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Effects of varying levels of cottonseed hulls on growth and metabolic indications of rumen development of dairy calves

Ryan Doescher
Louisiana State University and Agricultural and Mechanical College

EFFECTS OF VARYING LEVELS OF COTTONSEED HULLS ON GROWTH AND METABOLIC INDICATIONS OF RUMEN DEVELOPMENT OF DAIRY CALVES

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Ryan M. Doescher
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ABSTRACT

A study was conducted to determine the effects of varying levels of cottonseed hulls on growth and metabolic indications of rumen development of dairy calves. Sixty-four Holstein calves (Heifers, n=40; Bulls, n=24) were randomly assigned to one of four dietary treatments which included calf starters containing no cottonseed hulls (control; **C**), 10% cottonseed hulls (**10% CSH**), 15% cottonseed hulls (**15% CSH**), or 20% cottonseed hulls (**20% CSH**). Calves were fed their respective treatments beginning on day 6 until day 56 of age. Body weights were measured at birth and biweekly thereafter until day 56 of age. Wither and hip heights were measured beginning on day 14 and biweekly thereafter until day 56 of age. Feed intake and fecal scores were recorded twice daily through day 56. On days 14, 28, 42, and 56, rumen fluid was collected for analysis of pH, volatile fatty acids (**VFA**), and ammonia (**NH3**), and blood was collected for analysis of plasma urea nitrogen (**PUN**) and β-hydroxybutyrate (**BHBA**). There was no treatment effect on average daily starter intake, body weight, wither height, and hip height but a treatment effect on fecal scores. Calves consuming CSH had higher rumen pH than C. Rumen pH also decreased as calves aged. There was no treatment effect on rumen acetate, propionate, butyrate, and total VFA concentrations. A treatment effect on NH3 concentrations was observed, and NH3 concentrations decreased over time. There was no treatment effect on BHBA, but a main effect of sex was observed in which the males had greater BHBA levels. There was no treatment effect on PUN concentrations, but a main effect of sex was observed with females having greater PUN concentrations. Overall, incorporating cottonseed hulls into a calf starter showed no significant effect on growth and rumen development in Holstein dairy calves.

CHAPTER 1

INTRODUCTION

The proper management of replacement heifers is an essential component in the dairy industry. Dairy producers want to have replacement heifers reach a proper body weight and size as soon as possible for breeding. The sooner heifers are bred, the sooner they can enter the milking herd and become profitable (Bach et al., 2007). Attention to nutritional management of the neonatal calf is the first and most important step in successful heifer rearing. If proper nutritional management is not met, then a delay in rumen development could occur.

At birth, a calf is considered a monogastric animal due to the underdeveloped rumen. During this time, the calf is fed milk replacer until the calf can utilize solid feed. Though little rumen development occurs during the liquid feeding phase (Tamate et al., 1962; Van Soest, 1994), it is essential to offer the calf a solid feed, also known as a calf starter, to begin encouraging rumen development. If the calf is fed milk replacer longer than needed, the rumen wall and papillae will grow at a slower rate causing the rumen to be underdeveloped. With the rumen wall and papillae underdeveloped, the calf will have complications in digesting grain and forages later in life increasing the time to when the heifer can be bred (Heinrichs and Lesmeister, 2000). While milk replacer is essential for providing nutrients to the young dairy calf, it also has been shown that milk replacer consumption positively affects solid feed intake (Khan et al., 2007).

Rumen development involves both physiological and metabolic changes that take place within the rumen. These changes include physical change and volatile fatty acid (**VFA**) formation. In response to physical change, the rumen can increase in size from 30% of total stomach at birth to 80% of total stomach during adulthood (Van Soest, 1994). Also during this

time, muscular development and rumen weight increase due to the presence of solid feed in the rumen. Metabolic activity, such as the metabolism of VFA, is essential for proper papillae development. Though papillary growth does increase with concentrations of butyrate, propionate, and acetate, it was found that most of the growth occurs when butyrate is in greater concentration over propionate, and propionate is in greater concentration over acetate (Sander et al., 1959; Tamate et al., 1962). With more papillae present, it allows for a greater absorption of nutrients across the rumen wall.

Concentrates are considered a high-quality feed with high amounts of digestible energy (Van Soest, 1994). Roughages consist of high cell-wall contents with low net energy (Jurgen et al., 1997). As the cell wall content increases, the feed becomes bulkier. Both fiber and grains provides energy to the rumen microbes which will help encourage rumen development. Over the years, there has been an increased concern on whether or not to feed forages in calf starters before the calf's rumen has been fully developed. Though it has been reported that one should feed concentrates over forages to maximize early rumen development (Heinrichs and Lesmeister, 2000), it has also been reported that the presence of fiber in a calf starter can cause an increase in rumen development due to the calf's ability to digest the forage (Hibbs et al., 1953; Kincaid, 1980; Bartley, 1973). Also, Anderson et al. (1982) and Stobo et al. (1966) reported that calf starters containing forages increase average daily gain (**ADG**). With known evidence that forages are able to help promote rumen development, they may be used to replace some of the expensive concentrates in the calf starter making the feeding of forages and concentrates a cost effective method.

Cottonseed hulls (**CSH**) are the outer shell of cottonseed which contains effective fiber. It has been stated that cottonseed hulls have beneficial effects on dairy calves. Hill et al. (2009)

observed that the inclusion of 10% CSH in the calf starter increased dry matter intake, ADG, and development of rumen papillae. Murdock and Wallenius (1980) found that CSH present in a calf starter increased both calf starter intake and body weight gains during weeks 0-12. Miller et al. (1969) added 10% CSH to simplified and complex calf starters and found that the presence of CSH yielded greater dry matter intake and increased body weight gain. Van Horn et al. (1976) compared different calf starter rations containing 5%, 15%, or 25% CSH. Results showed that there was a greater increase in dry matter intake when 25% CSH was fed compared to 5% and 15% CSH. There was no significant difference in body weight growth between the three CSH rations.

Based on the previous information on CSH and fiber intake there is still little information present as to what is the appropriate amount of fiber, through the feeding of CSH in a calf starter, is needed to obtain the best results for calf growth and rumen development. The objective of this study was to determine the effects of varying levels of CSH on growth and metabolic indications of rumen development of dairy calves.

CHAPTER 2

REVIEW OF LITERATURE

Rumen Development

Background

Though much information is present on rumen development, rumen development will always be one of the most critical times in not just the calf's life but for the dairy producer, since these calves are the replacement heifers for the dairy operation. The calf's stomach is divided into four compartments. These compartments are known as the reticulum, rumen, omasum, and abomasum. It is known that it is essential for the calf to have a proper diet to encourage rumen development as soon as possible. Until the rumen develops, the main stomach compartment is the abomasum.

Rumen as a Rudimentary Structure

Beginning at birth, the calf is considered a monogastric animal. Though the reticulum, omasum, and rumen are present at this stage, they are nonfunctional (Heinrichs and Lesmeister, 2005). The abomasum is the main digestive organ and is also known as the true stomach. While milk or milk replacer is being fed, the presence of the esophageal groove causes the milk or milk replacer to bypass the rumen and enter the abomasum where digestion occurs. Once the young ruminant's rumen starts to develop, it will begin to transition from a monogastric digestive system to a fully developed ruminant (Heinrichs and Lesmeister, 2005). In order for the rumen to become fully developed, it must go through both physical and metabolic changes. For a calf to make the transition from a non-functioning rumen to a functioning rumen, a proper starter diet should be fed in order to encourage rumen development (Baldwin et al., 2004; Coverdale et al., 2004; Hamada et al., 1976; Warner et al., 1956).

Volatile Fatty Acids

The volatile fatty acids (**VFA**) (acetate, propionate, and butyrate) are the main energy source for ruminant animals (Brown et al., 1960; Martin et al., 1959). Martin et al. (1959) and Stobo et al. (1965) concluded that a calf is able to metabolize and utilize VFA as early as 3 weeks of age, and the amount of VFA produced will begin to increase with age (Anderson et al., 1987b). Rumen microbes are present and rumen fermentation is taking place when VFA are being produced. Lane and Jesse (1997) and Tamate et al. (1962) found that rumen papillae are developing when VFA are present within the rumen. When comparing the VFA, Sanders et al. (1959) and Tamate et al. (1962) reported butyrate and propionate caused increased papillae development over acetate.

β-Hydroxybutyrate

The rumen microbial population ferments carbohydrates to produce VFA. Butyrate is absorbed across the rumen wall and metabolized to ketones also known as β-hydroxybutryate (**BHBA**), which in turn will provide energy to the ruminant. Jurgens (1997) states that the rumen mucosa is able to absorb butyrate and oxidize it converting up to 50% of the butyrate to BHBA and producing 27 moles of ATP for energy. In young ruminants, BHBA is an indicator that rumen development is taking place due to the increase of metabolic activity within the rumen (Murdock and Wallenius, 1980; Quigley et al., 1991). Increased BHBA concentrations indicate that rumen mucosa is developing. In a young ruminant, little microbial fermentation is occurring therefore, there are minute amounts of ketones present (Baldwin et al., 2004). Quigley et al. (1991) noted that the production of blood BHBA increases as the amount of starter consumed increases in young ruminants. Calves that were between 0 and 4 weeks of age had lower levels of blood BHBA when compared to calves that were between 5 to 8 weeks of age. This was due

to the increase of metabolic activity of the rumen mucosa resulting from the increase in feed intake.

Ruminal Ammonia

Because ammonia (**NH3**) is a major metabolite found within the rumen, it has become a marker for rumen function. A ruminant is able to utilize non-protein nitrogen, such as urea, and convert it to NH3 which can be utilized by rumen microbes. This in turn will allow for microbial protein synthesis in which the protein can then be utilized by the ruminant (McDonald, 1952).

Anderson et al. (1987a) reported ruminal NH3 concentrations will decrease as the calf increases in age. This is due to the increase of the NH3 utilization by the rumen microbes as well as the absorption of NH3 across the rumen wall. This also corresponds to Beharka et al. (1998) who reported that when calves were fed a specific ground diet, there were higher NH3 concentrations at week 2 and lower NH3 concentrations at week 6 when compared to the calves who consumed an unground diet.

Rumen pH

The optimal pH for a mature ruminant for proper microbial growth is 6.7 (Van Soest, 1994) but it can range between 5.8 and 6.8. It has been determined that the rumen pH is dependent on the age of the calf and the physical form of the diet (Beharka et al., 1998; Hibbs et al., 1956). As microbial populations form within the rumen, production of butyrate, propionate, and lactate will cause a decrease in ruminal pH suggesting that rumen development is occurring due to the production of volatile fatty acids.

A study conducted by Hibbs et al. (1956) involved the feeding of different hay to grain ratios to Jersey and Holstein calves for 12 weeks. During the trial, the rumen pH was measured

on weeks 4, 6, 9, and 12 of age. It was reported that as the amount of hay to grain ratio increased, the rumen pH also increased. Hibbs et al. (1956) concluded that roughages can be used as a buffer when trying to establish a mature rumen during early rumen development.

Plasma Urea Nitrogen

Urea is a non-protein nitrogen source. When protein sources from feeds enter the rumen, microbial fermentation results in the production of NH3, carbon dioxide, and methane. NH3 is used for microbial protein synthesis or absorbed across the rumen wall and transported in circulation to the liver where it is converted into urea (Hayashi et al., 2006). The urea will be moved back to the rumen where microbes recycle it and utilize the urea for protein synthesis (Obara and Shimbayashi, 1980; Roseler et al., 1993). Nolan and Leng (1972) observed that urea enters the rumen through saliva flow and can be absorbed through the rumen wall. This is in agreement with Obara and Shimbayashi (1980) who reported that most of the urea found in the rumen resulted from diffusion through the rumen wall. After the microorganisms convert the urea into microbial proteins, the microorganisms pass into the abomasums where the microbial proteins are broken down into amino acids which are then absorbed by the small intestines of the ruminant (Hayashi et al., 2006).

