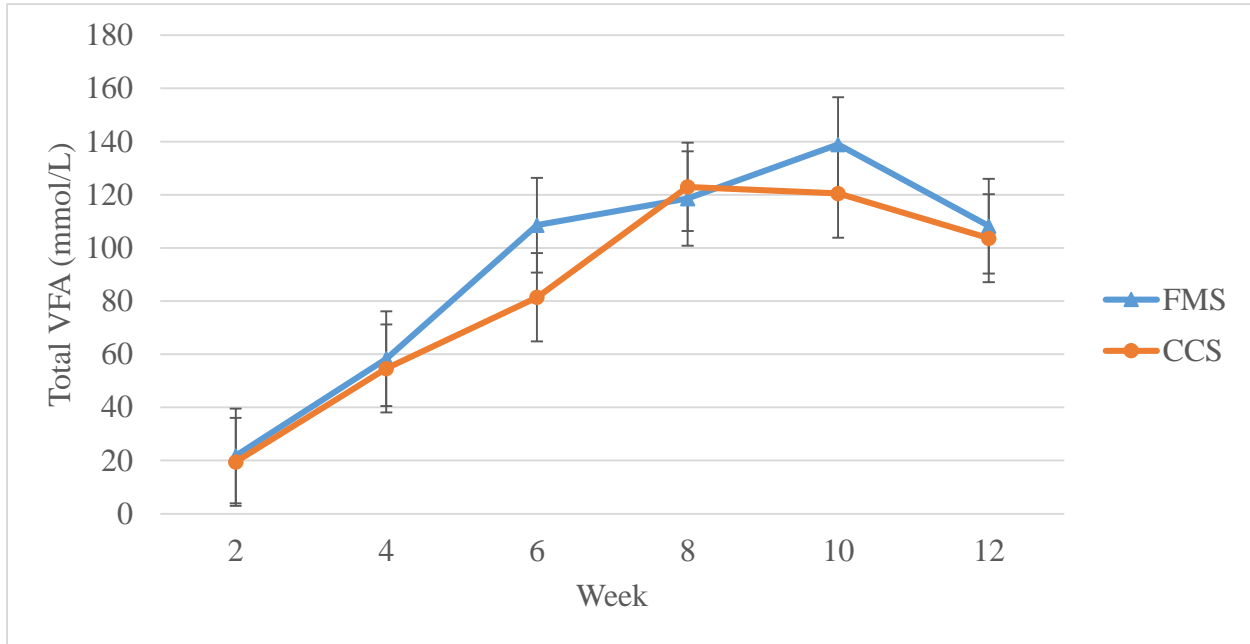


Figure 9. Least squares means of total volatile fatty acids (VFA) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 8.22.

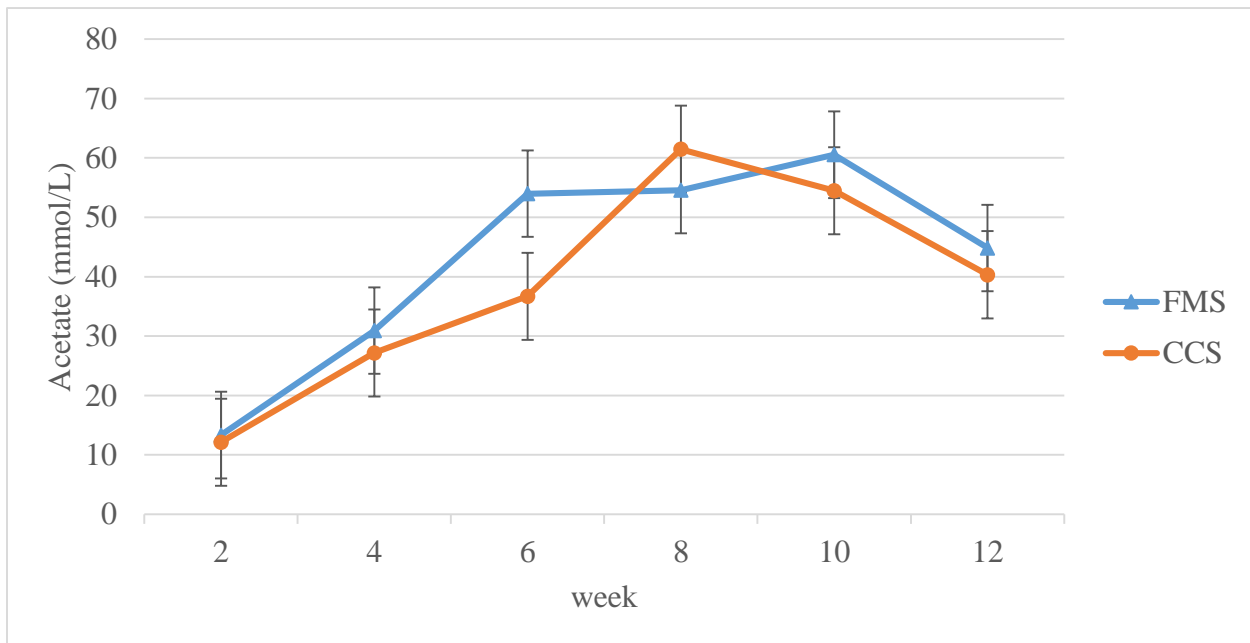


Figure 10. Least squares means of acetate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 4.23.

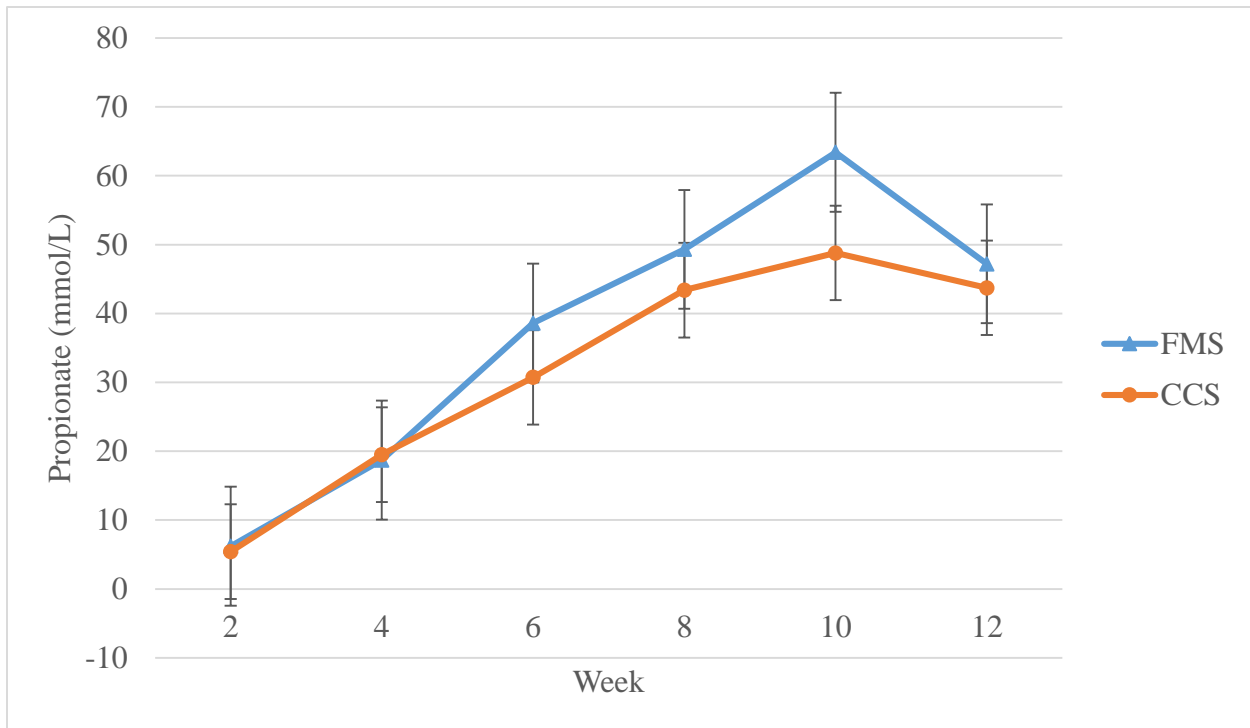


Figure 11. Least squares means of propionate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 3.49.

