ABSTRACT

Hill, Stephanie Rene. The Effects of Cottonseed Hulls Added to Diets With and Without Live Yeast or Mannanoligosaccharide in Young Calves (Under the direction of Dr. B. A. Hopkins and Dr. L. W. Whitlow).

The objective of this study was to investigate the effects of fiber in the form of cottonseed hulls (**CSH**) added to the starter and of live yeast (**YST**) or mannanoligosaccharide (**MOS**) added to milk, on growth, intake, rumen development, and health parameters in neonatal calves. Holstein (n = 116) and Jersey (n = 46) bull and heifer calves were assigned randomly at birth to one of six treatments. Calves continued on trial through 63 d. Bulls were castrated by 14 d. All calves were fed 3.8 L of colostrum daily for the first 2 d. Holstein calves were fed 3.8 L of whole milk and Jersey calves were fed 2.8 L of whole milk supplemented with either no additive, 4g YST, or 3g MOS daily through weaning at 42 d. Treatments included: 1) a corn/soybean meal based starter, 21% crude protein (**CP**), 6% acid detergent fiber (**ADF**), (**CON**), 2) a blend of 85% starter and 15% CSH, 18% CP, 15% ADF (**CON + CSH**), 3) starter and MOS (**CON + MOS**), 4) starter with CSH and MOS (**CON + CSH + MOS**), 5) starter and live yeast (**CON + YST**), and 6) starter with CSH and live yeast (**CON + CSH + YST**). Starter diets were offered from 1 d and daily amounts were increased by 0.09 kg when orts were 0 kg. Weekly measurements included body weight (**BW**), wither height, hip width, and dry matter intake from starter (**DMI**). Daily measurements included rectal temperatures, fecal, and respiratory scores. Twelve Holstein steers (2 per treatment) were killed for rumen tissue samples. Data were analyzed for the main effects of CSH, YST, and MOS. Average DMI was greater for Holstein calves consuming CSH diets (0.90 kg) than diets without CSH (0.75 kg). Body weight of Holstein calves on CSH treatments (54.9 kg) was greater (P<0 .05) than those fed diets without CSH (53.3 kg). Average daily gain was greater for Holsteins fed CSH diets (0.60 kg/d) than diets without CSH (0.54 kg/d). However, Holstein calves fed diets without CSH had a greater ($P < 0.05$) feed efficiency (0.65 kg feed/kg BW gain) than those fed CSH diets (0.71 kg feed/kg BW gain). There were no significant effects of YST or MOS on DMI, gain, or feed efficiency in Holstein calves (P >0.05). Holstein calves fed CSH diets had a lower ($P < 0.03$) fecal score (1.25) than those fed diets without CSH (1.34). Holstein calves fed CSH diets also had more narrow ($P < 0.01$) papillae (0.32 mm) compared to those fed diets without CSH (0.40 mm). There was no significant effect of additive on papillae length, width, or density. Surface area was not different across treatments or within sections of the rumen.

Jersey calves fed YST or MOS had greater (P < 0 .03) final BW (51.2 kg and 51 kg) than calves fed no supplement (47.5 kg). There were no significant effects of CSH, YST, or MOS on DMI, WH, or HW in Jersey calves ($P > 0.05$). Jersey calves fed YST supplement had a lower ($P < 0.05$) fecal score (1.26) compared to Jersey calves fed NA (1.46).

Cottonseed hulls did positively affect the growth and development of Holstein calves. This study indicates that cottonseed hulls are a suitable fiber

source for calf starter rations and that YST or MOS may have beneficial effects on calf responses to stress during the post-weaning period.

The Effects of Cottonseed Hulls Added to Diets With and Without Live Yeast or Mannanoligosaccharide in Young Calves

by

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BIOGRAPHY

Stephanie Rene Hill was born in Canton, North Carolina on August 14, 1979. She became interested in dairy nutrition while completeing her Bachelor of Science degree at N C State University. After graduation from Pisgah Senior High School in 1997, she completed her BS in Animal Science in 2000. She entered the Nutrition Program at N C State in 2001 in pursuit of a Master of Science Degree, under the direction of Dr. Brinton A. Hopkins and Dr. Lon W. Whitlow. She is a member of the American Dairy Science Association.

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Introduction

The formulation of dairy calf starter feeds to contain fiber has been a long-standing practice. Most commercial starters have a minimal amount of fiber (5% to 15 %) included; however, the sources include a variety of ingredients from forages such as alfalfa hay or concentrates such as soybean hulls. Several commercial mixes have less than 10% additional fiber and it is the decision of the producer to add some sort of roughage to the calf diet. Although protein and energy requirements for young calves have been published (NRC, 2001), requirements for fiber and its effects on growth, health, and rumen development are not yet clearly defined.

One fiber source used in the Southeast U. S. are cottonseed hulls, which are included in total mixed rations (**TMR**s) of lactating cows, dry cows and heifers without negative effects. Because cotton is a popular crop in the Southeast, dairy producers easily acquire the by-products of the plant, including the hulls. CSH provide a good source of effective and palatable fiber.

Another common addition to calf starters is antibiotics. With public health concerns growing over sub-clinical antibiotic use in agriculture, producers may soon face new regulations limiting use to clinical situations. However, the addition of antibiotics to calf starters can be important to maintain gut health in early life. Researchers have been faced with the concern of finding a replacement for antibiotics and have investigated probiotics, particularly live yeast cultures and yeast cell wall products. Two such products are *Saccharomyces* cerevisiae and oligosaccharides. The addition of *S. cerevisiae* has been shown to alter fermentation products in the digestive tract of adult and young ruminants. A recent study has shown this yeast to increase propionate and decrease the acetate to propionate ratio in rumen contents of older cows (Enjalbert, et al., 1999); others have shown an increase in butyrate and a decrease in propionate in young calves.

Mannanoligosaccharide is a part of the carbohydrate fractions of the yeast cell wall. Certain bacteria have a binding preference for particular carbohydrates (**CHO**) and when these CHO are fed in the diet, certain intestinal bacteria will attach themselves to the CHO and eventually make there way across the gut wall. One specific example is E. coli. E. coli has an affinity for mannose fractions. The addition of mannanoligosaccharide (**MOS**) to pre-ruminant diets provides an alternate mannose binding site for bacteria, such as E. coli. Because MOS is not digestible, attached bacteria leave the gut with the MOS.

Fiber in Calf Starters

Researchers have suggested fiber levels in calf starters range from no more than 5% crude fiber (Morrison et al., 1951) to not less than 15 % (Plaza et al., 1983). Kang and Leibholz (1973) found that including wheat straw at 19% of the diet will maximize weight gains and 22 % will maximize intakes. Kay et. al.