It was also noted by Hayashi et al. (2006) that the amount of recycled urea can help determine if rumen development is taking place. One should see an increase in the amount of recycled urea and less NH3 present as rumen development occurs. Overall, there should be more urea nitrogen present within the rumen once weaning has occurred. The presence of urea nitrogen in the blood can help determine if crude protein degradability is occurring. When high protein diets are fed, the amount of urea in the urine and blood begin to increase. Likewise when a diet low in nitrogen is fed, the amount of urea present within the urine and blood begin to

decrease due to the reutilization of urea.

Rumen Development

In the young calf, the rumen not only changes in physical size but the papillae will increase in both length and width. These changes can be greatly influenced by the physical form of the young ruminant's diet. The physical form of the diet will have an effect on the rumen microbial population which has become another important factor in rumen development. The microbial population consists of bacteria, protozoa, and anaerobic fungi. A symbiotic relationship is formed in which the ruminant provides the proper environment for the microbes, while the microbes provide end products for the ruminant from feeds that are consumed. In turn, the end products are absorbed by the rumen wall and used for energy (Yokoyama and Johnson, 1993). Rumen size, papillae development, and microbial development will complement each other allowing the young ruminant to transition into a mature ruminant.

Growth of the epithelial cells causes the rumen papillae to develop. As stated previously, the development of the papillae will allow the absorption of end products, such as butyrate, propionate, and acetate produced by the rumen microbes. In return, the absorption of these VFA will cause the rumen epithelial cells to develop (Heinrichs and Lesmeister, 2005; Lane et al., 2000). The consumption of dry feeds has been found to be a key factor in proper rumen development because of the microbial fermentation that takes place (Stobo et al., 1966). Dry feed has been found to increase the amount of bacteria present therefore increasing the amount of end products that are produced and absorbed (Beharka et al., 1998).

With the environment being the main cause for initial bacterial growth within the rumen, the presence of liquid, such as milk, in the rumen can slightly influence rumen development. Though the esophageal groove causes milk or milk replacer to bypass the rumen and enter the

abomasum, some liquid will still enter the rumen. The liquid that enters the rumen will begin to ferment, which will lead to low amounts of VFA production. This will help begin the rumen development process (Lane et al., 2000). Though milk or milk replacer can encourage rumen development, it is not enough to enhance rumen development over time.

Feedstuffs and Rumen Development

Diets have the greatest influence on rumen development. Rumen development has been reported to occur during the early stages of a calf's life if the proper diet is consumed by the calf. Though some bacteria are present at the time of birth, diet is used to influences microbial growth of bacteria. These bacteria will produce VFA which in turn cause the rumen to develop (Lengemann and Allen, 1955). The two feeds that have an influence on these changes are liquid and solid feeds.

Liquid feeds, such as milk replacer, are considered the main diet for neonatal calves but because of the presence of the esophageal groove, little rumen development occurs. Because the milk bypasses the rumen through the esophageal groove, metabolic activity within the rumen is decreased therefore reducing the absorption of VFA (Heinrichs and Lesmeister, 2005). Stobo et al. (1966) reported the rumen will increase in size as the calf increases with age. Though the calf may have an increase in rumen size when fed milk replacer, the rumen will still be underdeveloped when only milk or milk replacer is fed.

Solid feeds, such as starters or complete starters, are geared more for the development of the rumen in terms of metabolic activity and stimulation of the rumen. Solid feeds promote microbial proliferation within the rumen, which yields VFA to enhance rumen development. Solid feeds have also been reported to promote papillary development (Stobo et al., 1966). Solid feeds can consist of concentrates and/or forages. Though both concentrates and forages have

been linked to rumen stimulation, it seems that concentrates have the greatest effect on rumen development (Stobo et al., 1966; Warner et al., 1956).

Heinrichs and Lesmeister (2005) stated that concentrates have a greater impact on rumen development, such as papillae growth, than do forages. Heinrichs and Lesmeister (2000) reported that the feeding of concentrates can cause an increase in papillae length and improve the thickness of the rumen wall. The improved development of both the papillae and rumen wall will cause an increase in surface area, improving the absorption of nutrients. It was also reported that feeding concentrates will create the proper environment for rumen microbes to flourish. This will allow the microbes to produce butyrate which in turn will cause even greater rumen development.

Though much emphasis has been placed on the feeding of concentrates to young ruminants, one cannot forget the importance of feeding forages for rumen development. It was reported by Coverdale et al. (2004), Hill et al. (2009), and Kincaid et al. (1980) that early weaned calves fed forages consumed more feed and had improved weight gains. The increase in feed intake has been shown to lead to an increase in VFA concentrations (Coverdale et al., 2004). Tamate et al. (1962) noted forage consumption improved muscular development within the rumen due to the large particle size and bulk content of the forages. Both particle size and bulk content can cause an increase in the stimulation of rumen contractions allowing feedstuffs to move out of the rumen and into the intestines (Heinrichs and Lesmeister, 2005).

Forages

Background

It has been shown that fiber influences feed intake, passage rate, rumen function (Van Soest et al., 1991), and rumen activity of lactating dairy cows (Colenbrander et al., 1986). Forage

is defined as “a vegetative plant in a fresh, dried, or ensiled state which is fed to livestock such as pasture, hay, silage, or green chop” (Jurgens and Bregendahl, 2007). By feeding forages, it allows the cow to digest extra carbohydrates to help meet the energy requirements through VFA production and provide proper rumen function (Kendall et al., 2009). The fiber content can be best measured by neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**).

NDF is used to approximately measure the amount of cellulose, hemicellulose, and lignin in the plant cell wall and is less digestible when compared to nonstructural carbohydrates (NRC, 2001). The presence of these structural components helps determine energy intake and rumen fill. For example, high NDF will cause rumen fill due to the bulkiness of the fiber therefore causing a decrease in dry matter intake (NRC, 2001). NDF can be used as a buffering system because of the slow fermentation process in the rumen causing less acid production and increasing the production of saliva, which is also used as a buffer, due to the extra chewing of the forage. The NRC (2001) recommendation for the minimal amount of NDF is 25 percent of the dietary dry matter depending on the forage. Cows with lower energy requirements may have increased NDF concentrations.

ADF is an indicator of relative digestibility for structural carbohydrates (Fahey and Berger, 1993). ADF is part of the plant cell wall that is less digestible by the ruminant such as cellulose or lignin. When trying to determine the amount of fiber that should be fed, the amount of NDF should be considered over ADF. NDF can be best correlated with dry matter intake and rumen fill (Van Soest et al., 1991). The NRC (2001) recommends at least 17 percent ADF be present in the total diet for large dairy ruminants. This percentage could increase depending on the amount of NDF present in the diet. For instance, if the amount of NDF increases, the amount of ADF will also increase.

Forage in a Calf Feeding System

There have been multiple studies involving forages in a calf starter and whether or not the calf can benefit from the forages. It has been shown that when calves consume forages at an early age there is an increase in feed intake, improved weight gain, and improved rumen development (Anderson et al., 1982; Miller et al., 1969; Hill et al., 2009). Though some studies have shown similar results, others have shown that forages can have a negative effect on rumen development.

Kincaid (1980) fed Holstein calves, from d 3 through week 12 of age, a calf starter consisting of three different concentrate rations: a pelleted concentrate, 20% ground alfalfa and 80% concentrate fed together in a complete pelleted mix, pelleted concentrate and alfalfa pellets fed separately, or concentrates and long-stem alfalfa hay fed separately. Results showed that when the calves consumed the long stem alfalfa or the pelleted roughage rations they had improved weight gains due to increased feed intake. This was not in complete agreement with Hibbs et al. (1956), who fed three calf starter rations, each differing in hay to grain ratios (4:1; 3:2; 2:3). Significant increase in weight gain was seen when calves consumed starters that had higher concentrate to forage ratios.

Tamate et al. (1962) fed three different rations that consisted of a milk, hay, and grain ration; a milk ration; or a milk ration plus other substances that were placed into the rumen. The research consisted of 27 Holstein calves. Milk, hay, and grain ration was fed to seven of the 27 Holstein calves, and results indicated that there was rumen development with an increase in papillary and muscle development due to the hay that was given in the ration. In an experiment performed by Murdock and Wallenius (1980), three different types of calf starter rations were fed. These rations consisted of alfalfa hay, CSH, or alfalfa-beet pulp. Results showed the

inclusion of fiber sources did not affect rumen VFA indicating the fiber sources did not cause improved rumen development. There was an increase in calf growth when calves consumed starters containing CSH but this was attributed to feed acceptability.

Klein et al. (1987) evaluated the effects of four different dietary fiber sources in 40 Holstein heifers over a course of 12 weeks through the winter months. The treatments consisted of a pelleted prestarter and 10% alfalfa ration, a prestarter and 20% alfalfa ration, no prestarter and 10% alfalfa ration, or no prestarter and 20% alfalfa ration. Also, a similar experiment was conducted over a 10 week period during the spring months. Results indicated that calves that were on the prestarter and 20% alfalfa ration and the no prestarter and 10% alfalfa ration had greater body weights at 10 weeks of age. The calves given prestarter were weaned at 17 days of age indicating earlier rumen development over those that were not given a prestarter.

Cottonseed Hulls

Background

CSH are a byproduct of whole cottonseed once the oil has been extracted (Jurgens and Bregendahl, 2007). When concentrates and roughages started to increase in price, alternative types of feeds were used to alleviate the cost issue (Brown et al., 1977). This has led to the feeding of CSH to dairy cows. It was reported that when CSH were fed with undegradable intake protein there was an increase in feed intake and milk production (Gu et al., 1996). Though CSH are low in energy, they are considered to be a source of effective fiber and are highly palatable when fed to ruminants in a total mixed ration.

Rumen development is essential for the proper growth of dairy calves. With much emphasis placed on rumen development, many dairy producers are looking for improved

methods in developing the rumen. Though concentrates are needed for rumen epithelial development, fiber can be used for improved muscular development. This has led to the research of CSH in calf starters.

Cottonseed Hulls in a Calf Feeding System

It has been reported by Miller et al., (1969), Hill et al., (2009) and Van Horn et al., (1976) that CSH are beneficial to calves when consumed in a calf starter. However, the optimum amount of CSH to include in the diet to maximize rumen development is still not known.

Miller et al. (1969) fed 10% CSH to 32 dairy calves, including 16 Holstein heifers, 12 Holstein bulls, and 4 Jersey bulls, in a simplified and complex calf starter. Results showed an increase in feed consumption and weight gain when CSH was present in both the simplified and complex starter when compared to a simplified and complex starter containing no CSH. Though 10% CSH resulted in significant difference, it was still unclear if this was the optimum fiber level.

Hill et al. (2009) researched the use of CSH in a calf starter with the supplementation of live yeast or mannanoligosaccharide in milk for young claves. The study utilized 116 Holstein and 46 Jersey calves. The calves received 1 of 2 starter diets consisting of a corn-soybean meal-base starter or a 85% corn-soybean meal-base starter with 15% CSH. Results indicated an increase in dry matter intake and ADG in Holstein calves consuming the CSH starter. No significant effect on the Jersey calves was seen when fed the CSH starter. In another study involving CSH, Van Horn et al. (1976) included 15% CSH in a calf starter. The research consisted of 115 calves in which the calf starters contained 15% CSH or 30% citrus pulp or both. Researchers concluded there was a significant increase in weight gain for calves consuming the 15% CSH starter without the addition of the 30% citrus pulp. Research with the 15% CSH was

also compared to calf starters that contained 5% or 25% CSH. No significant difference in growth was reported, but there was a linear relationship between feed intake and CSH.

CHAPTER 3

MATERIALS AND METHODS

Animals and Dietary Treatments

Sixty-four Holstein calves, which consisted of 40 heifers and 24 bulls, were used in an eight week experiment to determine the effects of CSH on growth and rumen development. Each calf was born and raised between August, 2008 and March, 2009 and housed at the LSU Agriculture Center Research and Teaching Farm in Baton Rouge, Louisiana. The experimental protocol was approved by the IACUC (Institutional Animal Care and Use Committee) of the LSU Agricultural Center.

The calves were separated from their dams at birth, weighed, and housed individually in a 2.5-m^2 calf hutch with a 2.8-m^2 wire enclosure. All calves were placed on rock bedding until d 56. Each calf received 3.8-5.7 liters of colostrum from their dam and were vaccinated against the Rotavirus and Coronavirus (Calf Guard, Pfizer Animal Health, Lenexa, KS) on d 1prior to colostrum feedings.