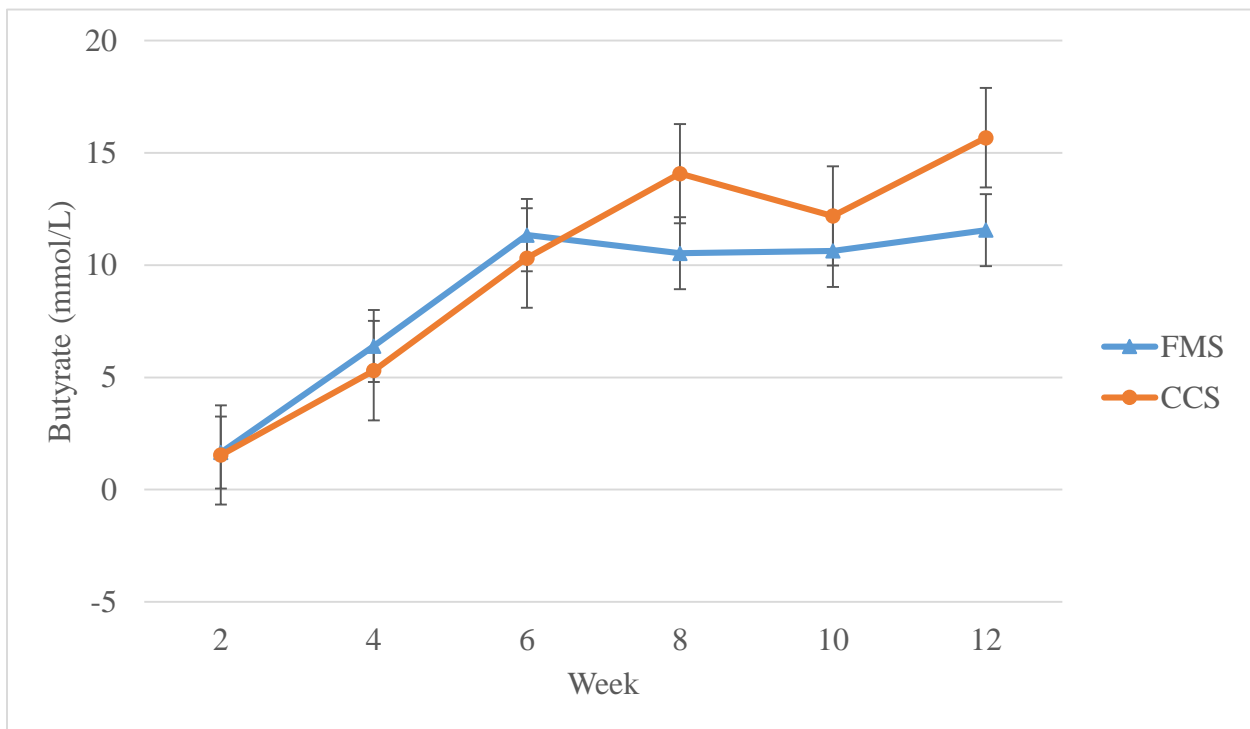


Figure 12. Least squares means of butyrate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 0.84.

Least squares means of molar percentages for acetate, propionate, and butyrate are presented in Table 4 and Figures 13, 14, 15, and 16. There were no treatment effects ($P > 0.05$) on acetate, propionate, and butyrate percentages. A main effect of time was noticed for molar percentage for acetate ($P < 0.001$), propionate ($P < 0.001$), and butyrate ($P < 0.001$). Acetate percentage decreased while propionate and butyrate increased with age. At the end of the trial, during weeks 10 and 12, percentage acetate and percentage propionate were similar; while percentage butyrate remained similar from week 6 to 12. These findings differ from Coverdale et al. (2004), who observed differences when ground, coarse commercial, and complete starters were fed; these researchers reported an increase in the molar percentage of acetate and a decrease in the molar percentage of propionate as forage increased in the diet. However, they also observed a decrease in percentage acetate in calves fed a ground diet. Even though particle size of the diet was not measured in this study, the mixing process of the FMS may have decreased overall particle size of the starter counteracting the effect that the chopped alfalfa hay could have in the rumen. In addition, high feed intake and low percentage of hay offered to calves may have caused the VFA production observed.

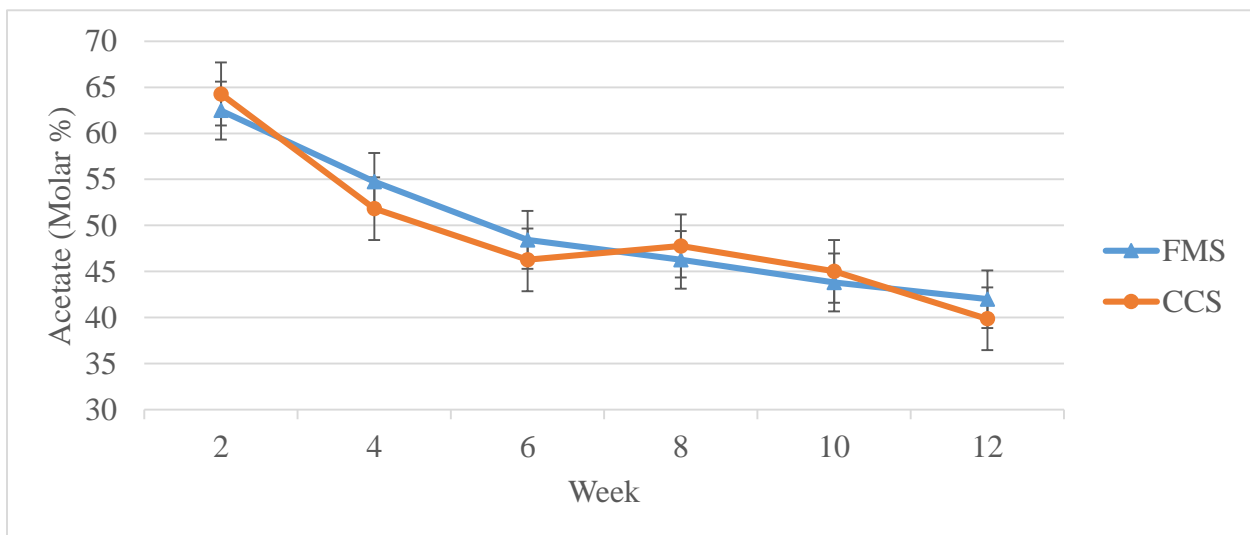


Figure 13. Least squares means of acetate (Molar %) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 2.03.