(1972) also found increased intakes when calves were fed high roughage diets. Whitaker et al. (1957) showed no significant differences in consumption of starter or hay or in body weight gains when calves were fed varying levels of fiber. Holsteins and Jersey calves were fed alfalfa hay ad libitum and starter ad libitum up to 1.8 kg daily. The diets contained 5, 9, or 13 % CF. Fiber levels did not affect intake or gains. Holsteins had higher intakes of hay and higher gains compared to Jerseys. The authors noted a negative correlation between hay consumption and starter consumption ($r = -0.55$).

The addition of bulky fiber to calf starters can lead to increased weights of gastrointestinal tracts, which can be mistaken for increased live weight gain. The increased weight of the tract can be due to gut fill or increased tissue weights. Increasing the fiber content of the diet may actually correlate to a decrease in live weight gain (Plaza et al., 1985). Jahn et al. (1969) designed a study to evaluate the effect of the ratio of starch to sugar and fiber on Holstein calves. Eight-week old calves were assigned to ten rations that had two ratios of starch to sugar (1:1 or 3:1) and five levels of fiber from 5 to 60 % of the diet. The sources of fiber were equal parts of rye, barley, and wheat straw. The starch to sugar ratio did not affect animal performance. The authors did show that actual live weight gains could be masked by an increase in gut fill when calves were fed different levels of ADF. The authors used the relationships of straw to ADF and straw to fill to derive an equation relating fill and ADF. Using this equation; $\hat{Y} = 8.33 + 0.41\ \text{ADF}$, where $\hat{Y}$ = fill as % live weight at slaughter and ADF = ADF as % DM of ration, gut fill (as a percent of live weight) was determined and then used to calculate the corrected body weight gain (**CBWG**). These calculations indicate that increased live weight gain was caused by increased fill in the gastrointestinal tract as the level of ADF increased. Voluntary intake also increased with percent ADF up to 32 % and then declined as ADF continued to increase. Strozinski and Chandler (1971) conducted a study using eight-week old calves and formulated an equation to relate gut fill to acid detergent lignin (**ADL**); $\hat{Y} = 3.3 + 2.5$ ADL, where $\hat{Y}$ = % fill at slaughter and ADL = ADL as % DM of the ration. This equation was compared to one calculated by Jahn et al. (1969) and it was determined that there is a 2.5 % increase in fill for every 1 % increase in ADL.

Other factors such as DMI and protein requirements are also affected by the level of roughage in the diet. Jahn et al. (1976) showed a greater DMI as fiber levels increased when protein was increased from 9 to 14.5 %. However, DMI decreased at protein levels above 14.5 % when ADF levels were high (11 and 25 % ADF). As the level of fiber increased, the CBWG decreased. Protein requirements also increased with increasing ADF (Table 1). At constant ADF levels, live weight gains increase with an increase in protein. The level of necessary fiber is not clearly defined by published research; however, completely eliminating fiber from the starter ration may cause unwanted metabolic changes which can affect the performance of calves (Miller et al., 1968).

Cottonseed Hulls

Factors that must be considered when incorporating by-product feeds into the rations of livestock include availability, cost, nutritive value, and potential negative effects. Producers must consider the economics of obtaining and processing the feed and its benefits. Cottonseed hulls (**CSH**) are a by-product feed that have been included in dairy rations in the Southeast U.S. and have shown beneficial effects on animal performance. They are a successful feed because, in this area, they are easily obtained, usually quite economical, and have been shown to increase palatability of the diet (Van Horn et al., 1983). There are some adverse characteristics of cottonseed hulls, including a possible negative effect on protein digestibility (Brown et al., 1976). Due to the bulkiness of the hulls, processing through mechanized feeding equipment may be difficult. In order to eliminate this problem, studies have been done to evaluate the effect of pelleted hulls in dairy cow rations. These studies have shown a more efficient feed utilization with the addition of CSH as evidenced by greater milk yield with decreased DMI (Vernlund et al., 1980). When compared with other high fiber by-product feeds, CSH produced the highest intakes (Van Horn et al., 1983). However, CSH did not perform as well as more fermentable fiber source. Total tract digestibilities of DM, OM, NDF, and ADF were less than 50% for sheep fed CSH and oat hulls compared to 70% for sheep fed corn fiber or soybean hulls (Hsu et al., 1987). Garleb et al., (1990) suggested that the low digestibility of CSH maybe due to the high content of lignin encrustation and crystallinity of cellulose which would bothprevent the fermentation of carbohydrate fractions of the cell wall. Although, much of the research on cottonseed hulls has focused on mature ruminants, similar results have been seen in young calves. Dairy producers in the southeast United States have been feeding CSH to young heifers and calves and have seen greater DMI and growth. Murdock and Wallenius (1980) conducted a study using Holstein heifer calves to evaluate the effects of different fiber sources on animal performance. Calves were on trial from 3 to 12 weeks of age and were fed rations containing alfalfa hay, cottonseed hulls, or alfalfa hay-beet pulp mix. Calves fed the cottonseed hulls had greater intakes compared to either of the other two treatments during the first four-week period as well as over the entire twelve-week study. The mean BW of calves fed alfalfa hay or alfalfa hay-beet pulp mix was lower than those fed CSH. However, there was no difference noted in feed efficiencies across ration treatments. Defoor et al. (2001) suggested that when CSH were compared to alfalfa hay, sudan silage, and wheat straw as a roughage source for finishing heifers, CSH had almost twice the roughage value of alfalfa hay. They also noted that DMI and NEg/kg of $BW^{.75}$ increased linearly with CSH. These authors concluded that increases in feed intake with CSH maybe related to energy dilution. Feeding CSH, with high NDF, may lead to a higher energy dilution, which might cause the animal to compensate by increasing intake. Moore et al. (1990) reported greater DMI intakes in steers fed CSH than in steers fed alfalfa, as well as a faster rate of passage from the rumen when steers were fed CSH compared to steers fed alfalfa or wheat straw. It was also noted that ruminal contents were more uniform, meaning there was not a separation of the solid and liquid phases within the rumen, when CSH diets were fed compared to wheat straw. This may contribute to the increase rate of passage seen with CSH. When steers were fed wheat straw there was definite stratification in the rumen. Formation of a dorsal mat may cause a delay in grain reaching the lower tract where it can be used by microbes to produce VFA. Research shows that cottonseed hulls may not be as fermentable as other fiber sources, and are less digestible in the rumen than alfalfa hay. Defoor et al. (2001) concluded that CSH have a higher roughage value than alfalfa, but could still be substituted at lower rates in the diets of finishing heifers. However, Moore et al. (1990) concluded that CSH did not improve nutrient utilization compared to other feedstuffs and may not be the best choice as a roughage source to beef steers. When considering diets of young calves, increased ADF may contribute to the abrasive value of the diet. Using CSH as the source of ADF may have beneficial effects if they are available and economical to the producer. Table 2 shows the effective fiber level of CSH compared to other feedstuffs, determined by chewing time in min/lb/DM.