Beginning on d 2 and 3 of life, calves were fed their dam's transition milk using a nipple bottle. Beginning on d 4 until d 42 (weaning), each calf was offered 20% protein, 20% fat milk replacer (reconstituted to 15% solids), containing decoquinate (Nutra Blend LLC, Neosho, MO). Each calf was fed milk replacer at 10% of birth weight at 0630 hours prior to the feeding of calf starter. Each calf was individually bucket trained when milk replacer was first fed and completely removed off milk replacer on d 42 of age. All calves consumed their milk replacer. Calves were randomly assigned, at birth, to one of four dietary treatments. Treatments included calf starter containing: 1) no cottonseed hulls **(C)**, 2) 10% cottonseed hulls (**10%CSH**), 3) 15%

CSH (**15% CSH**), and 4) 20% CSH (**20% CSH)**. The calf starter composition is presented in Table 1. Calves were offered their respective treatments *ad libitum* beginning on d 6. Each calf was fed starter twice daily at 0700 and 1600 hours. Each calf originally started with 113 g of starter in the AM, and feed refusal was weighed back before each new feeding. Starter was increased in 113 g increments when calves refused less that 36 g of feed per feeding. Water was offered *ad libitum* beginning on d 4. Calves were removed from the project at d 56 of age.

Table 1. Calf Starter Composition

Ingredients, % As Fed	Treatment			
	C	10% CSH	15% CSH	20% CSH
Corn (Rolled)	35.0	28.8	25.0	21.3
[1]KWC Custom Hfr-R	1.5	1.5	1.5	1.5
Pro-Lak	2.5	2.5	2.5	2.5
Friends HI-fat 14-5	10.0	10.0	10.0	10.0
[2]Rum/Vit.E/Lime Premix	1.0	1.0	1.0	1.0
Cargill Pellet Milk Plus	2.5	2.5	2.5	2.5
Dried Distillers Grain with solubles	9.0	9.0	9.0	9.0
Cottonseed Hulls	0.0	10.0	15.0	20.0
Sweet Stuff	5.0	4.2	3.9	3.5
Vegetable Oil	0.0	0.3	0.4	0.6
Protein Pellets (SBM 48)	12.3	12.3	12.3	12.3
Oats (Rolled or Crimped)	17.5	13.8	12.5	11.3
Molasses	3.8	4.3	4.5	4.8
Chemical Analysis				
Dry Matter, (%)	89.89	88.77	88.98	88.77
[3]TDN, (%DM)	79.6	76	74.2	72.5
Crude Protein, (%DM)	19.12	18.48	18.43	16.77
Neutral Detergent Fiber, (%DM)	19.49	26.77	30.26	34.66
Acid Detergent Fiber, (%DM)	7.86	13.67	16.56	19.3

[1]Kentwood Custom Heifer-R contains Monensin 2,400 g/ton, Calcium(Min) 15.00%, Calcium(Max) 18.00%, Phosphorus(Min) 5.75%, Salt(Min) 18.00%, Salt(Max) 21.00%, Magnesium(Min) 2.60%, Potassium(Min) 0.90%, Sulfur(Min) 1.00%, Cobalt(Min) 25 ppm, Copper(Min) 800 ppm, Iodine(Min) 80 ppm, Manganese(Min) 2,700 ppm, Selenium(Min) 20 ppm, Zinc (Min) 2,750 ppm, Vitamin A(Min) 200,000 IU/lb, Vitamin D-3(Min) 45,000 IU/lb, Vitamin E(Min) 1,000 IU/lb

[2]Rumensin/Vitamin E/Lime Premix contains (as fed) Soybean Meal 48 81.90%, Rumensin 80 0.60%, Vitamin E 20 5.00%, Ca Carbonate 12.50

[3]TDN = Total Digestible Nutrients calculated according to NRC (2001) using varying levels of CSH.

Sample Collection

Fecal scores were recorded twice daily at feeding. The scoring scale was based on fecal fluidity and ranged from one to four with 1 = normal, 2 = soft, 3 = runny, and 4 = watery (Larson et al., 1977). Body weights were recorded on d 1, 14, 28, 42, and 56 of age, and wither and hip heights were measured on d 14, 28, 42, and 56 of age. The calf starter's dry matter and nitrogen were determined by AOAC (1980) procedures. NDF and ADF were determined using an Ankom 200 fiber analyzer (Ankom Technology; Macedon, NY).

Rumen fluid was collected via stomach tube 4 hr post-feeding for analysis of VFA and NH3 on d 14, 28, 42, and 56 of age. Immediately after collection, rumen fluid pH was measured. After the pH was recorded, 1 mL of phosphoric acid (20% w/v) was added. All rumen fluid was stored at -20°C and protected from UV light until analysis.

Blood was collected via jugular venipucture on d 14, 28, 42, and 56 of age for analysis of BHBA and plasma urea nitrogen (**PUN**). Blood was collected in a 10 mL vacutainer tube containing sodium heparin. The blood was centrifuged for ten minutes at 600 x g and plasma was collected, protected from UV light, and stored at -20°C and until analysis.

Analytical Procedures

Ammonia Nitrogen

Acidified rumen fluid was thawed at room temperature and clarified by centrifuging at 30,000 x g for 20 min. The clarified ruminal fluid was separated from the sediment and analyzed for NH_4^+ using a modified phenol-hypochlorite reaction adapted from Broderick and Kang (1980) (Appendix A).

β-Hydroxybutyrate

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

Total Volatile Fatty Acids

A 4 mL sample of acidified ruminal fluid was combined with an internal standard for VFA quantification. The internal standard consisted of 1 mL of 25% (wt/wt) meta-phosphoric acid containing 10 g/L 2-ethylbutyric acid. The combined ruminal fluid and meta-phosphoric acid was centrifuged at 30,000 x g for 20 min. A GLC using a Shimadzu GC2010 equipped with a 15-m EC-1000 column with an internal diameter of 0.53 mm and a film thickness of 1.2 μm (Alltech Associates, Inc.; Deerfield, IL) was used to measure the concentrations of each VFA. Both the reagent preparation procedure and temperature gradient for VFA analysis was adapted from Grigsby et al. (1992) and Bateman et al. (2002) (Appendix C).

Plasma Urea Nitrogen

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

Statistical Methods and Calculations

Response variables measured daily were reduced to weekly means prior to analysis. All dependent variables were analyzed using the MIXED procedure (Littell et al., 1996) of SAS. For all variables measured over weeks, the model included treatment, sex, week, and their two- and three-ways interactions as fixed effects. Block within sex was included in the model as a random term. Weekly averages were analyzed as repeated measures using either a heterogeneous or homogeneous first-order auto regressive covariance structure. Calf within block by sex was the

subject of the repeated statement. The covariance structure was selected by choosing the best fitting model according to the Akaike Information Criterion.

Starter intake, body weight, wither height, hip height, BHBA and PUN at day 42 (preweaning) and at day 56 (postweaning) were analyzed including in the model treatment, sex, and its interaction as fixed effects. Block within sex was included into the model as a random term.

Three contrasts statements were built to test: 1) Linear 2) Quadratic and 3) Cubic effect of CSH. Values reported are least square means. Significance was declared at $P \leq 0.05$. When an interaction which included treatment was declared significant, a Tukey adjusted pair wise comparison was conducted among the selected means.

CHAPTER 4

RESULTS AND DISCUSSION

Performance Data

The least squares means for starter intake for calves fed C, 10% CSH, 15% CSH, and 20% CSH are presented in Table 2. Least square means for average daily starter intake per week for calves fed C, 10% CSH, 15%, CSH, and 20% CSH are presented in Figure 1. There was no effect of treatment ($P > 0.05$) on starter intake, but there was a significant effect of time ($P < 0.0001$) on starter intake over the entire trial. As calves increased in age, the amount of starter consumed increased. Both males and females responded similarly to each treatment. Our results compare with those of Coverdale et al. (2004) who reported a significant increase in feed intake over time when 7.5% bromegrass hay or 15% grass hay was used in calf starter. In contrast to the current study, several investigators experienced a significant increase in starter intake when CSH were present in calf starter (Murdock and Wallenius, 1980 (17.6% CSH); Hill et al., 2009 (15% CSH); Van Horn et al., 1976 (15% CSH); and Miller et al., 1969 (10% CSH)).

Least squares means for average body weight for calves fed C, 10% CSH, 15% CSH, and 20% CSH are presented in Table 2 and Figure 2. There was no treatment effect ($P > 0.05$) on the overall body weight of calves but a main effect of time ($P < 0.0001$) was present. As expected, body weight increased with age and feed consumption. These results are in agreement with Van Horn et al. (1976) in which body weight gain increased over time but there was no effect of including 15% CSH in the calf starter. Our results do not correspond with Miller et al. (1969) who observed a significant increase in weight gain when calves were fed 10% CSH in starter. Similarly, Coverdale et al. (2004) reported a significant increase in body weight when 7.5% bromegrass hay was fed with a ground calf starter.

Least squares means for wither and hip height for calves fed C, 10% CSH, 15% CSH, and 20% CSH are presented in Table 2 and Figures 3 and 4. Treatments had no effect ($P > 0.05$) on wither or hip height of calves but there was a main effect of time ($P < 0.0001$) in which both wither and hip height increased with age. Hill et al. (2009) reported similar results in calves fed starter containing 15% CSH when compared to no CSH.

Least squares means for weekly fecal scores for calves fed C, 10% CSH, 15% CSH, and 20% CSH are present in Table 2 and Figure 5. A sex by week interaction occurred ($P < 0.05$) in which the females had lower fecal scores. A main effect of treatment was present (linear; quadratic; $P < 0.05$) showing that as calves consumed the CSH their fecal scores improved when compared to the C. There was a main effect of week ($P < 0.0001$) in which fecal scores improved as calves aged. All fecal scores where within the ranges of typical healthy calves from d 0 to d 56. As the calves consumed more starter, their fecal scores decreased over time across all treatments. There were no health problems observed in the calves in the current study, therefore ruling out any effects that CSH may have had on calf gastrointestinal health.

Rumen Development Data

Least squares means for rumen pH of calves fed C, 10% CSH, 15% CSH, and 20% CSH are presented in Tables 3 and 4 and Figure 6. A sex by treatment interaction ($P < 0.05$) was observed (Table 4), in which the male calves on 20% CSH had a higher pH when compared to the males on C and 10% CSH. Rumen pH was affected by treatment (linear; $P < 0.05$) with calves fed 20% CSH having higher pH then those fed C (Table 3). Rumen pH decreased ($P < 0.0001$) over time for calves on all treatments (Figure 6). These results are in agreement with Hibbs et al. (1956) who fed three different hay to grain ratios (4:1; 3:2; 2:3) and reported an increase in ruminal pH as the amount of hay increased. Beharka et al. (1998) who fed 25%

Table 2. Least squares means for average daily starter intake, body weight (**BW**), wither height, hip height, and fecal scores for calves fed starters containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age.