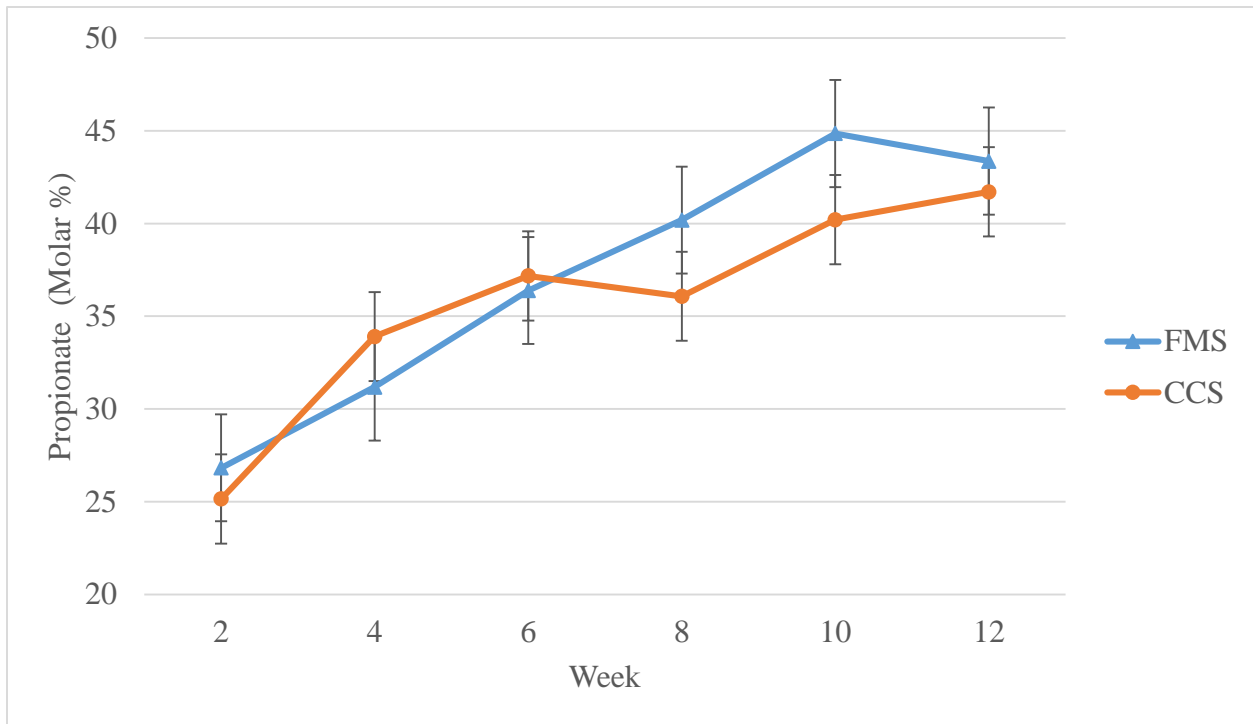


Figure 14. Least squares means of propionate (molar %) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 1.76.

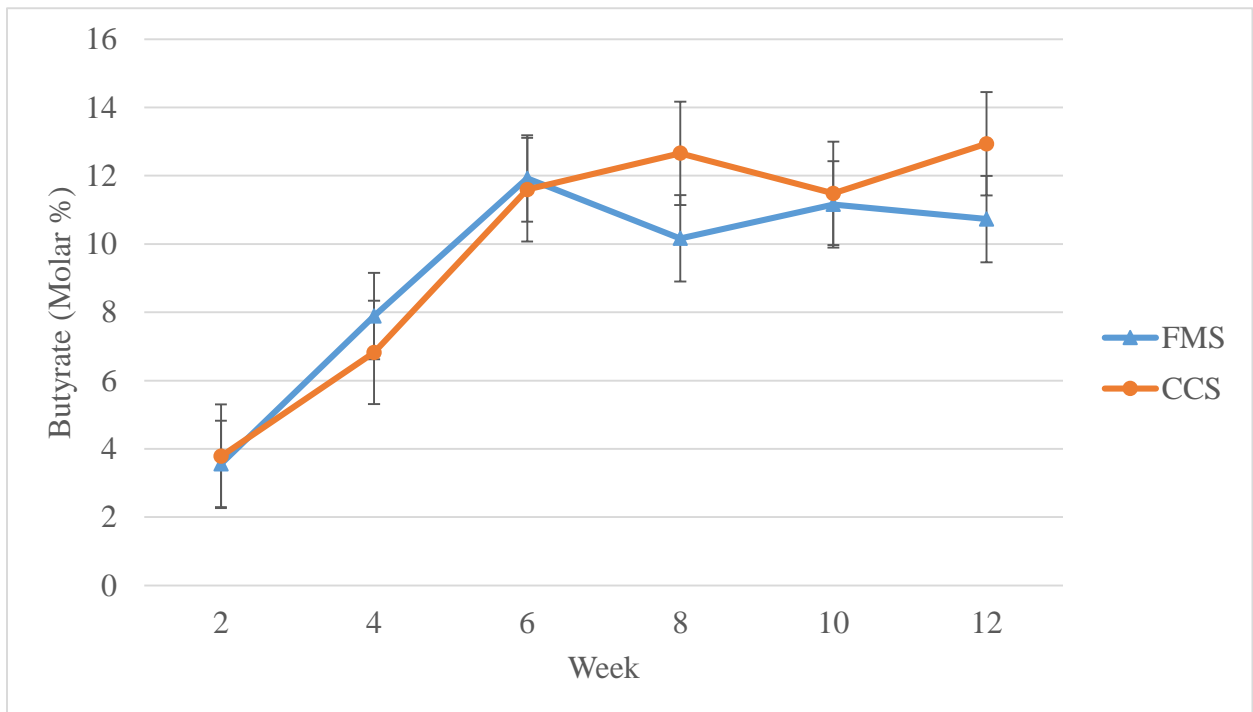


Figure 15. Least squares means of butyrate (molar %) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 1.78.