Starter and Rumen Development

The process of rumen development can be measured in three different phases: pre-ruminants, intermediate ruminants, and adult ruminants. Through all three stages there are noticeable changes in the anatomy, microbial population, and digestive function. There are two main theories about rumen development. The first is the ‘scratch theory’, or the idea that the addition of fibrous material to the diet provides abrasiveness and increases mucosal and muscular development. The second theory is rumen papillae develop due to chemical stimulation, particularly VFAs. Dry feed has an important function as a source of nutrition, but it also serves as an important tool in normal rumen development. Starter rations are typically high grain rations, which can increase VFA production in the young ruminant. Increased metabolic activity and blood flow, which is common with increases in butyrate (Stobo et al., 1965), contributes to greater papillae development. Advanced papillae development has a strong relationship with the growth of the whole animal (Stobo et al., 1965). Early studies have shown that calves given VFA solutions have greater papillae development than those given inert bulk (Flatt et al., 1958). VFA concentrations have been shown to increase in calves fed grain diets with little or no roughage (Klein et al., 1987). Proportions of VFAs can be altered depending on feedstuff. In a study conducted by Quigley et al. (1992) calves fed hay diets had higher propionate and lower butyrate. It was also noted that calves fed hay had lower ketones, which was probably related to decreased butyrate.

Roughage may also be fed with starter, as part of a mixed ration, or as a supplement. This roughage, in the form of hay or by-product feed, can facilitate the muscular, and sometimes to a lesser extent, mucosal development of the rumen. Harrison et al. (1960) fed Holstein heifers either high-hay or high-grain rations. The ratios of hay to concentrate were 9:1 or 1:9, respectively. The calves were on trial from 7 weeks of age to 16 weeks of age, at which time two calves from each group were slaughtered. At the same time, two other calves were reversed to a milk diet and fed ad libitum. At 38 weeks all remaining calves were slaughtered and observations were made on the anatomy of the digestive tracts. Two bull calves were fed all milk diets and two others were fed all milk and allowed free consumption of wood shavings. These bull calves were also slaughtered for anatomical observations. It was concluded that mucosal and muscular layers of the rumen develop independently and differently according to type of diet.

Calves fed high hay and high grain had marked papillae development compared to calves fed milk only diets. Good muscular development was noted in the bull calves fed milk and shavings. The most convincing piece of evidence in this study was that calves that were reversed to milk-only diets showed retrogression of papillae and muscle, however, muscular retrogression was at a slower rate. Similar results have been presented (Plaza et al., 1983) where calves consuming hay had thicker epithelium and fewer papillae per cm^2 than those consuming concentrate.

Particle size can contribute to the diet abrasive value (**DAV**) (McGavin and Morrill, 1976). In 1996, Greenwood, et al. designed a new method to measure DAV and conducted an experiment to determine if DAV and rumen development were related. In order to determine DAV, a mixer hook was evenly coated with paraffin and used to mix moistened feedstuffs at different particle sizes, including fine, intermediate, or coarse. DAV was measured according to the amount of paraffin that was abraded during the testing. They determined that DAV increased as particle size of the diet increased. The epithelium of calves fed the fine diet was darker compared to those fed a coarse diet. A third condition examined was the extent of keratinization of the papillae. Keratinization is a condition where the papillae begin to clump together and causes a decrease in surface area, and may lead to a decrease in absorption. In this study, a lower DAV resulted in an increase in the percentage of keratin layers on the papillae, a decrease in the percentage of metabolically active tissue, and an increase in the length of the papillae. Papillae shape may be altered by the content of the diet, but differences are also evident in different sacs of the rumen (Beharka et al., 1996).

Effects of Yeast Supplementation

Microbial feed additives may increase the efficiency of the rumen by altering fermentation products. Live yeast cultures provide many substrates for bacteria growth, including amino acids, B vitamins, and other organic acids. The benefits of feeding yeast may be due to either the utilization of their metabolites or to the interaction of the yeast and rumen microbes. Typically, yeast supplement are most likely to elicite responses in stressed animals. During times of stress, including growth stages, animals have higher nutrient requirements (Arambel and Kent, 1990). Phillips and von Tungelin (1985) fed yeast culture to post-stressed heifers and steers for four weeks. Animals receiving yeast had a greater DMI and ADG compared to control treatments.

A study by Chaucjeyras-Durand and Fonty (2001) showed that the inclusion of yeast in diets fed to gnotobiotically-reared lambs may have increased the rate at which cellulolytic bacterial species propagated in the rumen. The authors suggested that this increase of growth rate was due to the ability of viable yeast cells to scavenge oxygen from the rumen. Cellulolytic species are extremely oxygen sensitive. Cellulolytic species deceased when the rumens of these lambs were exposed to oxygen during fitting of the cannula. The cellulolytic population remained stable in the rumens of the lambs fed a yeast supplement.

Yeast Supplementation and Rumen Fermentation and N Utilization

Enjalbert et al. (1999) showed a decrease in rumen ammonia with the addition of a yeast supplement. These authors suggested that decreased protein degradation in the rumen might be responsible; however, other authors have suggested that a decrease in ammonia could be due to increased ammonia utilization and therefore greater microbial N flow to the lower tract. Harrison et al. (1988) also showed less variability in ruminal ammonia with the inclusion of yeast and a decrease in ammonia concentration in lactating cows. Erasmus et al. (1992) showed an increase in the rate of passage of microbial N in lactating cows fed yeast and showed that yeast altered the amino acid pattern to the lower tract, increasing the flow of lysine and methionine, the two main limiting amino acids in dairy cattle rations. Greater lysine and methionine flow may be the result of increased microbial protein production in the rumen coupled with the excellent concentrations of these AA in microbial CP. The authors concluded that a change in the amino acid pattern may contribute to the observations of increased milk production with yeast supplementation. Conversely, Putnam et al. (1997) found no effects of yeast supplementation on individual amino acid profile, amount of microbial protein, or its flow to the lower tract, but they did show a slightly higher escape of dietary protein to the duodenum.