	Treatment					
Item	C	10% CSH	15% CSH	20% CSH	SEM[1]	P-Value
Starter Intake, g/d						
Preweaning, d 42	790.21	908.97	1042.75	960.95	111.31	0.35
Postweaning, d 56	2040.46	2333.80	2255.95	2477.10	165.68	0.31
Entire Trial	816.33	894.90	918.84	918.40	89.99	0.80
BW, kg						
Preweaning, d 42	59.68	60.76	60.72	58.50	2.02	0.84
Postweaning, d 56	69.21	71.01	71.25	68.77	2.57	0.87
Entire Trial	56.04	56.63	56.73	54.72	1.80	0.83
Wither Height, cm						
Preweaning, d 42	84.41	84.16	85.09	83.47	0.83	0.43
Postweaning, d 56	86.18	86.70	87.94	85.71	0.82	0.12
Entire Trial	82.63	82.58	83.59	81.69	0.74	0.20
Hip Height, cm						
Preweaning, d 42	88.63	88.31	88.63	87.23	0.83	0.45
Postweaning, d 56	90.56	91.00	91.27	89.30	0.84	0.27
Entire Trial	86.68	86.67	87.15	85.74	0.80	0.48
[2]**Fecal Score**[abc]						
Entire Trial	2.41	2.23	2.21	2.30	0.07	0.02

SEM[1] = Standard error of mean

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

[a]Main effect of treatment ($P < 0.05$)

[b]Linear effect due to CSH ($P < 0.05$)

[c]Quadratic effect due to CSH ($P < 0.05$)

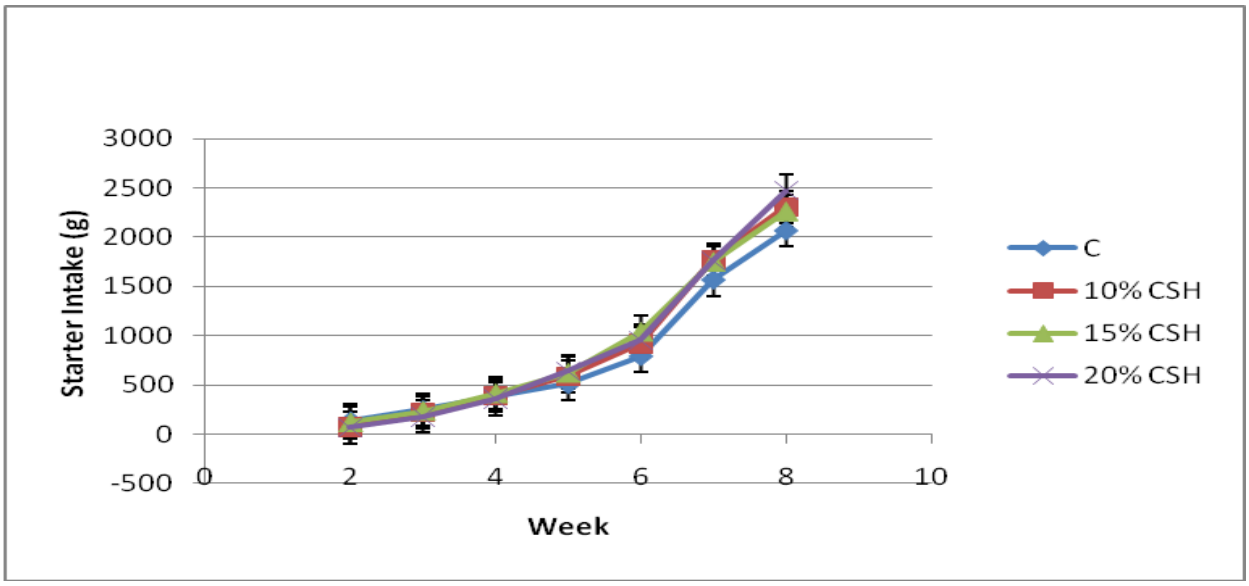

Figure 1. Least squares means for weekly average of calf starter intake for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 163.55

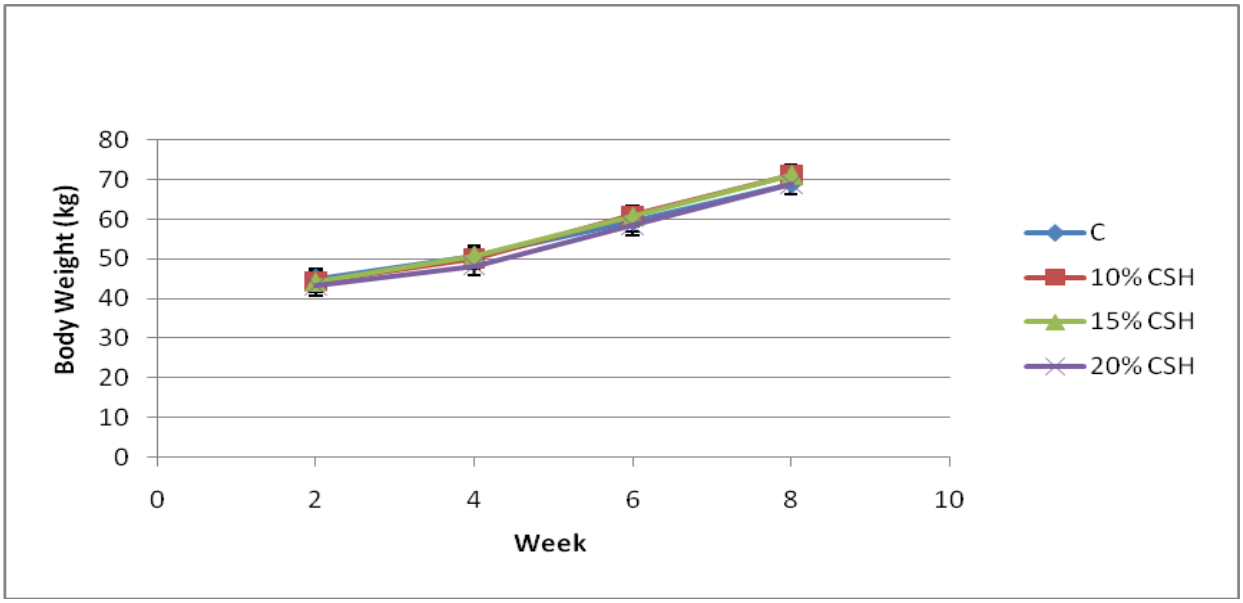

Figure 2. Least squares means for biweekly average of body weight for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 2.55

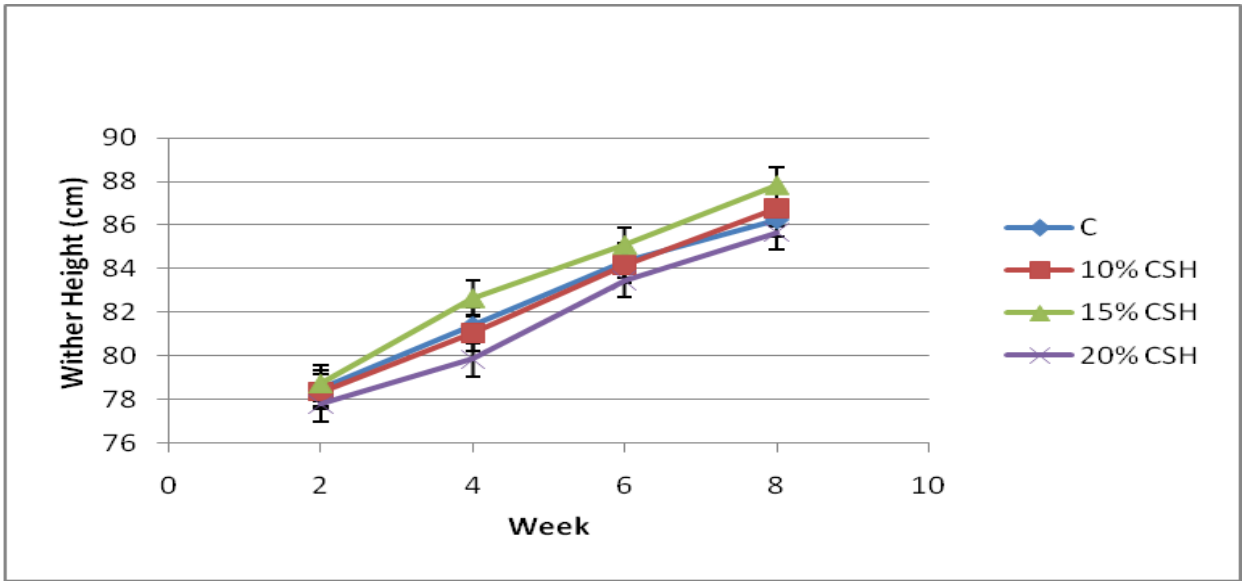

Figure 3. Least squares means for biweekly average of wither height for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 0.80

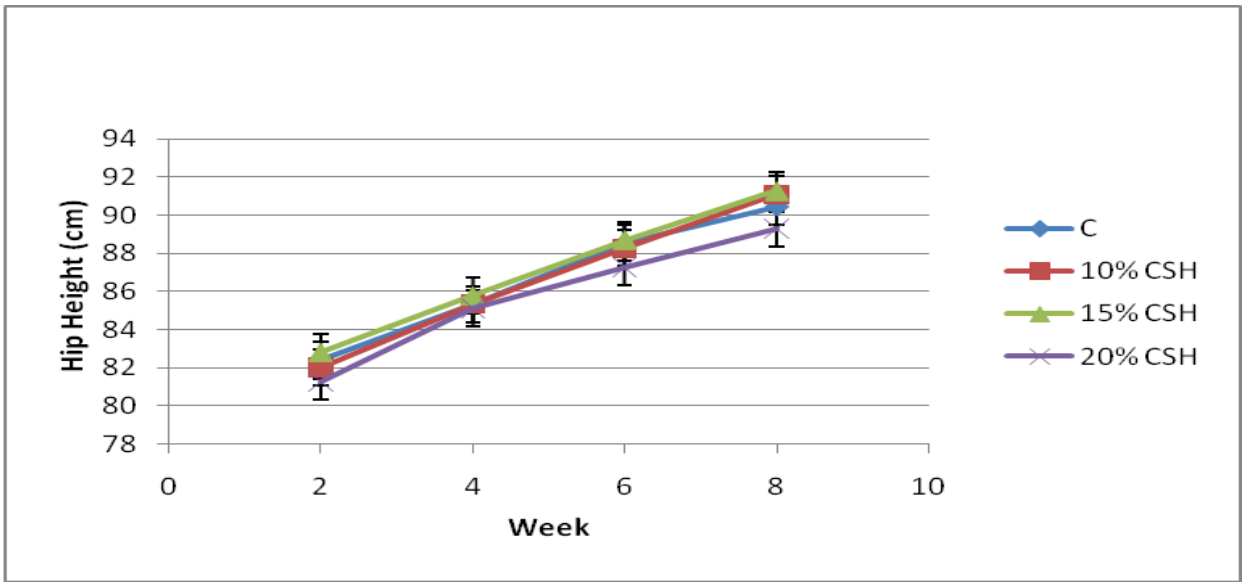

Figure 4. Least squares means for biweekly average of hip height for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 0.95

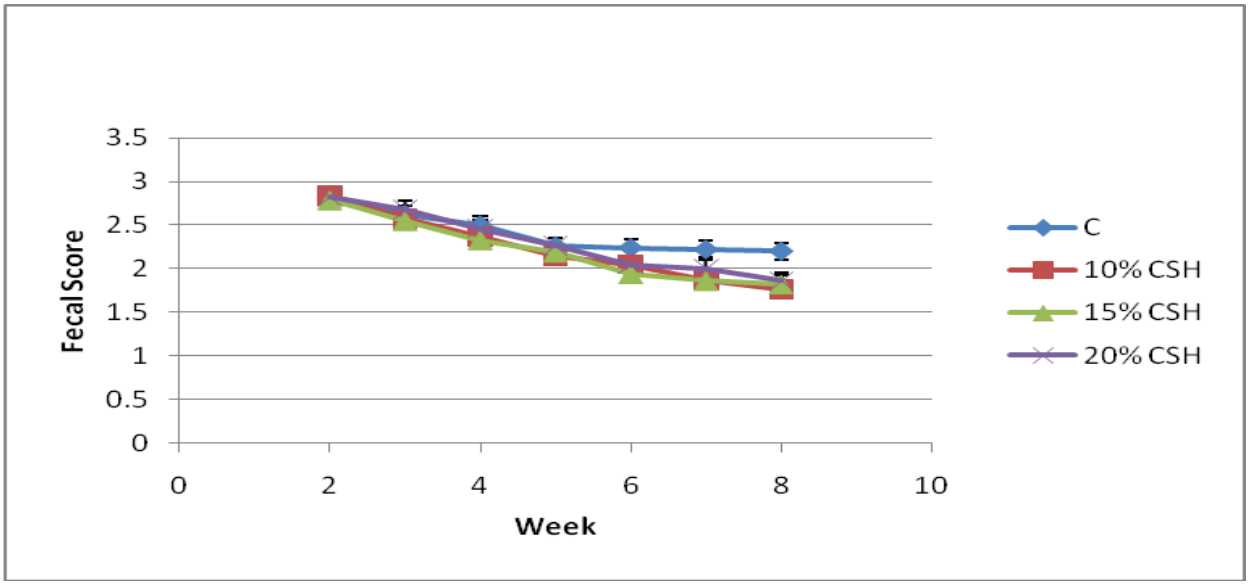

Figure 5. Least squares means for weekly average of fecal scores for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 0.10