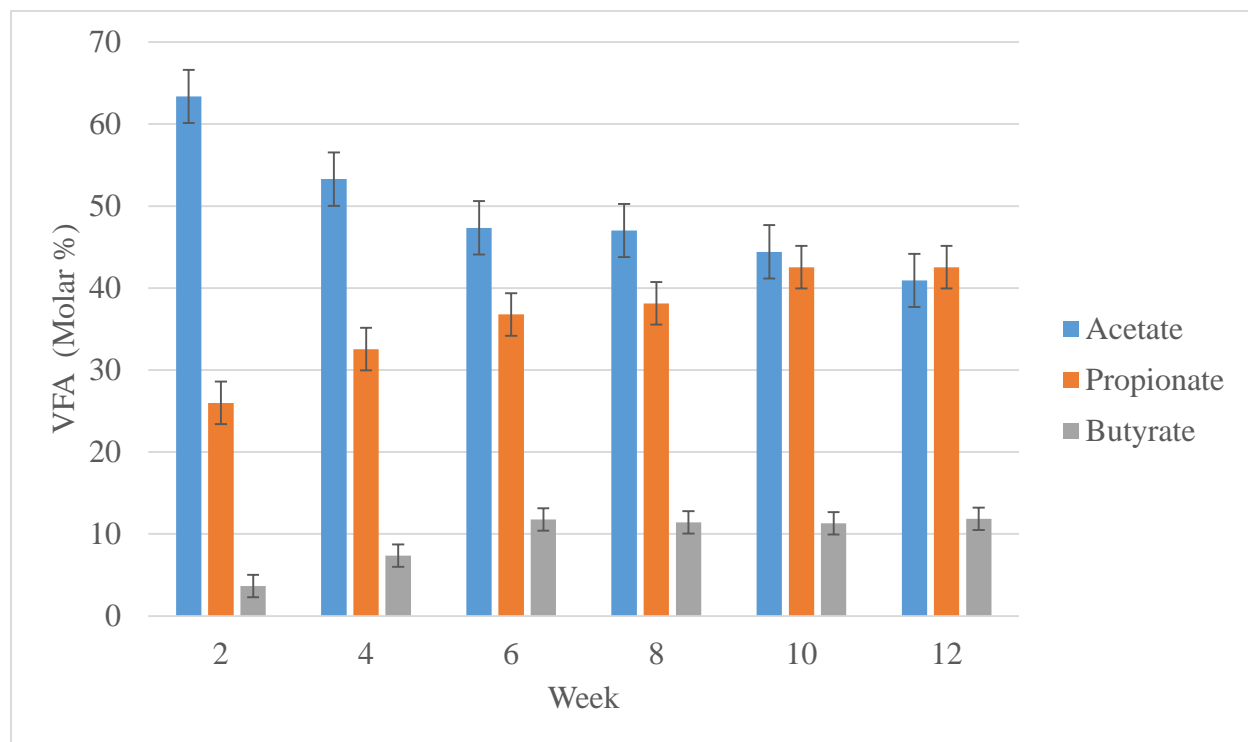


Figure 16. Least squares means of overall VFA percentages, for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$).

Least square means of ruminal ammonia (NH_3) concentrations are presented in Table 4 and Figure 17. Calves fed CCS had higher ammonia concentrations ($P = 0.03$) than calves fed FMS. In contrast Beharka et al. (1998) did not observed treatment effects on NH_3 when ground or unground diets containing 25% alfalfa hay and 75% grain mix were fed to the calves. There was not treatment by week interaction ($P = 0.19$) for ruminal ammonia in this study. In contrast, Beharka et al. (1998) observed a treatment by age interaction. In their study, NH_3 concentrations were greater in calves fed a ground diet compared to those fed an unground diet. At week 6, however, calves fed the ground diet had lower NH_3 concentrations than those in the unground diet. There was a main effect of week ($P < 0.001$) with NH_3 concentrations increasing with age. It is known that NH_3 should decrease with age due to the fact that NH_3 is being used by microbial population, and it is absorbed by developed papillae on the rumen wall (Godfrey, 1961; Bryant and Robinson, 1962; and Anderson et al., 1987). The increased ammonia concentrations

observed in this trial, especially in calves fed the CCS cannot be explained because more information about the CCS is needed as well as a deeper study of rumen papillae and microbial population.

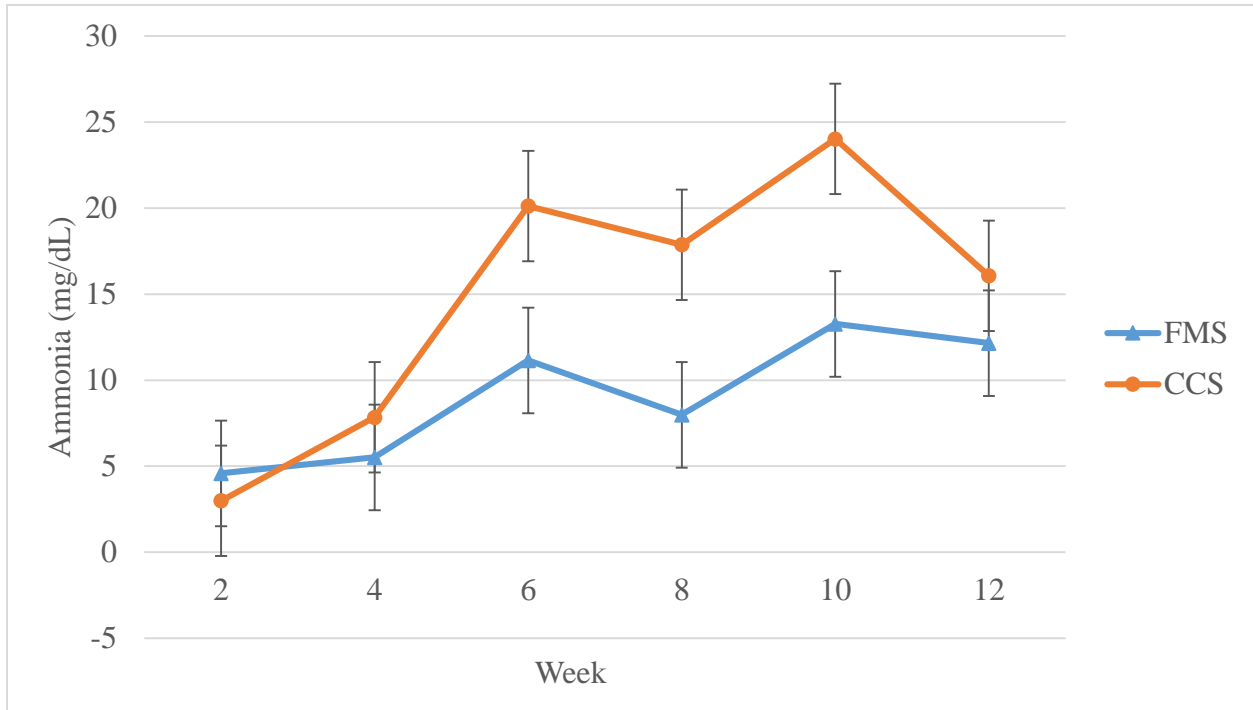


Figure 17. Least squares means of Ammonia for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$).

Least squares means of plasma urea nitrogen (PUN) concentrations are presented in Table 4 and Figure 18. PUN concentrations were affected by treatment ($P < 0.05$). A treatment by week interaction ($P < 0.05$) was observed. At weeks 6 and 8 calves fed CCS had greater PUN concentrations and at week 12 PUN was higher in calves fed FMS. Main effect of week ($P < 0.001$) was observed in this study. PUN increased with age as expected, being the greatest peak at weeks 8 and 10 for calves fed CCS and FMS respectively. This is indicative of initiation of urea recycling and absorption observed when the rumen is developing (Hayashi et al., 2006). However, after these peaks, PUN concentrations began to decrease specially in those calves fed the CCS which is not normal after weaning. Postweaning PUN should keep increasing as rumen

development takes place (Davis and Drackley, 1998). If compared with ruminal ammonia concentrations which showed a decrease at week 8 for both treatments and an increase in the following weeks with exceptions of its decrease observed at week 12 for calves fed the CCS diet, we can explain that PUN concentrations peaks observed and its decrease by the end of the trial correspond with the pattern observed on NH₃ concentrations.