Yeast supplementation has been shown to alter ruminal VFA production and concentrations, including increasing acetate to propionate ratios and decreasing methane production. Enjalbert et al. (1999) showed an increase in the molar percentage of propionate and a decrease in the acetate to propionate ration (A: P). Reports of changes in VFAs have been conflicting. Piva et al. (1993) showed no significant differences in VFA, but acetate and A: P tended to be higher in cows supplemented with yeast. In contrast, Harrison et al. (1988) found that cows fed a yeast supplement had a higher molar propionate level and lower molar acetate, resulting in a decreased A:P. In the same study, yeast supplementation increased concentrations of branched chain acids (isobutyrate, isovalerate, and valerate). The authors concluded that yeast serves to stabilize rumen fermentation.

Quigley et al. (1992) found that yeast supplementation may also affect lactate production in the rumen. Jersey calves were fed experimental diets with the inclusion of either sodium bicarbonate or yeast. Calves that were fed yeast cells had decreased amounts of ruminal lactate at 4h post-feeding. Plasma lactate declined with feeding, but tended to be lower when calves were fed yeast compared to bicarbonate.

Effects of Yeast on Health Parameters

Seymour et al. (1994) suggested that yeast has a beneficial effect on the overall gut health of dairy calves. They reported that yeast had a positive effect on fecal scours as well as feed to gain ratio. Data were analyzed so that a daily score greater than 3 on a scale of 1 to 4, with 1 being normal, was rated as a case of fecal scours. In period 2, during the transition to dry feed, calves fed yeast had a lower DMI, but showed a better feed to gain ratio, indicating that the yeast may have helped the calves adapt to dry feed. In period 3, after transition to dry feed, calves fed yeast showed a lower percentage of fecal scours and a lower incidence of abnormal body temperature. The authors speculated that Cr supplied by the yeast may have improved the immune response; however, Cr was not assayed.

Effects of Supplementation of Mannanoligosaccharide

Oligosaccharides are made from isomerization of disaccharides, enzymatic hydrolysis of polysaccharides, or by direct extraction from the cell wall of yeasts. The type of carbon backbone to which they adhere typically classifies these structures. Mannanoligosaccharides are derived from yeast cell walls, while fructooligosaccharides are formed by the transfructosylation of sucrose, or hydrolysis of inulin (Iji et al., 2001).

The mode of action of MOS and other oligosaccharides in animal diets is not well known; Ofek et al. (1977) suggested that mannans act as high affinity ligands for pathogenic bacteria and offer a source of competitive exclusion. Pathogens with mannose specific Type-1 fimbriae adsorb to MOS instead of attaching to the intestinal cell wall and are removed from the intestine (Ferket et al., 2001). Gnoth et al. (2000) examined the digestibility of human oligosaccharides (HMO) found in breast milk. In order to determine if HMO were digested in the small intestine of humans, the authors used three common intestinal enzymes salivary amylase, porcine pancreatic amylase, and brush border membrane vesicles (**BBMV**). Digestion with salivary amylase or pancreatic amylase did not degrade the structure; however, after 2h incubation with BBMV, slight modifications were detected. This reinforces the idea that MOS is poorly digested and will carry the attached pathogens out of the gastrointestinal tract.

Oligosaccharides may also have a positive effect on the gut health of animals. In human research, oligosaccharides have served as probiotics, enhancing the non-pathogenic microbes in the intestine. In animal research, they aid in eliminating pathogenic bacteria and reducing incidence of disease (Spring, 1998). In swine and poultry diets MOS has improved performance perhaps due to the high levels of production stress in these animals and the resultant benefit of reducing other stressors such as the stress of intestinal organisms (Spring, 1998).

MOS is derived from the cell wall of S. *cerevisiae* however, either may be an advantageous supplement in young ruminants. It is possible that effects of both additives may be similar.

Summary

The addition of fiber to diets has the potential to increase intakes, body weights, and improve rumen development in calves. Feeding CSH produced increased intakes in lactating and dry cows, heifers, and calves. CSH may not always improve nutrient utilization; however, studies suggest that CSH are a suitable roughage source when they are economical to the producer.

Mannanoligosaccharide is a major component of yeast cell wall. Feeding either MOS or a viable yeast culture may influence rumen fermentation, nitrogen metabolism, or gut health in dairy cattle. There have been positive effects of including MOS or yeast in cattle diets, but research results are conflicting and results may be influenced by the type of diet and degree of animal stress. The use of MOS or yeast may be preferred over that of antibiotics because the additives promote growth of beneficial bacteria in the gastrointestinal tract. More research must be conducted in order to produce appropriate recommendations on supplementation with these products.

In conclusion, the proper growth and health of calves is essential to any dairy operation. The inclusion of fiber in calf starter rations is important in order to realize the growth potential of these animals, by improving rumen development and gut health. The addition of yeast additives may also have a positive effect on rumen development, growth of the animal, and animal health.

The objective of this study was to investigate the effects of fiber in the form of CSH added to the starter and of live yeast (**YST**) or mannanoligosaccharide added to milk, on growth, intake, rumen development, and health parameters in neonatal calves.

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Running Head : Cottonseed hulls, additional fiber, yeast supplementation, and rumen development

The Effects of Cottonseed Hulls Added to the Diet With and Without Live Yeast or Mannanoligosaccharide in Young Calves

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Abstract

The objectives of this study were to investigate the effects of fiber in the form of cottonseed hulls (**CSH**) added to the starter and of live yeast (**YST**) or mannanoligosaccharide (**MOS**) added to milk, on growth, intake, rumen development, and health parameters in neonatal calves. Holstein (n = 116) and Jersey (n = 46) bull and heifer calves were assigned randomly within gender at birth, to one of six treatments. Calves continued on trial through 63 d. Bulls were castrated by 14 d. All calves were fed 3.8 L of colostrum daily for the first 2 d. Holstein calves were fed 3.8 L of whole milk and Jersey calves were fed 2.8 L of whole milk supplemented with either no additive, 4g YST, or 3g MOS daily through weaning at 42 d. Treatments included: 1) a corn/soybean meal based starter, 21% crude protein (**CP**), 6% acid detergent fiber (**ADF**), (**CON**), 2) a blend of 85% starter and 15% CSH, 18% CP, 15% ADF (**CON + CSH**), 3) starter and MOS (**CON + MOS**), 4) starter with CSH and MOS (**CON + CSH + MOS**), 5) starter and live yeast (**CON + YST**), and 6) starter with CSH and live yeast (**CON + CSH + YST**). Starter diets were offered from day 1 and daily amounts were increased by 0.09 kg when orts were 0 kg. Weekly measurements included body weight (**BW**), wither height, hip width, and dry matter intake from starter (**DMI**). Daily measurements included rectal temperatures, fecal, and respiratory scores. Twelve Holstein steers (2 per treatment) were killed for rumen tissue samples. Data were analyzed for the main effects of CSH, YST, and MOS. Average DMI was greater for Holstein calves consuming CSH diets (0.90 kg) than diets without CSH (0.75 kg). Body weight of Holstein calves on CSH treatments (54.9 kg) was

greater than those fed diets without CSH (53.3 kg) (*P*<0 .05). Average daily gain was greater for Holstein calves fed CSH diets (0.60 kg/d) than diets without CSH (0.54 kg/d) (*P* < 0.05). However, Holstein calves fed diets without CSH had a greater feed efficiency (0.65 kg feed/kg BW gain) than those fed CSH diets (0.71 kg feed/kg BW gain) (*P* < 0.05). There were no significant effects of YST or MOS on DMI, gain, or feed efficiency in Holstein calves (*P* >0.05). On a 5 pint scale, Holstein calves fed CSH diets had a lower fecal score (1.25) than those fed diets without CSH (1.34) (*P* < 0.03).