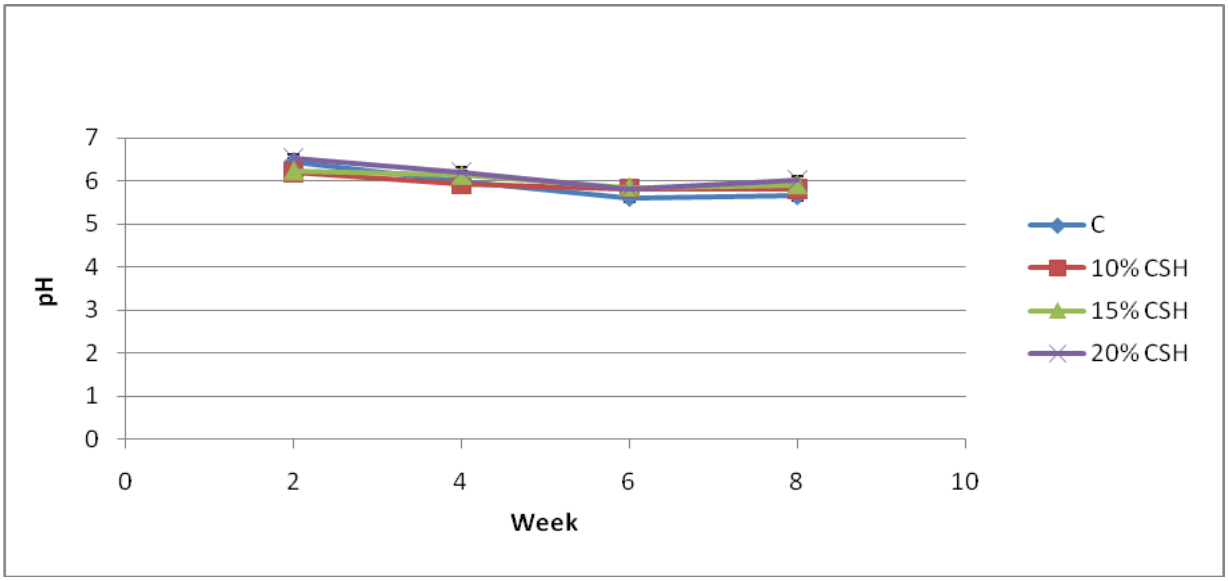

Figure 6. Least squares means for biweekly average of rumen pH for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.001$). SEM = 0.102

Table 3. 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, and NH3 (mg/dL) for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age.

	Treatment					
Item	C	10% CSH	15% CSH	20% CSH	SEM[1]	P-Value
Rumen pH[AB]	5.92[b]	5.94[ab]	6.03[ab]	6.14[a]	0.06	0.03
Acetate, mmol/L	34.81	42.20	39.15	34.58	3.10	0.15
Propionate, mmol/L	28.44	34.21	30.82	28.33	3.09	0.48
Butyrate, mmol/L	4.62	5.89	5.43	5.43	0.61	0.37
Total VFA, mmol/L	72.70	86.48	80.18	72.13	7.63	0.23
Acetate, molar %	50.42	50.05	51.83	52.10	1.02	0.32
Propionate, molar %	37.36	37.49	36.72	35.82	1.00	0.35
Butyrate, molar %	6.29	7.02	6.54	6.96	0.41	0.53
NH3, mg/dL[AB]	9.59	10.15	7.58	6.98	0.86	0.01

SEM[1] = Standard Error of the Mean

[A]Main effect of treatment ($P < 0.5$)

[B]Linear effect due to CSH ($P < 0.5$)

[a,b]Means with a row with different superscripts differ ($P < 0.05$)

Table 4. Least squares means for performance and metabolic indications of rumen development for male vs. female calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age.

Item	Treatment									
	C		10% CSH		15% CSH		20% CSH		SEM[1]	P-Value
	M	F	M	F	M	F	M	F		
n	6	10	6	10	6	10	6	10		
Starter Intake, g/d	870.20	762.46	847.34	942.46	968.85	868.84	819.59	1017.21	142.21	0.48
Rumen pH[A]	5.86[b]	5.92[ab]	5.85[b]	6.02[ab]	6.05[ab]	6.01[ab]	6.28[a]	6.00[ab]	0.071	0.03
Acetate, mmol/L[B]	31.70	37.92	35.17	42.23	31.65	46.65	23.49	45.68	4.80	0.02
Propionate, mmol/L[B]	22.92	33.97	27.85	40.58	23.86	37.77	17.34	39.31	4.89	0.58
Butyrate, mmol/L[B]	4.11	5.13	5.52	6.25	4.17	6.69	3.88	6.99	0.96	0.05
Total VFA, mmol/L[B]	66.34	79.06	71.93	101.02	63.46	96.91	48.75	95.51	9.47	0.01
Acetate, Molar %	51.62	49.21	49.11	50.98	52.12	51.53	54.21	49.99	1.62	0.13
Propionate, Molar %	35.29	39.42	36.63	38.36	35.61	37.83	33.61	38.03	1.57	0.46
Butyrate, Molar % [A]	5.74	6.85	7.92	6.11	6.31	6.77	6.31	7.62	0.66	0.03
NH3	8.37	10.81	10.01	10.29	7.32	7.84	6.31	7.66	1.35	0.75
BHBA	0.19	0.15	0.19	0.14	0.18	0.17	0.19	0.19	0.02	0.5
PUN	11.77	14.89	12.90	14.82	12.50	14.16	11.24	14.41	1.05	0.17

SEM[1] = Standard Error of the Mean
[A] Sex by treatment interaction ($P < 0.05$)
[B]Main effect of sex ($P < 0.05$)
[a,b,c]Means within a row with different superscripts differ ($P < 0.05$)

alfalfa hay at different particle sizes, reported a decreased in ruminal pH with age of calf regardless of the fiber level in the diet. Rumen pH and age of calf are considered inversely related; therefore as the calf increases in age the rumen pH will decrease as the rumen begins to develop. It was expected that the rumen pH would increase slightly as the consumption of CSH increased due to the change of the rumen microbial population and fiber content. It is unknown as to why the sex by treatment interaction occurred. Fiber should have similar effects on the rumen of both male and female animals.

Least squares means of rumen concentrations of acetate, propionate, and butyrate for calves fed C, 10% CSH, 15% CSH, and 20% CSH are presented in Tables 3 and 4 and Figures 7, 9, and 11. A sex by week interaction occurred for propionate ($P < 0.05$) in which the female calves produced more propionate over time. There was no effect of treatment ($P > 0.05$) on acetate, propionate, and butyrate but a quadratic response ($P < 0.05$) for acetate was observed. A significant effect of time for acetate ($P < 0.0001$), propionate ($P < 0.0001$), and butyrate ($P < 0.0001$) was present showing that VFA concentrations increased as the rumen developed. These results are in agreement with Coverdale et al. (2004) and Beharka et al. (1998) who saw similar results when forages were fed in a complete calf starter. VFA concentrations increased as starter intake increased especially during postweaning. These results are in agreement with Greenwood et al. (1997), Klein et al. (1987), Coverdale et al. (2004), and Laborde et al. (2008) who have all reported an increase in VFA concentration when starter intake increased. There was a main effect of sex for acetate ($P < 0.05$), propionate ($P <0.0001$), butyrate ($P < 0.05$) with the females producing more acetate, propionate and butyrate. The increased concentration of the VFA in female calves could be attributed to more females on the study and should have little biological significance. For total VFA production, there was a sex by week interaction ($P < 0.05$) which

the female calves produced more VFA over time. Overall, there was no treatment effect on total VFA concentrations ($P > 0.05$), but there was a main effect of time ($P < 0.0001$) and sex ($P < 0.0001$) with the females having higher total VFA concentrations. The increase in total VFA concentrations over time is in agreement with Coverdale et al. (2004) who fed 7.5% bromegrass hay or 15% hay in a calf starter.

Least squares means of molar percentages for acetate, propionate, and butyrate for calves fed C, 10% CSH, 15% CSH, and 20% CSH are present in Tables 3 and 4 and Figures 8, 10, and 12. There was a sex by treatment interaction ($P < 0.05$) for molar percent butyrate present; however when pair wise comparison were made, using a Tukey adjustment, the treatments means were not significantly different from each other. A main effect of time was observed for molar percent acetate ($P < 0.0001$), propionate ($P < 0.0001$), and butyrate ($P < 0.05$). Coverdale et al. (2004) observed a similar increase in molar percent acetate and a decrease in molar percent propionate as the amount of forage increased in calf starter diets.

Least squares means of rumen NH3 concentrations for calves fed C, 10% CSH, 15% CSH, and 20% CSH are present in Table 3 and 4 and Figure 13. A treatment effect (linear; $P < 0.05$) on rumen NH3 concentrations was present with calves on treatments C, 10% CSH, and 20% CSH. In contrast, Beharka et al. (1998) experienced no treatment effect for NH3 concentrations when 25% alfalfa hay was fed in a calf starter. There was a main effect of week ($P < 0.0001$) present in which as the calves aged and the rumen began to develop, the NH3 levels decreased as evidence by higher levels of NH3 during the preweaning period compared to the postweaning period. The increased NH3 levels before weaning can be attributed to the consumption of milk replacer (Anderson et al., 1987b) and lack of rumen fermentation.

Least squares means of BHBA for calves fed C, 10% CSH, 15% CSH, and 20% CSH are

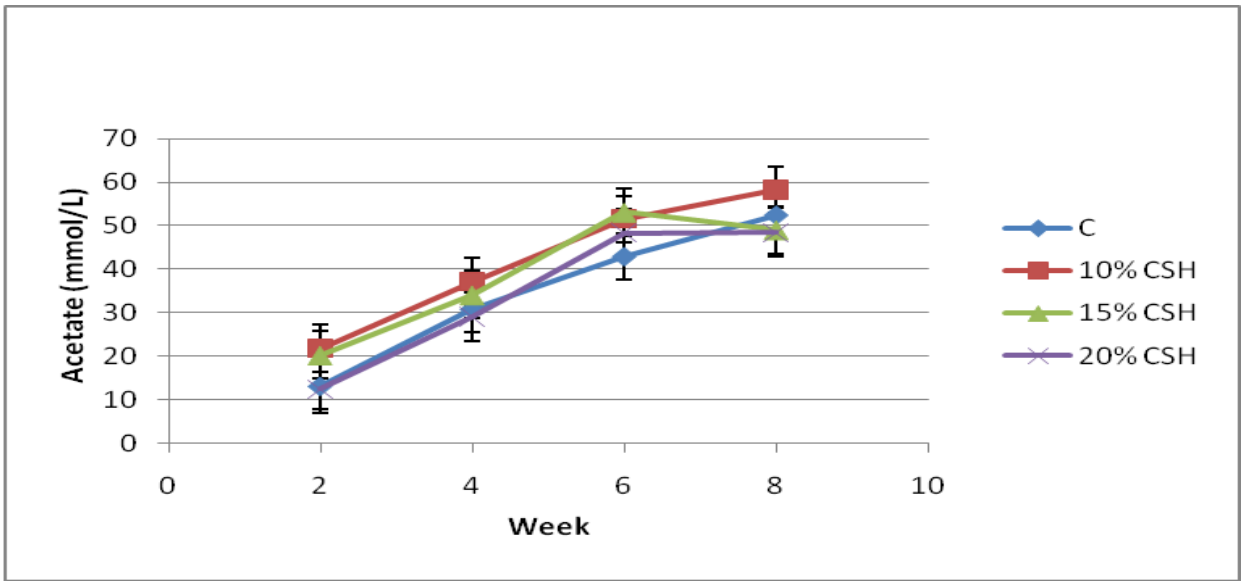

Figure 7. Least squares means for biweekly average of acetate concentrations for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 5.61