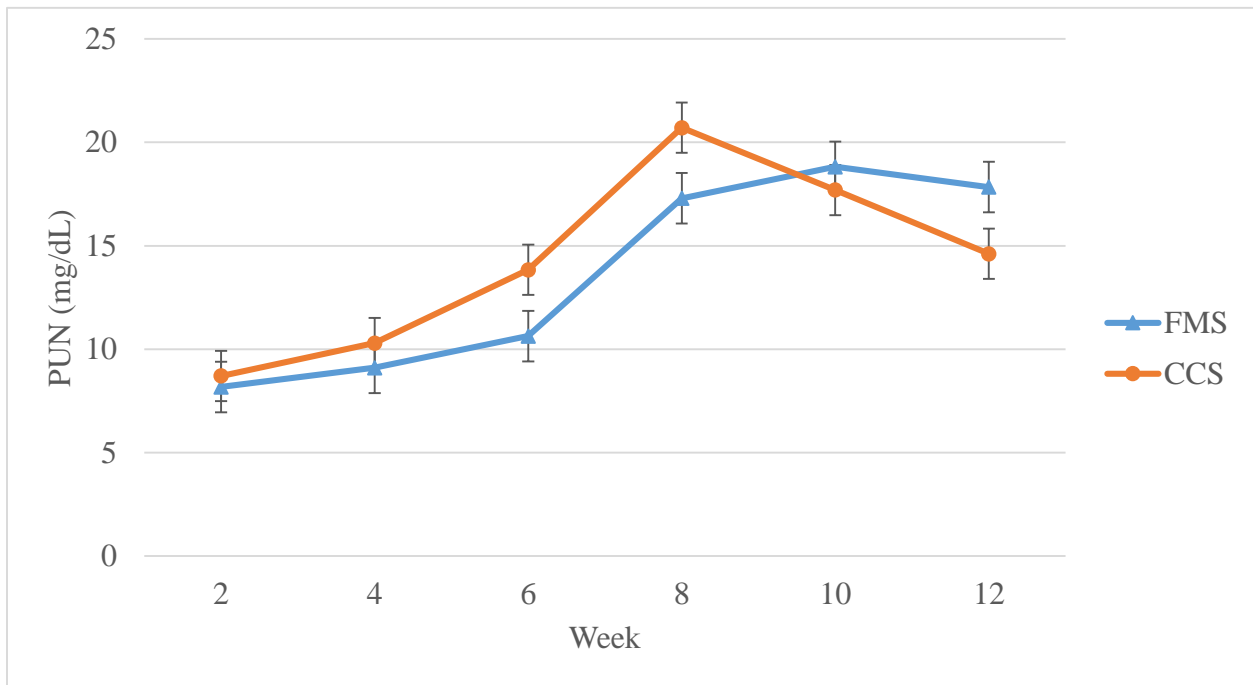


Figure 18. Least squares means of plasma urea nitrogen (PUN) for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Treatment by week interaction ($P < 0.05$). Main effect of week ($P < 0.001$). SEM 1.22.

Least squares means of β -hydroxybutyrate (BHBA) are presented in Table 4 and Figure 19. There was no treatment effect ($P > 0.05$) on plasma BHBA over the entire trial. Plasma BHBA increased over time ($P < 0.001$). This is comparable to Coverdale et al. (2004) and Quigley et al (1990) who also observed increased BHBA over time with highest concentrations postweaning. However, BHBA concentrations obtained after weaning cannot be compared with other researcher's findings, because levels in our study were relatively lower than those observed in other studies. Coverdale et al. (2004) obtained mean concentrations of BHBA after weaning

up to 0.26 mg/dL. Additionally, Doescher et al. (2007) observed values as high as 0.27 mg/dL by week 10. In our study, the highest BHBA levels observed were after weaning but these were 0.18 mg/dL by week 12.

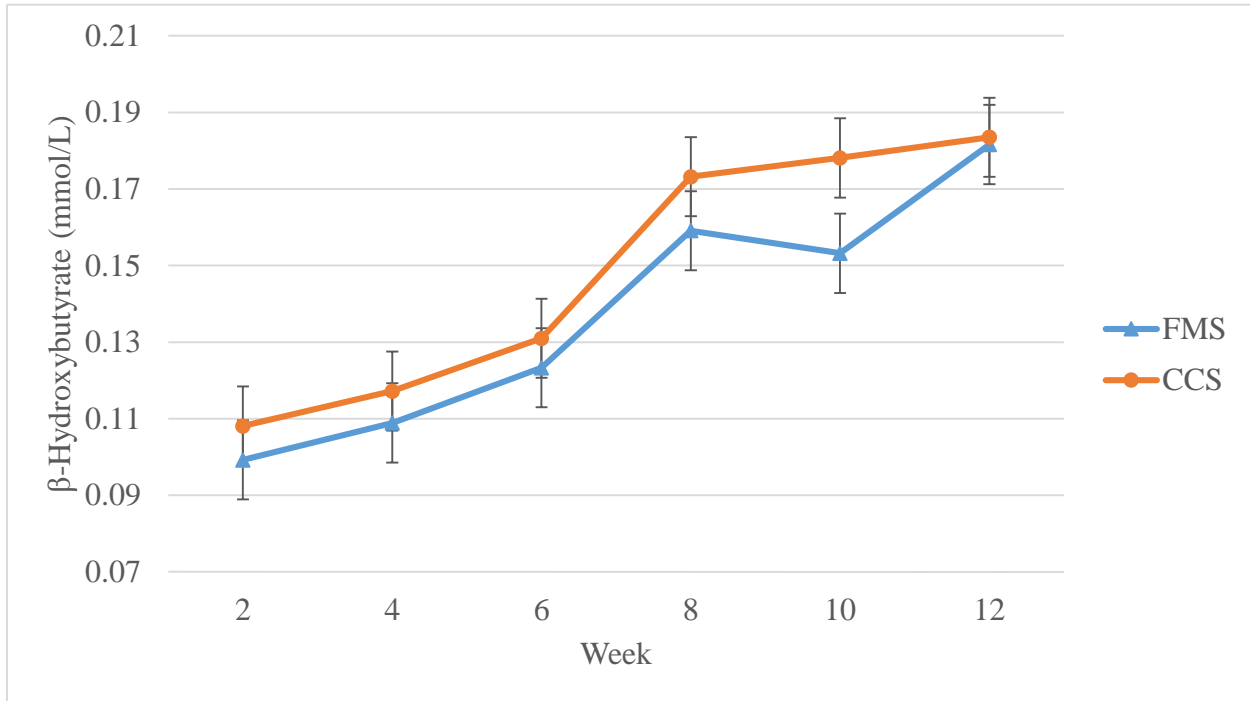


Figure 19. Least squares means of β -hydroxybutyrate for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 0.01.

Least squares means of plasma glucose concentrations are presented in Table 4 and Figure 20. There was no treatment effects ($P > 0.05$) on plasma glucose concentrations, but a main effect of week ($P < 0.05$) was observed. Levels of glucose decreased with age until week 8 as expected. However, glucose concentrations increased at week 10 and 12 for both treatments. These increases in glucose do not correspond to the findings of other researchers who observed concentrations of 76 mg/dl by week 9 on calves conventionally weaned or early weaned. (Quigley et al., 1991). Should be low, because the majority of highly digestible carbohydrates are fermented by the microbial population. However, in this particular study high starter intake was observed, which may have allowed higher amounts of non-structural carbohydrates to escape the

rumen Carbohydrate digestion and absorption in the small intestine would promote increased plasma glucose concentrations.