Holstein calves fed CSH diets also had more narrow papillae (0.32 mm) compared to those fed diets without CSH (0.40 mm) (*P* < 0.01). There was no significant effect of additive on papillae length, width, or density.

Jersey calves fed YST or MOS had greater final BW (51.2 kg and 51 kg) than calves fed no supplement (47.5 kg) (*P* < 0 .03). There were no significant effects of CSH, YST, or MOS on DMI, WH, or HW in Jersey calves (*P* > 0.05). Jersey calves fed a YST supplement had a lower fecal score (1.26) compared to Jersey calves fed NA (1.46) (*P* < 0.05).

(**Key Words**: cottonseed hulls, fiber, rumen development, yeast)

Abbreviation Key: CSH = cottonseed hulls; MOS = mannanoligosaccharide; YST = yeast.

Introduction

The formulation of dairy calf starter feeds to contain fiber has been a long-standing practice. Most commercial starters have a minimal amount of fiber (5% to 15 %) included; however, the sources include a variety of ingredients from forages such as alfalfa hay or concentrates such as soybean hulls. Although protein and energy requirements for young calves have been published (NRC, 2001), requirements for fiber and its effects on growth, health, and rumen development are not clearly defined. The NRC (2001) recommends that calves be fed dry feed from an early age and suggests that long hay is not as beneficial to developing rumen mucosa as starters with adequate levels of digestible fiber. Some producers make hay available to young calves although this is not uniformly recommended. Dry feed has an important function as a source of nutrition, but it also serves as an important tool in normal rumen development. Starter rations are typically high grain rations, which can increase VFA production in the young ruminant. Increased metabolic activity and blood flow, which is common with increases in butyrate (Stobo et al., 1965), contributes to greater papillae development. Advanced papillae development has a strong relationship with the growth of the whole animal (Stobo et al., 1965). Early studies have shown that calves given VFA solutions have greater papillae development than those given inert bulk (Flatt et al., 1958). VFA concentrations have been shown to increase in calves fed grain diets with little or no roughage (Klein et al., 1987). Proportions of VFAs can be altered depending on feedstuff. In a study conducted by Quigley et al. (1992) calves fed hay diets had higher propionate and lower butyrate. It was also noted that calves fed hay had lower ketones, which was probably related to decreased butyrate.

Harrison et al. (1960) concluded that mucosal and muscular layers of the rumen develop independently and differently according to type of diet. Calves fed high hay and high grain had marked papillae development compared to calves fed milk only diets. Good muscular development was noted in calves fed milk and wood shavings. The most convincing piece of evidence in this study was that calves that were reversed to milk only diets showed retrogression of papillae and muscle, however, muscular retrogression was at a slower rate.

Factors that must be considered when incorporating by-product feeds into the rations of livestock include availability, economics, nutritive value, handling, processing, and potential negative effects. Cottonseed hulls are a by-product feed that have been included in dairy rations in the Southeast U.S. and have been beneficial for animal performance. They are a successful feed because, in this area, they are easy to handle, palatable, high in roughage value, stimulate intake, and are economical (Van Horn et al., 1983). Currently, CSH are effectively included in total mixed rations (TMRs) of lactating cows, dry cows and heifers. Defoor et al. (2001) suggested that when CSH were compared to alfalfa hay, sudan silage, and wheat straw as a roughage source for finishing heifers, CSH had almost two times the roughage value of alfalfa hay. When compared with other high fiber by-product feeds, CSH produced the highest intakes (Van Horn et al., 1983). Moore et al. (1990) reported greater DM intakes in steers fed

CSH than in steers fed alfalfa hay, as well as a faster rate of passage from the rumen when steers were fed CSH compared to steers fed alfalfa hay or wheat straw. It was also noted that ruminal contents were more uniform, meaning there was not a separation of the solid and liquid phases within the rumen, when CSH diets were fed compared to wheat straw. This may contribute to the increased rate of passage seen with CSH.

With growing public health concerns about sub-clinical antibiotic use in agriculture, producers may soon face new regulations limiting use to clinical situations. However, the addition of antibiotics to calf starters can be important to maintain gut health in early life. Researchers have been faced with the task of finding a replacement for antibiotics and have investigated probiotics, particularly live yeast cultures and yeast cell wall products. Probiotics are defined as viable, naturally occuring organisms (NRC, 2001). They are typically fed during times of stress however, their mode of action has not been well defined. Yoon and Stern (1995) categorized the mode of action of probiotics into the following: stimulation of desirable microbial growth in the rumen, stabilization of the pH, altered ruminal fermentation pattern and end products, increased nutrient flow post-ruminally, increased nutrient digestibility, and alleviation of stress though enhanced immune response. Two such products are *Saccharomyces cerevisiae* and oligosaccharides. The addition *of S. cerevisiae* has been shown to alter fermentation products in the digestive tract of adult and young ruminants. A recent study has shown this yeast to increase propionate and decrease the acetate to propionate ratio in the rumen contents of older cows (Enjalbert, et al., 1999); others have shown an increase in butyrate and a decrease in propionate in the rumen contents of young calves.

Oligosaccharides are a part of the carbohydrate fractions of the yeast cell wall. Certain bacteria have a preference for particular carbohydrates (**CHO**) and when these carbohydrates are fed in the diet, certain intestinal bacteria will attach themselves to the CHO wall and eventually make there way across the gut wall. One specific example is E. coli. E. coli has an affinity for mannose fractions. The addition of mannanoligosaccharide to pre-ruminant diets provides an alternate mannose binding site for bacteria, such as E. coli. Because MOS is not digestible, attached bacteria are excreted in the feces with the MOS.