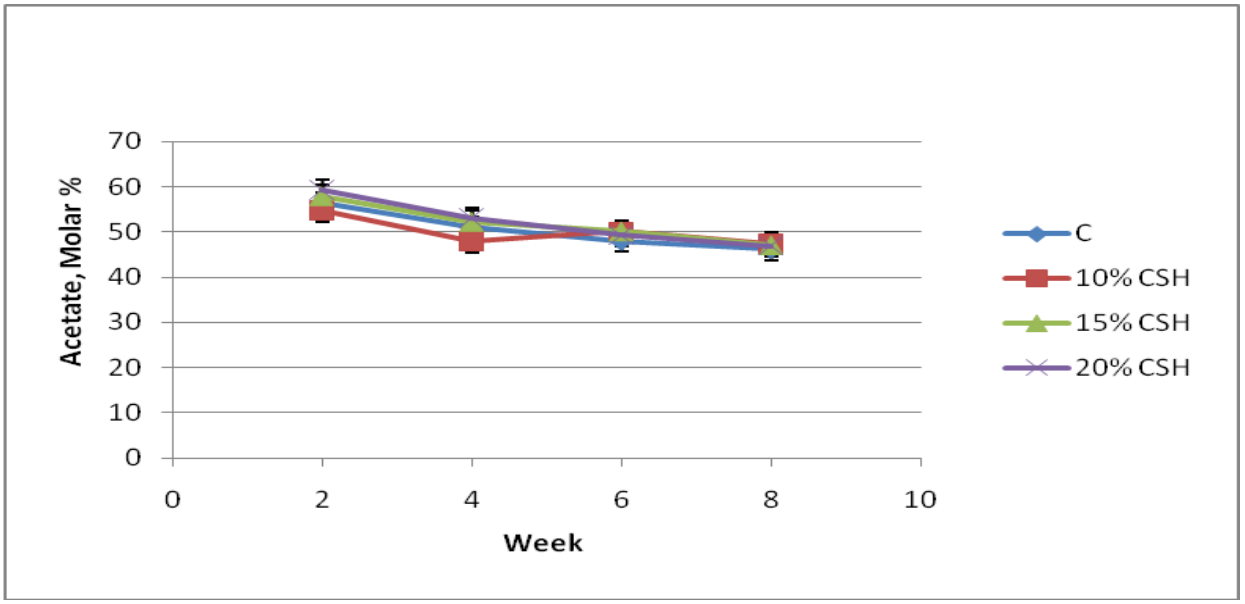

Figure 8. Least squares means for biweekly average of molar percent of acetate for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 2.41

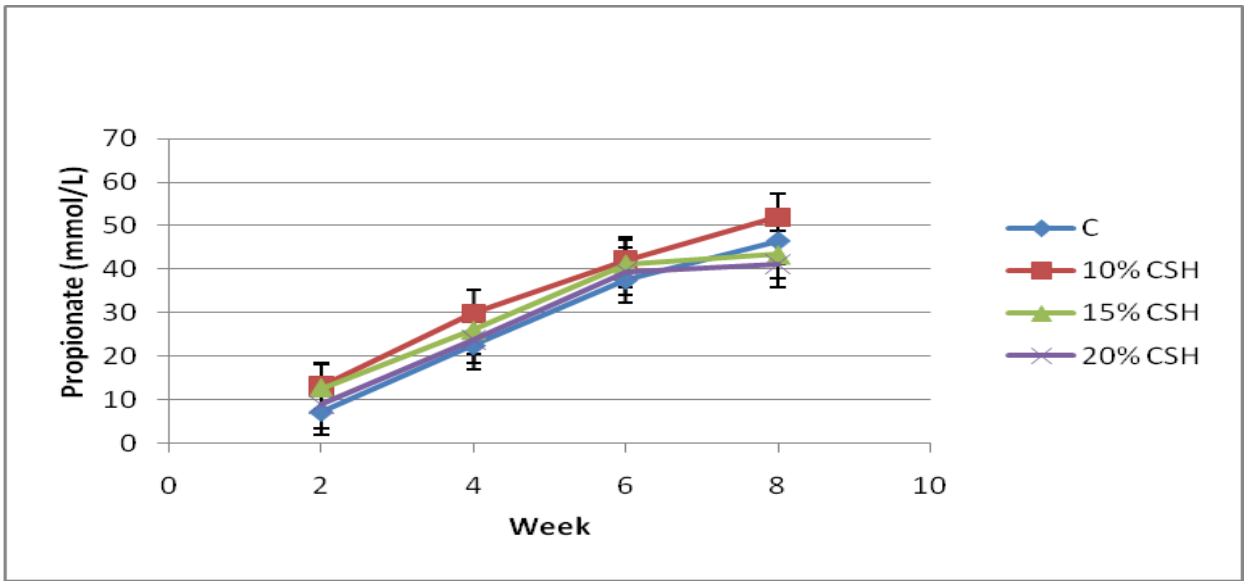

Figure 9. Least squares means for biweekly average of propionate concentrations for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 5.46

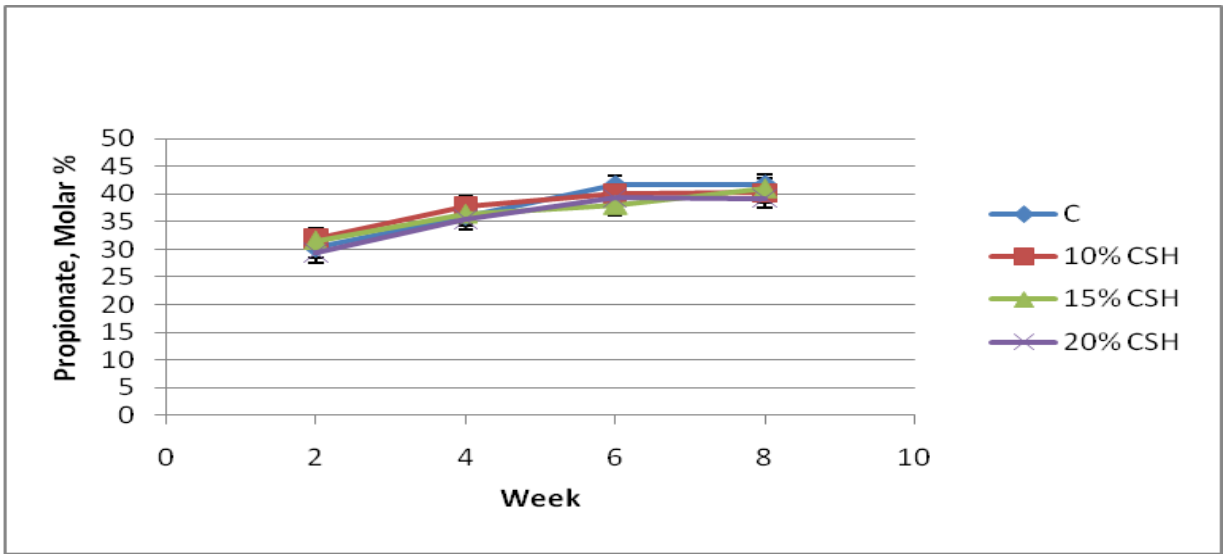

Figure 10. Least squares means for biweekly average of molar percent of propionate for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 1.80

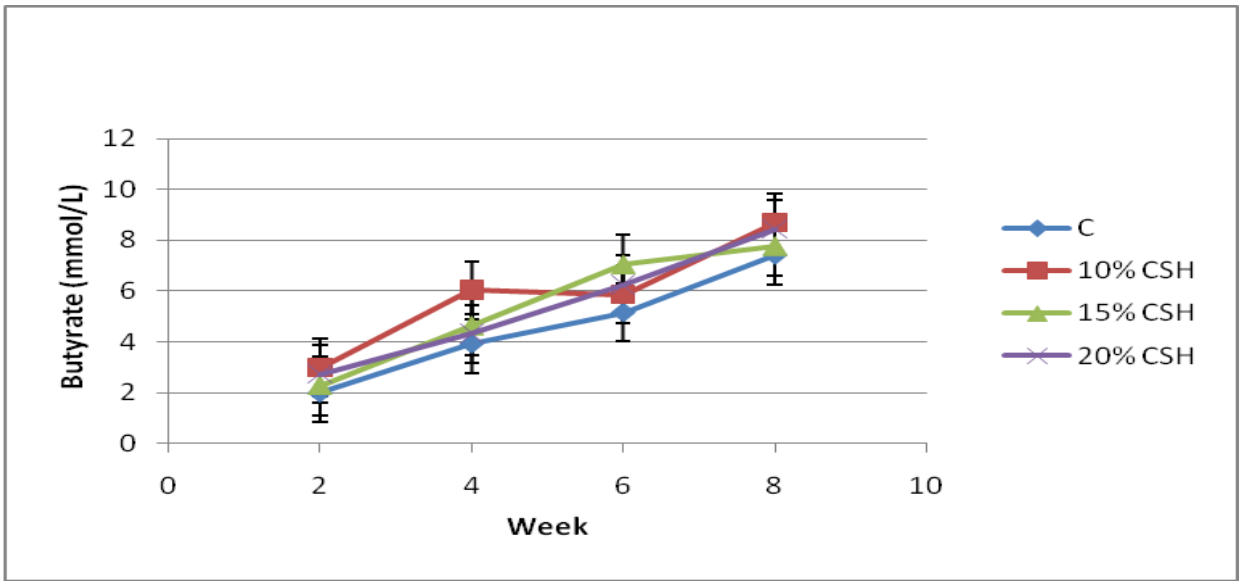

Figure 11. Least squares means for biweekly average of butyrate concentrations for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 1.17

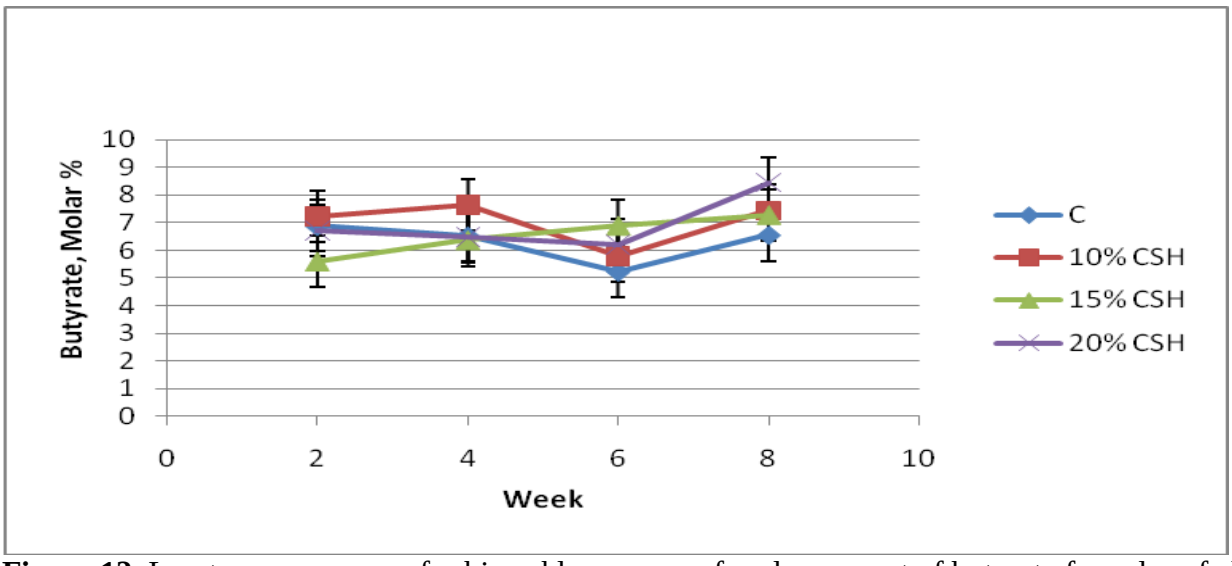

Figure 12. Least squares means for biweekly average of molar percent of butyrate for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.05$). SEM = 0.93