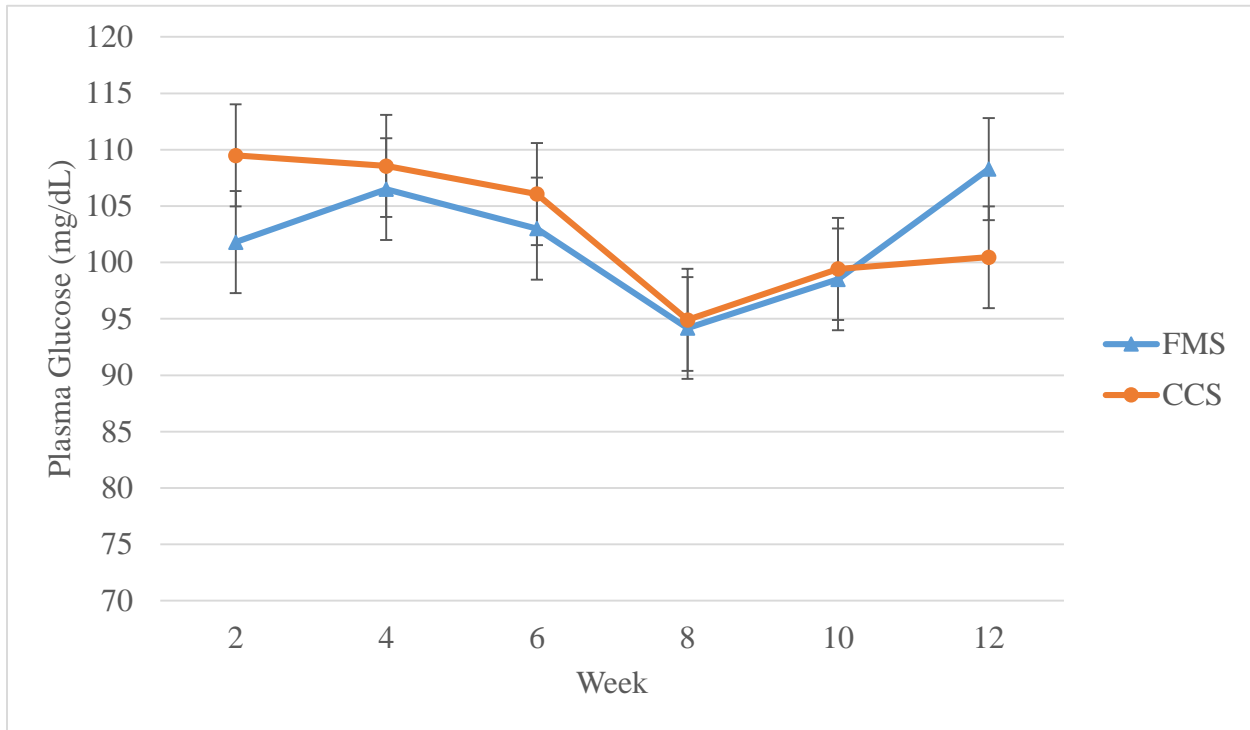


Figure 20. Least squares means of plasma glucose for calves fed farm mixed starter (FMS) and commercial calf starter (CCS). Main effect of week ($P < 0.001$). SEM 2.76

SUMMARY AND CONCLUSIONS

Summary

A study was conducted to evaluate performance, blood metabolites, and rumen development of Holstein calves fed commercially prepared or farm mixed calf starter. Twenty-four male Holstein calves were used in a 12 week study. Calves were randomly assigned to one of two treatment diets. Treatment diets included commercial calf starter (CCS) and farm mixed starter (FMS) offered free choice beginning on day 4. Calves were offered milk replacer (20% CP; 20% fat), Calves were housed in individual calf hutches with wire enclosures. Milk replacer was reduced to half on day 42 and calves were weaned at day 49. Water was offered free choice throughout the trial.

Body weight, hip height, wither height, hip width, and body length were measured weekly. Milk replacer intake, starter intake, and fecal scores were recorded daily. Blood samples, and rumen fluid samples were collected every two weeks beginning at week 2. Blood samples were analyzed for PUN, plasma glucose, and β -hydroxybutyrate concentrations. Rumen fluid samples were analyzed for pH, VFA, and NH_3 .

Feed intake tended to be greater ($P = 0.08$) for calves fed FMS than calves fed CCS. There was a treatment by week interaction, with calves fed FMS having greater ($P < 0.05$) starter intake during weeks 11 and 12. Postweaning BW was greater ($P < 0.05$) for calves fed FMS compared to calves fed CCS. There was a treatment by week interaction, as calves fed FMS were heavier ($P < 0.05$) from week 8 to 12 than calves offered CCS. Feed efficiency was similar ($P > 0.05$) among treatments throughout the 12 week trial. Average daily gain was greater ($P < 0.05$) for calves fed FMS. There were no treatment effects ($P > 0.05$) on body length, wither height, hip height, hip width, and fecal scores.

Ruminal pH decreased ($P < 0.001$) with age and was higher ($P = 0.031$) for calves fed FMS than calves fed CCS. There were no treatment effects ($P > 0.05$) on acetate, propionate, butyrate, and total VFA concentrations, as well as in their molar percentages. As expected with rumen development VFA increased ($P < 0.05$) with age. In contrast with normal development, rumen NH_3 concentrations increased ($P < 0.001$) with age and were higher ($P = 0.03$) for calves fed CCS. Plasma BHBA and plasma glucose concentrations were not affected ($P > 0.05$) by treatment. Concentrations of PUN were greater ($P < 0.05$) for calves fed CCS at weeks 6 and 8 were higher at week 12 in calves fed FMS. As expected PUN and BHBA increased ($P < 0.001$) with age. Plasma glucose concentrations decreased ($P < 0.001$) as calves aged with the exception of the increase observed at weeks 10 and 12.

Conclusions

It was intended for this study to determine if commercially prepared calf starter, as compared to calf starter mixed on the farm, would improve performance and rumen health of dairy calves. Data obtained from this research suggest that FMS, which contained chopped alfalfa hay as a fiber source, resulted in greater starter intake when fed ad libitum. Although BW gain was greater with FMS, feed efficiency was similar to that observed in calves fed CCS. Differences in intake and body weight gain were observed after calves were weaned, but no differences in skeletal growth were observed.

The weaning process is critical for dairy calves. For this reason, a healthy well developed rumen is needed at weaning so calves will be capable of surviving on solid feeds. Both diets supported VFA production, general rumen development, and overall calf performance. These data suggest that farm mixed starter, when formulated and prepared correctly, can equal commercial calf diets for general calf performance and rumen development.