The primary objectives of this study were: 1) to investigate the effects of CSH, as a fiber source, added to the starter on intake, growth, and health and rumen development measures and 2) to investigate the effects of live yeast (YST) or MOS, added to the milk, on the same parameters in both Holstein and Jersey calves.

Materials and Methods

Fifty-six (56) Holstein calves and 46 Jersey calves from the North Carolina State University Lake Wheeler Road Dairy Educational Unit (**DEU**) and 60 Holstein calves from the North Carolina Department of Agriculture Piedmont Research Station-Dairy Unit (PRS) were randomly assigned at birth to one of six treatments. Treatments included: 1) a corn/soybean meal based starter, 21%

crude protein (**CP**), 6% acid detergent fiber (**ADF**), (**CON**), 2) a blend of 85% starter and 15% CSH, 18% CP, 15% ADF (**CON + CSH**), 3) starter and MOS (**CON + MOS**), 4) starter with CSH and MOS (**CON + CSH + MOS**), 5) starter and live yeast (**CON + YST**), and 6) starter with CSH and live yeast (**CON + CSH + YST**). At birth, calves at DEU were individually housed in hutches; at PRS calves were individually housed in either hutches or pens in an open barn. All bull calves were castrated by elastration by 14 d of age and all calves were dehorned by cauterization at 42 d of age. Calves remained in hutches or pens until 63 d of age. Calves were fed 3.8 L of colostrum once daily for the first 2 days on trial. Holsteins were then bottle fed 3.8 L of whole milk once daily and Jerseys were bottle fed 2.8 L of whole milk once daily until weaning at 42 d. The milk contained either no additive, 4g of yeast, or 3g of MOS. The additive was supplied in the milk at the dosages suggested by the manufacturer, Saf-Agri Corp. Minneapolis MN. The additive was mixed with 15cc of warm water in the bottle to ensure that it was properly dissolved and then milk was added. Calves were offered experimental starter from day 1 on trial and water, ad libitum. Starter was formulated to meet NRC crude protein (CP) and energy requirements. Cottonseed hulls were added at the expense of the whole diet to produce a 85:15 ratio of starter:CSH (Table 1). Calves were offered 0.09 kg/d of starter from 1 d and daily amounts were increased by 0.09 kg/d when orts were equal to 0 kg.

Sample Collection and Analysis

Body weight (**BW**), wither height (**WH**), and hip width (**HW**) were all measured weekly in the a.m. Rectal temperatures and respiratory and fecal scores were determined each a.m. by the same two members of the farm staff (Table 2). Feed samples were collected weekly and composited by month. Orts were weighed twice a week and samples were taken twice weekly and composited by month. Daily intakes were calculated from orts. Feed and orts samples were analyzed for DM and CP according to (AOAC) and NDF and ADF according to Van Soest (1967). Chemical analyses of feed are listed in Table 1.

Rumen Development Measures

At the North Carolina State University College of Veterinary Medicine-Necropsy Lab, 12 Holstein steers, 2 per experimental treatment, were euthanized at 63 days of age by jugular venipuncture with sodium pentobarbitol at 1 mg/ 4.5 kg body weight. An incision was made along the ventral midline to expose the gastrointestinal tract. Once exposed, the tract was ligated at the cardiac sphincter and again at the pyloric sphincter and removed from the abdominal cavity. The tract was washed and photographed, using an Olympus digital camera. The four different sections of the rumen (cranial, caudal, dorsal, and ventral) were marked from the exterior with the rumen positioned on its ventral side. The reticulum was removed and an incision was made at the entrance to the cranial sac and continued in a lateral direction until the interior was exposed. Rumen fluid samples were collected by straining contents through doublelayered cheesecloth into collection tubes. The tubes were then placed in a cooler, and within 2 h were measured for pH, and frozen for later determination of VFA concentrations.

Rumen tissue was rinsed clean with cold water and photographed with a 35mm camera. Five pieces, each three to four inches, were cut from each section and attached to tongue depressors to prevent shrinkage in preservative. The samples were fixed in Trump's solution (McDowell and Trump, 1976) for at least 72 hours and then processed for histology slides and Scanning Electron Microscopy (**SEM**). Using histology slides and Optimus 6.1 software, papillae length and width were measured. Number of papillae per cm^2 was measured using digital images from SEM. Once the images were acquired, acetate sheets were placed on top of the image and each papillae was marked and counted.

Statistical Analysis

This experiment used a factorial arrangement of treatments with CSH (with and without), additive type (MOS, YST, or NA), sex, and location as the class variables and all possible interactions were examined. The two breeds were analyzed separately, however, a breed comparison was done using animals at one location (NCSU-DEU) only. When analyzing Holstein data, location was removed from the model when it was not significant, but was significant for BW, average daily gain (**ADG**), and respiratory scores. Data with multiple measures per calf were analyzed by repeated measures ANOVA using the MIXED procedure of SAS. Feed efficiency, ADG, health and rumen measures were subjected to ANOVA using the GLM procedure of SAS. Birth weight was defined as a covariate for BW analyses including ADG. Significance was declared at $P < 0.05$.

Results and Discussion

Holstein Calves

Growth and Intake Data

Dry matter intake was greater for calves fed the CSH diets (0.90 kg/d) compared to calves fed non-CSH diets (0.75 kg/d)(Table 4). Crude protein intake was not different for calves fed CSH diets compared to those fed non-CSH diets because the greater DMI for calves fed CSH diets compensated for a lower CP concentration in the CSH diets, which resulted from the 15 % dilution by added CSH. Jahn et al., (1976) reported a 42 % increase in protein requirements when calves were fed diets with ADF levels similar to the diets in this study. There is an evident associative effect of CSH on increasing DMI (Van Horn et al., 1984), therefore the addition of CSH (as an ADF source) may aid the calf in meeting the increased protein requirement due to an increase in DMI. Acid detergent fiber intake was higher for calves fed the CSH diet, which is expected considering the high ADF content of CSH (NRC, 2001) (Table 4). Calves fed CSH diets had higher ($P < 0.05$) BW at weeks 7, 8, and 9 compared to calves fed non-CSH diets (Figure 1). In evaluating the DMI of the CSH diet, the intake is 15 % CSH and 85

% base starter and therefore is equivalent to 0.14 kg of CSH (CSH intake kg = DMI kg x 0.15) and 0.76 kg of base starter (DMI kg – CSH intake kg). This greater DMI was directly related to and accounts for the higher BW in calves fed CSH diets. These results were similar to those observed by Miller et al. (1968) who reported greater DMI when calves were fed CSH as supplemental fiber compared to those fed no supplemental fiber. Body weights or DMI were not affected by additive type. Hip width and WH were not significantly affected by starter or additive type (Table 5). Jahn et al. (1969) speculated that gut fill could be responsible for increased BW observed in calves fed high roughage diets. Although we did not measure gut fill in this study, increased energy intake could account for increased BW gain. Increased fiber content of the diet will increase the bulk in the rumen, particularly when feeding hay as a roughage source. However, CSH have been shown to produce more homogenous digesta, indicated by a lack of separation of the solid and liquid phases in the rumen, which may eliminate bulkiness while still providing effective fiber. This may also increase the rate of passage from the rumen, thereby allowing for greater DMI when feeding CSH (Moore et al., 1990).