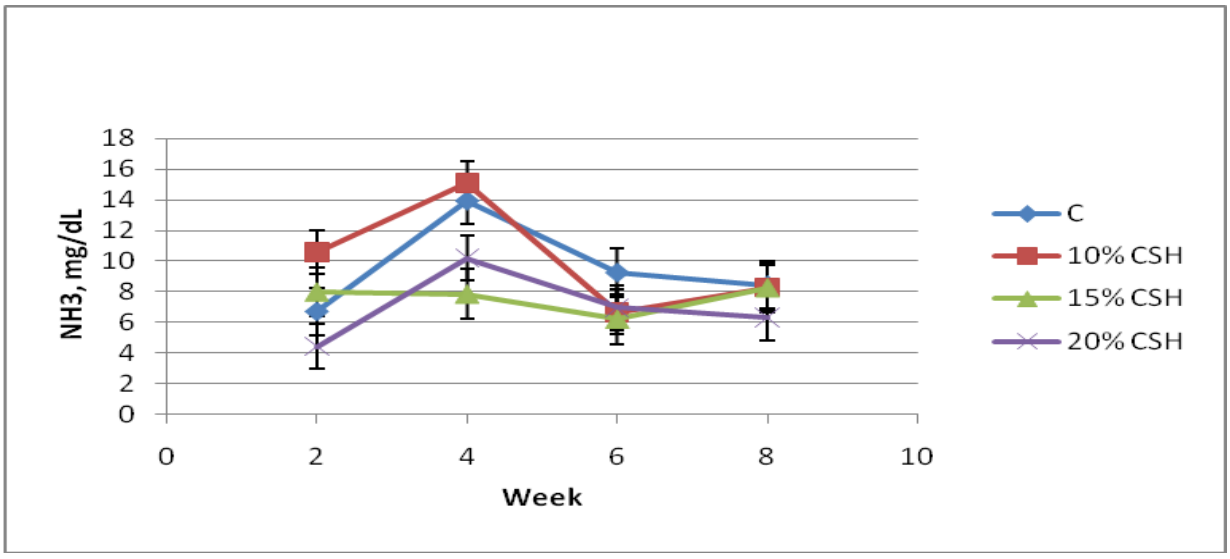

Figure 13. Least squares means for biweekly average of NH3 for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 1.62

presented in Tables 4 and 5 and Figure 14. There was no effect of treatment ($P > 0.05$) on plasma concentrations of BHBA over the entire trial, but there was an increase in plasma BHBA concentrations over time ($P < 0.0001$). These results are similar to Coverdale et al. (2004) who reported an increase in BHBA with age when 7.5% bromegrass hay or 15% hay was fed in a calf starter. In contrast, Quigley et al. (1992) observed a decrease in BHBA levels when calves consumed hay *ad libitum*. During the preweaning period, calves had lower concentrations of BHBA than the postweaning period. A possible reason for this is the reduction of microbial fermentation in the rumen during the preweaning period (Baldwin et al., 2004). Therefore, as calves are weaned, there is an increase in microbial fermentation which increases the conversion of butyrate to BHBA through rumen absorption.

The least squares means of PUN for calves fed C, 10% CSH, 15% CSH, and 20% CSH are presented in Tables 4 and 5 and Figure 15. Over the entire trial, there was no effect of

treatment ($P > 0.05$). This is in agreement with Klein et al. (1987) who observed no difference in PUN levels when alfalfa hay was fed to calves on different weaning systems. There was a main effect of time ($P < 0.0001$) with higher PUN levels during the postweaning period than the preweaning period. Therefore, PUN levels increased as calves increased with age. This is in agreement with Hayashi et al. (2006) who also observed an increase in PUN concentrations as the calves aged. This effect is due to the recycling of urea and the absorption of urea as the rumen begins to develop. The increased PUN concentrations correspond with the reduced NH3 levels. As NH3 levels decrease, PUN levels should increase due to the synthesis of PUN from NH3 through the liver (Hayashi et al., 2006). A main effect of sex ($P < 0.05$) during the preweaning period and over the entire trial was also observed with the female calves having higher PUN levels. All PUN levels were within a normal range therefore the increased PUN levels in the females could be attributed to more females on the study and should have little biological significance in this study.

Table 5. Least squares means for BHBA (mmol/L) and PUN (mg/dL) for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age.

	Treatment					
Item	C	10% CSH	15% CSH	20% CSH	SEM[1]	P-Value
BHBA, mmol/L						
Preweaning, d 42	0.16	0.15	0.18	0.15	0.02	0.52
Postweaning, d 56	0.27	0.25	0.27	0.33	0.03	0.17
Entire Trial	0.17	0.16	0.18	0.19	0.01	0.59
PUN, mg/dL						
Preweaning, d 42	14.22	14.68	15.20	13.45	1.24	0.78
Postweaning, d 56	14.47	15.46	14.78	14.98	1.05	0.91
Entire Trial	13.33	13.86	13.33	12.83	0.67	0.68

SEM[1] = Standard Error of the Mean

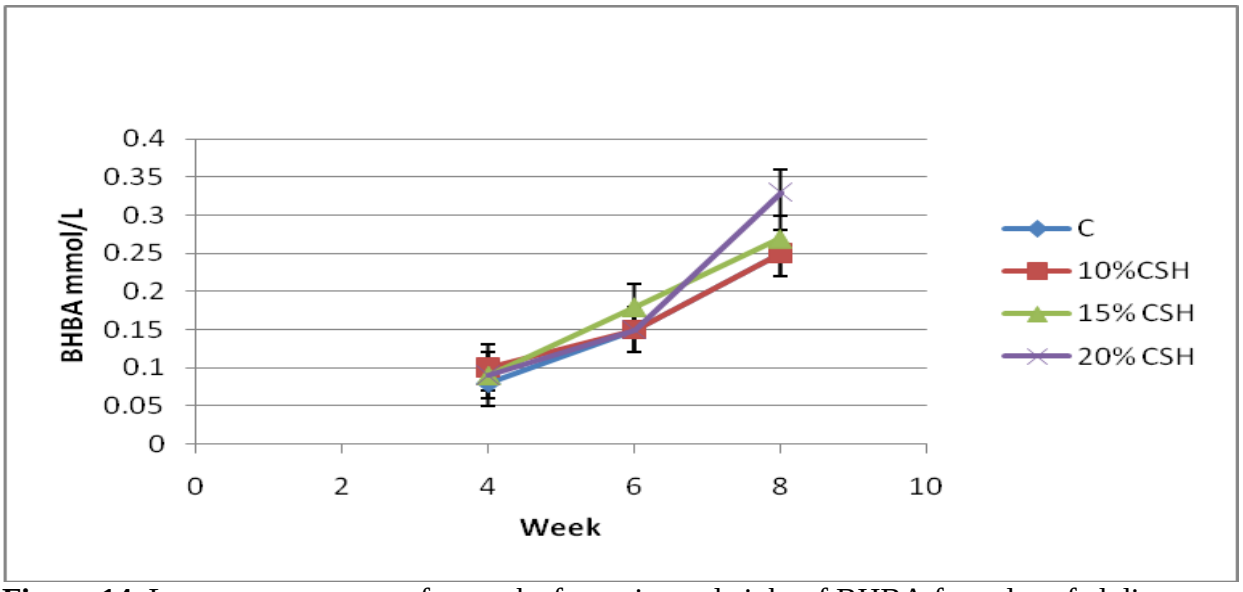

Figure 14. Least squares means for weeks four, six, and eight of BHBA for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 0.034

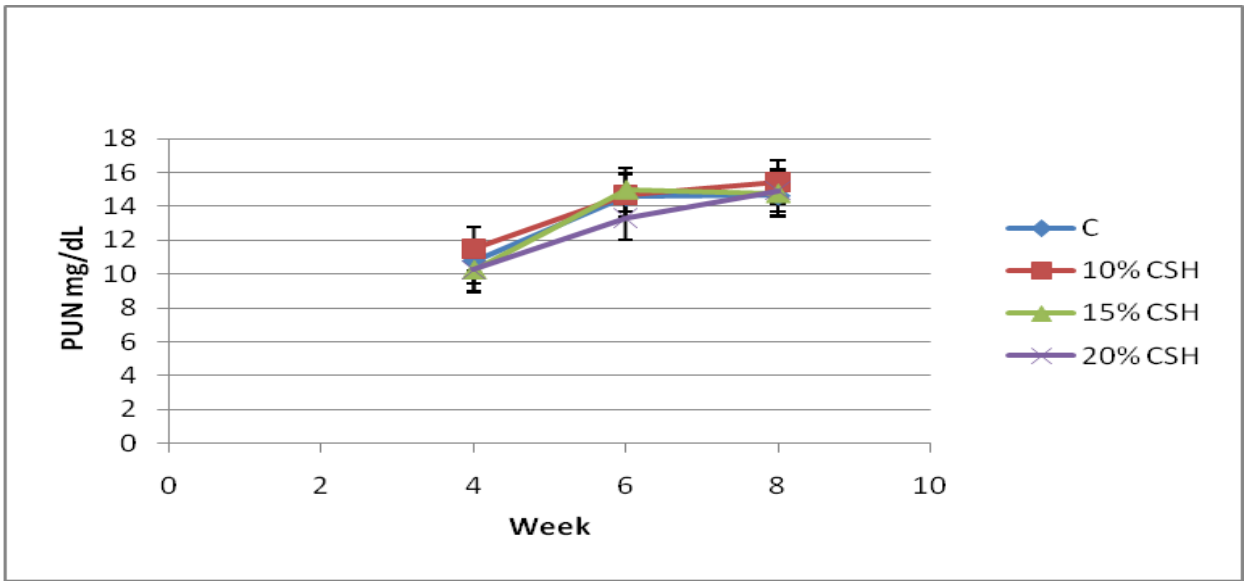

Figure 15. Least squares means for weeks four, six, and eight of PUN for calves fed diets containing no cottonseed hulls (C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), and 20% cottonseed hulls (20% CSH) through d 56 of age. There was a main effect of time ($P < 0.0001$). SEM = 1.31

CHAPTER 5

SUMMARY AND CONCLUSIONS

Summary

A study was conducted to determine the effects of varying levels of CSH on growth and metabolic indications of rumen development of dairy calves. Sixty-four Holstein calves (heifers n=40; bulls n=24) were randomly assigned to one of four calf starter treatments which included no cottonseed hulls (control), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), or 20% cottonseed hulls (20% CSH). Calves were separated from their dams on d 0, weighed, vaccinated, and individually housed in a 2.5m^2 calf hutches with a 2.8 m^2 wire enclosure on rock bedding until d 56 of age.

Feed intake and fecal scores were recorded twice daily beginning on d 6 until d 56 of age. Body weights were measured at birth and biweekly thereafter until d 56 of age. Wither and hip height were measured biweekly beginning on d 14 until d 56 of age. Rumen fluid was collected biweekly beginning on d 14 through d 56. Rumen fluid was analyzed for pH, VFA, and NH3. Blood was collected biweekly starting on d 14 through d 56 for the analysis of BHBA and PUN.

The overall mean average daily starter intake was not affected ($P > 0.05$) by the inclusion of 10% CSH, 15% CSH, or 20% CSH in the calf starter. Though the fecal scores were affected ($P < 0.05$) by the incorporation of CSH in the calf starter, all fecal scores were within normal range for healthy calves. The overall mean of body weight, wither height, and hip height were not affected ($P > 0.05$) with the inclusion of CSH in calf starter.

There was a significant treatment effect ($P < 0.05$) on rumen pH in which the addition of CSH in calf starter caused the rumen pH to increase. Although a sex by treatment ($P < 0.05$) interaction occurred, rumen pH was still within normal ranges and should have little biological

significance in this study. There was no treatment effect ($P > 0.05$) on acetate, propionate, butyrate, and total VFA, however there was an effect of sex ($P < 0.05$) for acetate, propionate, and butyrate in which the female calves had greater concentrations then the male calves. A sex by week interaction occurred for propionate and total VFA production. There was a main effect of sex ($P < 0.0001$) for molar percentage of propionate which propionate decreased over time. Also, there was a sex by treatment interaction ($P < 0.05$) for molar percent of butyrate. Although differences were observed between both male and female calves, all VFA levels were within normal range and should have little biological significance on this study. There was a treatment effect ($P < 0.05$) on NH3 concentrations with the calves on C, 10% CSH, and 20% CSH treatments having lower NH3 levels.