REFERENCES

- Anderson, K. L., T. G. Nagaraja, & J. L. Morrill. 1987. Ruminal metabolic development in calves weaned conventionally or early. *J. Dairy Sci.* 70:1000-1005.
- Asai, T. 1973. Developmental processes of reticulo-rumen motility in calves. *Jap. J. Vet. Sci.* 35: 239-252.
- Bateman, H. G., C. C. Williams, & Y. H. Chung. 2002. Effects of supplemental zinc in high quality diets on ruminal fermentation and degradation of urea in vitro and in vivo. *Prof. Anim. Sci.* 18:363-367.
- Becker, R. B., P. T. Dix Arnold, & S. P. Marshall. 1951. Development of the bovine stomach during fetal life. *J. Dairy Sci.* 34(4): 329–332.
- Beharka, A. A., T. G. Nagaraja, J. L. Morrill, G. A. Kennedy, & 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.
- Beiranvand, H., G. R. Ghorbani, M. Khorvash, A. Nabipour, M. Dehghan-Banadaky, A. Homayouni, & S. Kargar. 2014. Interactions of alfalfa hay and sodium propionate on dairy calf performance and rumen development. *J. Dairy Sci.* 97(4): 2270-2280.
- Broderick, G. A. & J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64-75.
- Brownlee, A. 1956. The development of rumen papillae in cattle fed on different diets. *British veterinary journal.* 112(9): 369-375.
- Bryant, M. P. & I. M. Robinson. 1962. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. *J. Dairy Sci.* 46 (2): 150–154.
- Bull, L. S., L. J. Bush, J. D. Friend, B. J. Harris, & E. W. Jones. 1965. Incidence of ruminal parakeratosis in calves fed different rations and its relation to volatile fatty acid absorption. *J. Dairy Sci.* 48(11): 1459-1466.
- Castells, L., A. Bach, G. Araujo, C. Montoro, & M. Terré. 2012. Effect of different forage sources on performance and feeding behavior of Holstein calves. *J. Dairy Sci.* 95: 286–293.
- Church, D. C. 1983. Digestive physiology and nutrition of ruminants. Vol. 1: Digestive physiology 2nd ed. O & B Books. Inc. p. 34.
- Church, D. C. 1988. The ruminant animal digestive physiology and nutrition. Waveland Press, Inc. p. 25, 139, 176, 177, 290.

- Coverdale, J. A., H. D. Tyler, J. D. Quigley, & J. A. Brumm. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. *J. Dairy Sci.* 87:2554-2562.
- Davis, C. L., & J. K. Drackley. 1998. The development, nutrition, and management of the young calf. Iowa State University Press, Ames, IA.
- Doescher, R. M. 2010. Effects of varying levels of cottonseed hulls on growth and metabolic indications of rumen development of dairy calves. Baton Rouge, LA. Louisiana State University.
- Drackley, J. K., 2008. Calf nutrition from birth to breeding. *Vet. Clin. Food Anim.* 24: 55-86.
- Flatt, W. P., R. Warner, & J. K. Loosli. 1958. The influence of purified materials on the development of the ruminant stomach. *J. Dairy Sci.* 41:1593–1600.
- Godfrey, N. W. 1961. The functional development of the calf II. Development of rumen function in the calf. *J. Agr. Sci.* 57(2): 177.
- Grigsby, K. N., M. S. Kerley, J. A. Paterson, & J. C. Weigel. 1992. Site and extent of nutrient digestion by steers fed a low-quality bromegrass hay diet with increment levels of soybean hull substitution. *J. Anim. Sci.* 70:1941-1949.
- Harrison, H. N., R. G. Warner, E. G. Sander, & J. K. Loosli. 1960. Changes in the tissue and volume of the stomachs of calves following the removal of dry feed or consumption of inert bulk. *J. Dairy Sci.* 43(9): 1301-1312.
- Hayashi, H., M. Kawai, I. Nonaka, F. Terada, K. Katoh, & 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. Pouden, & 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.
- Hill, S. R., B. A. Hopkins, S. Davidson, S. M. Bolt, D. E. Diaz, C. Brownie, T. Brown, G.B Huntington, & L. W. Whitlow. 2009. The addition of cottonseed hulls to the starter and supplementation of live yeast or mannanoligosaccharide in the milk for young calves. *J. Dairy Sci.* 92:790-798.
- Hodgson, J. 1971. The development of solid food intake in calves. 1. The effect of previous experience of solid food, and the physical form of the diet, on the development of food intake after weaning. *Animal Production.* 13: 15.

- Jami, E., A. Israel, A. Kotser, & I. Mizrahi. 2013. Exploring the bovine rumen bacterial community from birth to adulthood. *ISME Journal*, 7(6): 1069-1079.
- Kerley, M., A. Kinser, J. Firkins, G. J. Fahey, & L. Berger. 1985. Effects of roughage particle size on site of nutrient digestion and digesta flow through the gastrointestinal tract of sheep fed corncob-concentrate diets. *J. Anim. Sci.* 61(2): 504-513.
- Khan, M. A., D. M. Weary, & M. A. G. von Keyserlingk. 2011. Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. *J. Dairy Sci.* 94: 1071–1081.
- McCann J. C. Wickersham T. A. & Loor J. J. 2014. High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. *Bioinformatics and Biology Insights* 8: 109–125.
- Murdock, F. R. & R.W. Wallenius. 1980. Fiber sources for complete calf starter rations. *J. Dairy Sci.* 63:1869-1873.
- Nocek, J. E. 1997. Bovine acidosis: implications on laminitis. *J. Dairy Sci.* 80: 1005–1028.
- Nocek, J. E., J. H. Herbein, & C. E. Polan. 1980. Influence of ration physical form, ruminal degradable nitrogen and age on rumen epithelial propionate and acetate transport and some enzymatic activities. *J. Nutr.* 110: 2355–2364.
- 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, & 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., L. A. Caldwell, G. D. Sinks, & 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.
- Reid, R. L. Studies on the carbohydrate metabolism of sheep. vi. inter-relation between changes in the distribution of levels of glucose and in the levels of volatile fatty acids in the blood of lambs. 195. *Australian J. Agr. Res.* 4(2): 213-223.
- Sander, E.G., R. G. Warner, H. N. Harrison, & J. K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of ruminal mucosa in the young calf. *J. Dairy Sci.*421600.
- Stobo, I. J. F., J.H. B. Roy, & H. J. Gaston. 1966. Rumen development in the calf. 1. The effect of diets containing different proportions of concentrates to hay on rumen development. *Br. J. Nutr.* 20:171-188.

- Stobo, I. J. F., J. H. B. Roy, & H. J. Gaston. 1966. Rumen development in the calf. 2*. The effect of diets containing different proportions of concentrates to hay on digestive efficiency. *Br. J. Nutr.* 20:171-215.
- Suárez, B., C. Van Reenen, N. Stockhofe, J. Dijkstra, & W. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. *J. Dairy Sci.* 90:2390–2403.
- Suarez-Mena, F., Heinrichs, A., Jones, C., Hill, T., & Quigley, J. 2016. Straw particle size in calf starters: Effects on digestive system development and rumen fermentation1. *J. Dairy Sci.* 99: 341–353.
- Tamate, H., A. D. McGilliard, N. L. Jacobson, & R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. *J. Dairy Sci.* 45:408-420.
- 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., W. P. Flatt, & J. K. Loosli. 1956. Dietary Factors influencing the development of the ruminant stomach. *J. Agric. Food Chem.* 4:788-792.
- Weary, D. M., J. Jasper, & M. J. Hötzel. 2008. Understanding weaning distress. *Applied Animal Behaviour Science.* 110: 24-41.
- Zitnan, R., J. Voigt, U. Schonhusen, J. Wegner, M. Kokordova, H. Hagemeister, M. Levkut, S. Kuhla, & , A. Sommer. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. *Arch. Anim. Nutr.* 51: 279-291.