Calves fed non-CSH diets had lower ADG (0.54 kg/d) than those fed CSH diets (0.60 kg/d). Calves fed CSH diets had higher ADG, however, they had a lower feed efficiency. Calves fed non-CSH diets had a feed efficiency of 0.35 kg gain/kg intake while calves fed CSH diets had a feed efficiency of 0.30 kg gain/kg intake. The greater DMI and dilution of the energy content by the addition of CSH contributed to the lower feed efficiency. These results are similar to those of

Miller et al. (1968) where calves fed a simplified starter, similar to the non-CSH diet fed in this study, had a lower feed to gain ratio compared to those fed the starter and CSH, indicating a lower feed efficiency when feeding CSH. Yeast or MOS supplementation did not have an effect on ADG or feed efficiency (Table 5).

Health Parameters

Calves fed CSH diets had a lower fecal score (1.25) than calves fed non-CSH diets (1.34). According to the scale used in this study (Table 2), a lower fecal score would indicate a lower incidence of scours. Fecal scores were also analyzed as the number of days with a score greater than 2. Although it was not significant, there was a trend (P = 0.17) for calves fed non-CSH diets to have a higher percentage of days above 2 (6.05 %) compared to calves fed CSH diets (4.46 %). Rectal temperature and respiratory scores were not significantly different by CSH treatments and there were no effects of additive type on fecal or respiratory scores or body temperatures. All health parameters were within normal limits throughout this study and of 162 calves there was only one death, which was not related to the study indicating that these calves were under low stress, which may have influenced the effect of additive.

Rumen Development Measures

Papillae length, width, density, and surface area were measured. Calves that were fed CSH diets had more narrow papillae ($P < 0.05$) and had greater papillae density ($P < 0.03$) compared to calves fed the non-CSH diets. There were no effects of additive on length, width, density, or surface area.

Papillae development was different in separate sections of the rumen (Table 6). The dorsal section had greater papillae density compared to all other sections. The papillae in the dorsal section were more narrow compared to those in the ventral section ($P < 0.03$), but were not different from those in the cranial or caudal sacs. The dorsal section also had the shortest papillae compared to all other sections. These results suggest that because the dorsal area of the rumen is not typically exposed to rumen contents, it would have minimally developed papillae. Although the density was greater, the papillae in this section were shorter and less wide than those in other sections.

Because this section of the rumen does not have substantial contact with digesta, this tissue may not be exposed to any great extent to the volatile fatty acids or feed particles that are necessary for papillae development. Due to the amount of variance related to this section, the values obtained for length, width, density, and surface area were removed from the statistical analysis (Hill et. al., 2004).

Volatile fatty acids were measured from rumen fluid samples. Butyrate, propionate, and acetate are the principle VFAs that affect mucosal development (Van Soest, 1982). There were no differences in acetate, butyrate, propionate concentrations, or the acetate to propionate ratio across treatments. However, calves fed MOS or YST had greater concentrations of valerate (6.4 mM and 5.8 mM) compared to calves fed NA (3.7 mM). Quigley et al, (1992) showed an increase in ruminal acetate and butyrate and a decrease in propionate when feeding yeast, which conflicted with the results from this study, however, the additives provided in this study were delivered as part of the milk which may have passed directly to the abomasums resulting in little or no ruminal effects.

Jersey Calves

Growth and Intake Data

The type of starter, with or without CSH, did not have a significant effect on DMI, BW, HW, WH, ADG, or FE in Jersey calves (Table 7 and 8). However, Jersey calves did show a response to the additive. Calves that were fed either MOS or YST had greater BW (51 kg and 51.2 kg) at weeks 7, 8, and 9 on trial than those fed NA (47.5 kg) (Figure 2).

Health Measures

Fecal score, rectal temperature, and respiratory score were not affected by starter type in Jersey calves. However, calves that were fed YST additive had a lower fecal score (1.26) compared to those fed NA (1.46), but were not different form those fed MOS (1.35). In this study, a lower fecal score would indicate a lower incidence of scours (Table 2). Rectal temperatures were lower (37.9°C) in calves fed the YST additive compared to those fed NA (38.1°C) at the P = 0.06 level.

Conclusions

This study indicates that the amount of fiber in a calf ration does affect growth, intake, and rumen development. Rations containing 15 % CSH as a fiber source were adequate to produce normal intake and gains in both Holstein and Jersey calves. The addition of CSH increased DMI in Holsteins and directly contributed to increased ADG. Differences were also noted in rumen development when calves were fed CSH.

This study also indicates that the addition of MOS or YST may be beneficial to Jersey calves, particularly during the transition period from milk to dry feed. The animals used in this study had normal health parameters, which indicate a low stress level and that may have influenced the affects of the additives.

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Appendix

Technique for the Dissections and Analysis of the Reticulorumen in the Young Calf

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Introduction

Researchers have suggested fiber levels in calf starters range from no more than 5% crude fiber (Morrison et al, 1951) to not less than 15 % (Plaza et al., 1983). The process of rumen development can be measured in three different phases: pre-ruminants, intermediate ruminants, and adult ruminants. Through all three stages there are noticeable changes in the anatomy, microbial population, and digestive function. There are two main theories about rumen development. The first being the 'scratch theory', or the idea that the addition of fibrous material to the diet provides abrasiveness and increases mucosal development. The second theory is that rumen papillae develop due to chemical stimulation, particularly VFAs. Dry feed has an important function as a source of nutrition, but it also serves as an important tool in normal rumen development. Starter rations are typically high grain rations, which can increase VFA production in the young ruminant. Increased metabolic activity and blood flow, which is common with increases in butyrate (Stobo et al., 1965), contributes to greater papillae development. Advanced papillae development has a strong relationship with the growth of the whole animal (Stobo et al., 1965). Early studies have shown that calves given VFA solutions have greater papillae development than those given inert bulk (Flatt et al., 1958). VFA concentrations have been shown to increase in calves fed grain diets with little or no roughage (Klein et al., 1987). Proportions of VFAs can be altered depending on feedstuff. In a study conducted by Quigley et al. (1992) calves fed hay diets had higher propionate and lower butyrate. It was also noted that calves fed hay had lower ketones, which was probably related to decreased butyrate. At feeding, acetate was greater than propionate and butyrate, which indicates that VFA proportions change, with time, after feeding.