There was no effect ($P > 0.05$) of CSH on plasma BHBA production. A main effect of sex ($P < 0.05$) was present in which the males had higher levels of BHBA when compared to the females. There was no significant treatment effect ($P > 0.05$) on PUN production but a main effect of sex ($P < 0.5$) was observed. Both BHBA and PUN concentrations were within normal ranges.

Conclusions

These data suggest that the addition of CSH in calf starter rations seems to have little effect on calf growth and rumen development. According our study, the only effects that CSH have on a young dairy calf is ruminal pH, fecal scores, and NH3 concentrations. It seems that the increase in ruminal pH could possibly be due to increased saliva flow from consuming CSH therefore buffering the rumen. Improved fecal scores through the feeding of CSH may have a positive effect on gut health but extensive research is needed. Though there was a treatment

effect on NH3 concentrations, rumen development was not affected by the addition of CSH therefore the benefit of CSH on NH3 is not apparent.

REFERENCES

Anderson, M. J., M. Khoyloo, and J. L. Walters. 1982. Effect of feeding whole cottonseed on intake, body weight, and reticulorumen development of young holstein calves. J. Dairy Sci. 65:764-772.

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

Anderson, K. L., T. G. Nagaraja, J. L. Morill, T. B. Avery, S. J. Galitzer, and J. E. Boyer. 1987b. Ruminal microbial development in conventionally or early-weaned calves. J. Anim. Sci. 64:1215-1226.

AOAC. 1980. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists. George Banta Company, Inc., Menasha, WI.

Bach, A., A. Gimenez, J. L. Juaristi, and J. Ahedo. 2007. Effects of physical form of a starter for a dairy replacement calves on feed intake and performance. J. Dairy Sci. 90:3028-3033.

Baldwin, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87:(E. Suppl.):E55-E65.

Bartley, E. E. 1973. Effects of a self-fed pelleted mixture of hay and calf starter on the performance of young dairy calves. J. Dairy Sci. 56:817-820.

Bateman, H. G., C. C. Williams, and 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.

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

Broderick, G. A. and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in rumen fluid and in vitro media. J. Dairy Sci. 63:64-75.

Brown, W. H., F. M. Whiting, B. S. Daboll, R. J. Turner, and J. D. Schuh. 1977. Pelleted and nonpelleted cottonseed hulls for lactating dairy cows. J. Dairy Sci.60:919-923.

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

Colenbrander, V. F., D. L. Hill, M. L. Eastridge, and D. R. Mertens. 1986. Formulating dairy rations with neutral detergent fiber. 1. Effect of silage source. J. Dairy Sci. 69:2718-2722.

Coverdale, J. A., H. D. Tyler, J. D. Quigley, and 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.

Fahey, Jr., G. C., and L. L. Berger. 1993. "Protein Metabolism of Ruminant Animals." *Ruminant Animal: Digestive Physiology and Nutrition*. Waveland Press, Inc. pp227-297.

Greenwood, R. H., J. L. Morrill, E. C. Titgemeyer, G. A. Kennedy. 1997. A new method of measuring diet abrasion and its effect on the development of the forestomach. J. Dairy Sci. 80:2534-2541.

Grigsby, K. N., M. S. Kerley, J. A. Paterson, and J.C. Weigel. 1992. Site and extent of nutrient digestion by steers fed a low-quality bromegrass hay diet with incremental levels of soybean hull substitution. J. Anim. Sci. 70:1941-1949.

Gu, S. C., B. R. Moss, and J. C. Lin. 1996. "Cottonseed Hulls as a Feed Source for Dairy Cattle." *Highlights of Agricultural Research*. Alabama Agricultural Experiment Station. 43: 3.

Hamada, T., S. Maeda, and K. Kameoka. 1976. Factors influencing growth of rumen, liver, and other organs in kids weaned from milk replacers to solid foods. J. Dairy Sci. 59:1110-1118.

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

Heinrichs, A. J. and K.E Lesmeister. 2005. "Rumen Development in the Dairy Calf." *Calf and Heifer rearing*. Nottingham: Nottingham University Press. pp53-65.

Heinrichs, A. J. and K.E Lesmeister. "Why you should hold off on feeding forage to calves." Hoard's Dairyman. Sept 25, 2000.

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

Hibbs, J. W., W. D. Pounden, and H. R. Conrad. 1953. A high roughage system for raising calves based on the early development of rumen function. I. effect of variations in the ration on growth, feed consumption, and utilization. J. Dairy Sci. 36:717-727.

Hill, S. R., B. A. Hopkins, S. Davidson, S. M. Bolt, D. E. Diaz, C. Brownie, T. Brown, G.B Huntington, and 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.

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

Jurgens, M. H. and K. B. Bregendahl. 2007. "Feedstuffs Used in Livestock Diets." *Animal Feeding and Nutrition*. Kendall/Hunt Publishing Company. pp 258-259.

Kendall, C., C. Leonardi, P. C. Hoffman, and D. K. Combs. 2009. Intake and milk production of cows fed diets that differed in dietary neutral detergent fiber and neutral detergent fiber digestibility. J. Dairy Sci. 92:313-323.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, J. K. Ha, H. G. Lee, Y. J. Choi. 2007. Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:876-885.

Kincaid, R. L. 1980. Alternate methods of feeding alfalfa to calves. J. Dairy Sci. 63:91-94.

Klein, R. D., R. L. Kincaid, A. S. Hodgson, J. H. Harrison, J. K. Hillers, and J. D. Cronrath. 1987. Dietary fiber and early weaning on growth and rumen development of calves. J. Dairy Sci. 70:2095-2104.

Lane, M. A. and B. W. Jesse. 1997. Effect of volatile fatty acid infusion on development of the rumen epithelium in neonatal sheep. J. Dairy Sci. 80:740-746.

Lane, M. A., R. L. Baldwin, and B. W. Jesse. 2000. Sheep rumen metabolic development in response to age and dietary treatments. J. Anim. Sci. 78:1990-1996.

Laborde, J. M. 2008. Effects of probiotics and yeast culture on rumen development and growth of dairy calves. MS Thesis. Louisiana State University, Baton Rouge.

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

Lengemann, F. W. and N. N. Allen. 1955. The development of rumen function in the dairy calf. I. Some characteristics of the rumen contents of cattle of various ages. J. Dairy Sci. 38:651-656.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® System for Mixed Models. pp 31□63. SAS Inst. Inc., Cary, NC.

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

McDonald, I. W. 1952. The role of ammonia in ruminal digestion of protein. J. Biochem. 51:86-90.

Miller, W. J., Y. G. Martin, and P. R. Fowler. 1969. Effects of addition of fiber to simplified and to complex starters fed to young dairy calves. J. Dairy Sci. 52:672-676.

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

National Research Council. 2001. "Nutrient Requirements of the Young Calf." *Nutrient Requirements of Dairy Cattle*. National Academy Press. pp 36-40.

Nolan, J. V. and R. A. Leng. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Br. J. Nutr. 27:177-194.

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

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

Quigley, J. D., T. M. Steen, and S. I. Boehms. 1992. Postprandial changes of selected blood and ruminal metabolites in ruminating calves fed diets with or without hay. J. Dairy Sci. 75:228-235.

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

Sander, E. G., R. G. Warner, H. N. Harrison, and J. K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. J. Dairy Sci. 42:1600-1605.

SAS. 2003. SAS/STAT User's Guide (Version 9.1.3 Service Pack 4). SAS Inst. Inc., Cary, NC.

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

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

Van Horn, H. H., M. B. Olayiwole, C. J. Wilcox, Barney Harris Jr., and J. M. Wing. 1976. Effects of housing, milk, feeding management, and ration formulation on calf growth and feed intake. J. Dairy Sci. 59:924-929.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

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

Warner, R. G., W. P. Flatt, and J. K. Loosli. 1956. Dietary Factors influencing the development of the ruminant stomach. J. Agric. Food Chem. 4:788-792.

Yokoyama, M. T. and K. A. Johnson. 1993. "Microbiology of the Rumen and Intestine." *The Ruminant Animal: Digestive Physiology and Nutrition*. Waveland Press, Inc. pp125-144.

APPENDIX A

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 of running this assay. Phenol is a cancer-causing agent 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 nitroferricyanide (sodium nitroprusside) in 1.5 L of distilled H_2O (**dH_2O**). 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 dH_2O and store in brown glass bottle. Phenol needed if 29.7g. Use goggles when measuring phenol and be careful. Phenol can cause burn when it comes into contact with skin.

Hypochlorite Reagent

Dissolve 15g of sodium hydroxide in approximately 2 L of dH_2O. Add 113.6 g of disodium phosphate heptahydrate ($Na_2HPO4•7H_2O$) to this solution using mild heating and mixing. After the disodium phosphate has mixed, allow the solution to cool. After cooling, add 150 mL of commercial bleach (5.25% sodium hypochlorite, 131.25 mL if using 6% bleach) and mix thoroughly. Bring solution to 3 L by adding dH_2O. Filter solution through #1 filter paper and store in polyethylene bottle protected from light.

Ammonia Standard Solution

A stock solution of 100m*M* (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 m*M* concentration desired in working standard to 100 mL total using dH_2O.

Procedure

1) Sample of ruminal fluid will need to be diluted with dH_2O prior to analysis to bring the concentration of NH_3 into the working range of this assay. Therefore, mix 0.5 mL of clarified ruminal fluid with 4.5 mL of dH_2O and use these samples for the reaction.
2) Add 0.5 mL of sample or standard into test tube (use dH_2O for blanks).
3) Add 2.5 mL 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 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.**

APPENDIX B

β-HYDROXYBUTYRATE COLORMETRIC ASSAY

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

Reagents:

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

Procedure:

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

Calculation

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

APPENDIX C

ANALYSIS OF VOLATILE FATTY ACIDS IN RUMINAL FLUID

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

Reagents

1) 25% (wt/vol) metaphophoric acid (fluka #79615) solution containing 2 g/L of 2-ethyl butyric acid (216.5 µL 2-EB to 100 mL m-phos acid solution; Aldrich #10, 995-9).
2) VFA standard
    a. Add the following volumes of acids to a 100-mL volumetric flask and fill volume with dH_2O. Store in refrigerator when not in use.

MW	Acid	Volume (µL)	Conc (g/L)	Conc (m*M*)
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

Centrifuge strained ruminal fluid at 30,000 x g for 20 min (this step may be skipped).

1) Mix 4 mL of rumen fluid supernatant with 1 mL of m-phosphoric acid solution containing 2-EB.
2) Allow to stand in ice bath for 30 min (this step may be skipped).
3) Centrifuge at 30,000 x g for 20 min.
4) Remove the supernatant for GC analysis.
5) 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 provide a correction factor of 1.25).

For use on Shimadzu GC, samples should be in 2 mL autosampler vials. The optimal vials that we have used are ordered from Cole-Parmer. They are Target autosampler vials (#A98810-00). These are 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.

APPENDIX D

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 labeled; unknown, control, standard, blank.
2) Add 0.010 ml (10 µL) of sample to its 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 for 10 minutes at room temperature 2- 26°C).
4) Add 2.0 mL of BASE RGT, mix and incubate at 37°C for 5 min. (Alternative: React for 10 minutes at room temperature 2- 26°C).
5) Set the wavelength of the photometer at 630nm and zero the photometer with the BLANK. Read and record the absorbances of all vials and proceed to the Calculation with Example below.

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

Calculation:

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

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

VITA

Ryan Michael Doescher was born in November 1984, in Metairie, Louisiana, to Eric and Sandra Doescher. After graduating from Brother Martin High School, in New Orleans, in May 2003, he began his undergraduate studies in animal sciences at Louisiana State University in August 2003. In December 2007, he received his Bachelor of Science degree in animal science. After graduating, he began his graduate studies at Louisiana State University in dairy calf nutritional physiology. He will receive the degree of Master of Science in May of 2010.