APPENDIX A. BOVINE SERUM/PLASMA IgG TURBIDIMETRIC ASSAY

Ref: Bovine Serum/Plasma IgG. Catalog # 102. Midland Bioproducts Corporation®. 800 Snedden Drive PO Box 309, Boone, Iowa.

This method uses a turbid meter MBC QTII®, this meter must be updated downloading procedure from the website www.midlandbio.com.

Procedure:

1. Allow the sample and reagent vial to warm to room temperature (Approx. 20 Min.)
2. Insert the zero vial into the MBC QTII®. Align the ▼ on the vial with the ▲ on the adapter to obtain a continuous beeping and view ***** across the display. If ***** and beeping is not observed, the vial may need to rotate right or left to initiate the zero feature. Cover the vial. When completed “0.000” “ZERO”, and “0 Program” will be displayed. Remove the zero vial.
3. Add 10 µL of sample to the reagent vial. Do not allow the pipet tip to touch the side of the tube or the reagent. Re-cap and mix the contents by inverting several times. Wipe off any liquid or smudges from the exterior of the vial.
4. Incubate the reagent vial containing the sample for 5 minutes at room temperature.
5. Invert the vial several times to mix the contents, then insert it into the MBC QTII®. Align the ▼ on the coded vial with the ▲ on the adapter. Rotate the vial right or left to initiate the measurement if necessary. Cover the vial.
6. The MBC QTII® will display the result in mg/dL. Record the value or log the data into the instrument.

Limitation procedure:

This method is linear to bovine IgG level of 1700 mg/dL. The MBC QTII® will indicate if a sample is out of range with the message “OVERRNG” or “UNDERRNG”. Samples higher than 1700 mg/dL should be re-assayed after dilution with 0.9% saline. The results should then be multiplied by the dilution factor.

Expected Values:

Normal Values are 300 to 1700 mg/dL of IgG in calves, however, distribution may vary between local populations.

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 (10 μ l) of sample to it corresponding vial.
- 3) Add 0.5 mL of ENZYME RGT to all vials, mix gently, and incubate at 37°C for five minutes. (Alternative: React to 10 minutes at room temperature 2-26°C).
- 4) Add 2.0 mL of BASE RGT, mix gently and incubate at 37°C for 5 min. (Alternative: React to 10 minutes at room temperature 2-26°C).
- 5) Set the wave length of the photometer at 630 nm and zero the photometer with the BLANK. Read and record the absorbance 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;

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

APPENDIX C. 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 μ L of working reagent into each well of a 96 well plate. Incubate 10 min at 25° C.
3. Add 5 μ L of standard and unknowns in duplicate into appropriate wells. Incubate 10 min at 25° C
4. Read and record absorbance of all wells at 505 nm. Subtract blank reading from absorbance values.

Calculation:

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

APPENDIX D. 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 reagent to all tubes and place in a 37°C heating bath for at least five minutes.
- 2) Add 0.01ml (10µl) of sample to respective tubes. Mix and incubate at 37°C for exactly five minutes.
- 3) After incubation, zero spectrophotometer with the reagent blank. Read and record absorbance of all tubes at 500nm (500-520nm).
- 4) To determine results see calculations.

Calculations:

A= Absorbance

$$\frac{A(\text{Patient}) \times \text{Concentration of Standard}}{A(\text{Standard})} = \text{Glucose (mg/dl)}$$

APPENDIX E. 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. 18:363-67.

Reagents:

1) 25% (wt/vol) metaphosphoric acid (fluka #79615) acid solution containing 2 g/L of 2- ethyl butyric acid (216.5 μ 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 dH₂O.

MW	Acid	Volume (μ 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 (4 mL 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-Primer. 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 H₂/air flame.
- 6) Helium is used as the carrier gas with a spitless injection at a flow of 60 mL/min.

APPENDIX F. PHENOL-HYPOCHLORITE ASSAY FOR AMMONIA

Adapted from Broderick and Kang. J. Dairy Sci. (1980) 63:64

CAUTION: Wear gloves and protective clothing when mixing these reagents or running this assay. Phenol is a cancer-causing reagent and will burn the skin. WEAR GLOVES. This procedure allows for the use of repipets or pipetors. After reading, all waste material should be treated as hazardous waste and contained in bottles. All tubes and/or cuvettes must be rinsed before discarding.

Phenol Reagent

Dissolve 0.15g of sodium nitroferrocyanide (sodium nitroprusside) in 1.5 L of distilled H₂O (dH₂O). Add 33 mL (90% w/v) phenol (measured in a graduated cylinder) and mix thoroughly. Bring solution to final volume of 3 L by addition of dH₂O and store in a brown glass bottle. Phenol needed is 29.7g. Use goggles when measuring phenol and be careful. Phenol can cause burn when it comes into contact with skin.

Hypochlorite Reagent

Dissolved 15g of sodium hydroxide in approximately 2 L of dH₂O. Add 113.6g of disodium phosphate heptahydrate (Na₂HPO₄·7H₂O) to this solution using mild heating and mixing. After the disodium phosphate has mixed, allow the solution to cool. After cooling, add 150mL of commercial bleach (5.25% sodium hypochlorite, 131.25 mL if using 6% bleach) and mix thoroughly. Bring solution to 3 L by adding dH₂O. Filter solution through #1 filter paper and store in polyethylene bottle protected from light.

Ammonia Standard Solution

A stock solution of 100mM (170mg/dL) ammonia can be prepared by dilution 0.6607g of ammonium sulfate (dry overnight before use) to 100 mL with 0.1N HCl.

Working standards can then be made from the stock solution. Dilute 1 mL of stock solution per mM concentration desired in working standard to 100 mL total using dH₂O.

Procedure

- 1) Sample of rumen fluid will need to be diluted with dH₂O prior to analysis to bring the concentration of NH₃ into the working range of this assay. Therefore, mix 0.5 mL of clarified ruminal fluid with 4.5 mL of dH₂O and use these samples for the reaction.
- 2) Add 0.05 mL of samples or standard into test tube (use dH₂O for blanks).
- 3) Add 2.5 mL of phenol reagent to all tubes then mix on vortex.
- 4) Add 2.0 mL hypochlorite reagent to all tubes then mix on vortex.
- 5) Place in 95°C water bath for 5 min. Place marbles on top of each tube before inserting in water bath to prevent condensation from falling into the tubes.
- 6) After cooling, read samples on a spectrophotometer at 630 nm wave-length.
- 7) Dispose of all waste material in accordance with the hazardous waste regulations of your institution. This means that the PHENOL cannot be discarded in the municipal sewer without proper authorization.

VITA

Ruth Marisol Orellana Rivas was born in Siguatepeque, Honduras. She graduated from Instituto Dr. Genaro Muñoz Hernández in December of 2007. She began her bachelor's degree in January of 2008 at Universidad Nacional de Agricultura at Catacamas, Honduras. In December of 2011, she received her Bachelor of Agricultural Sciences. In January of 2014, she began her graduate studies at the School of Animal Sciences at Louisiana State University in dairy calf nutrition. After graduating, she will begin working toward a Doctoral degree at the Department of Animal and Dairy Sciences at University of Georgia, in Tifton, Georgia.