Roughage may also be fed with starter, as part of a mixed ration, or as a supplement. This roughage, in the form of hay or by-product feed, can facilitate the muscular, and sometimes to a lesser extent, mucosal development of the rumen. Harrison et al. (1960) fed Holstein heifers either high-hay or high-grain rations and slaughtered calves for anatomical observations. It was concluded that mucosal and muscular layers of the rumen develop independently and differently according to type of diet.

Particle size can contribute to the diet abrasive value (**DAV**) (McGavin and Morrill, 1976). In 1996, Greenwood, et al. designed a new method to measure DAV and conducted and experiment to determine if DAV and rumen development were related. In order to determine DAV, a mixer hook was evenly coated with paraffin and used to mix moistened feedstuffs at different particle sizes, including fine, intermediate, or coarse. DAV was measured according to the amount of paraffin that was abraded during the testing. They determined that DAV increased as particle size of the diet increased. The epithelium of calves fed the fine diet was darker compared to those fed a coarse diet. A third condition examined was the extent of keratinization of the papillae. Keratinization is a condition where the papillae begin to clump together and causes a decrease in surface area, and may lead to a decrease in absorption. In this study, a lower DAV resulted in an increase in the percentage of keratin layers on the papillae, a decrease in the percentage of metabolically active tissue, and an increase in the length of the papillae. Papillae shape may be altered by the content of the diet, but differences are also evident in different sacs of the rumen (Beharka et al., 1996).

In order to evaluate the effects of different ration types on rumen development, techniques must be used to remove, dissect, and analyze tissue. This paper discusses a technique used to evaluate rumen development in young calves fed cottonseed hulls (**CSH**) as a source of fiber. The method used allowed for proper and consistent examination of the different sacs of the rumen (dorsal, ventral, cranial, caudal), photography, as well as preparation for scanning electron microscopy and histology.

Materials and Methods

Fifty-six (56) Holstein calves from the North Carolina State University Dairy Educational Unit were randomly assigned at birth to one of six treatments. Treatments included: 1) a corn/soybean meal based starter, 2) a blend of 85% starter and 15% CSH, 3) starter and MOS , 4) starter with CSH and MOS , 5) starter and live yeast, and 6) starter with CSH and live yeast. Holsteins were bottle fed 3.8 L of whole milk once daily until weaning at 42 d. The milk contained either no additive, 4g of yeast, or 3g of MOS.

At the North Carolina State University College of Veterinary Medicine-Necropsy Lab, 12 Holstein steers, 2 per experimental treatment, were euthanized at 63 days of age by jugular venipuncture with sodium pentobarbitol at 1 mg/ 4.5

kg body weight. An incision was made along the ventral midline to expose the gastrointestinal tract. Once exposed, the tract was ligated at the cardiac sphincter and again at the pyloric sphincter and removed from the abdominal cavity. The tract was washed and the exterior photographed (Figures 1 and 2). The four different sections of the rumen (cranial, caudal, dorsal, and ventral) were marked from the exterior with the rumen positioned on its ventral side. The reticulum was removed and an incision was made at the entrance to the cranial sac and continued in a lateral direction until the interior was exposed. Rumen tissue was rinsed clean with cold water and photographed according to McGavin and Morrill (1976) (Figure 3). Five pieces, each three to four inches, were cut from each section and attached to tongue depressors to prevent shrinkage in preservative (Figure 4). Because the method of dissection left the dorsal section in two halves, tissue was cut on both sides of the incision line. The samples were fixed in Trump's solution (McDowell and Trump, 1976) for at least 72 hours and then processed for histology slides and Scanning Electron Microscopy (**SEM**). Samples fixed in this solution have the ability to be stained and processed by conventional means and be embedded in paraffin blocks. The blocks do not have the brittle characteristics of those fixed in 2 % or higher glutaraldehyde. Tissue samples were also processed for histology slides.

Using histology slides and AxioVision 4.0 software (Carl Zeiss Vision Imaging System, Thornwood, NY 10594), papillae length and width were measured (Figure 5). Number of papillae per cm^2 was measured using digital images from SEM (Figure 6). Once the images were acquired, acetate sheets were placed on top of the image and each papillae was marked and counted.

In order to calculate surface area (**SA**) certain assumptions were made. Papillae were considered to be cylindrical in shape and measured width was assumed equal to the radius of the papillae. Equations 1 and 2 were used to determine SA. Rumen measures were subjected to ANOVA using the GLM procedure of SAS.

Equation 1. $\text{Surface area}_{\text{papillae}} = 2 \times W \times \pi \times L + 2\pi \times W^2$, where W = width in mm and L= length in mm.

Equation 2. $\text{Surface Area}_{\text{section}}$ = Average SA/section X × Average density of section X, where X can be cranial, caudal, ventral or dorsal.

Results and Discussion

Calves that were fed non-CSH diets (0.32) had more narrow papillae ($P < 0.01$) compared to calves fed CSH diets (0.40). There was a trend ($P = 0.14$) for calves fed CSH diets (48.4) to have more dense papillae compared to calves fed non-CSH diets (36.5). Papillae length was not affected by CSH or additive type. Additive did not have effects on width, or density. Surface area was not different across treatments ($P < 0.20$).

The dorsal section of the rumen was noted to have very underdeveloped papillae. Because this section of the rumen does not have substantial contact with digesta, this tissue may not be exposed to any great extent to the volatile fatty acids or feed particles that are necessary for papillae development. Due to the amount of variance related to this section, the values obtained for length, width, density, and surface area were removed from the statistical analysis.

The following results were obtained when the dorsal section was removed from analysis. Calves that were fed CSH diets had more narrow papillae (0.33 mm) compared to calves fed non-CSH diets (0.42) (P < 0.05). Calves fed CSH diets also had more dense papillae (45.8/ cm2) compared to calves fed the non-CSH diets (28.9/cm^2) (P < 0.01). There was a trend (P = 0.03) for calves fed no additive to have greater papillae density (46.3/cm^2) compared to those fed MOS (29.8/cm^2). There were no effects of additive or cottonseed hulls on length. Surface area was not different across treatments or within section (P < 0.20). A correlation procedure was performed to analyze the relationship between papillae width and density. The correlation between these two variables was negative (-0.26) indicating that although papillae of the calves fed CSH may be less wide, there was a greater density. Likewise, calves fed non-CSH diets had wider papillae but fewer per cm^2. The lack of difference in surface area between treatments may be influenced by this negative correlation.

Terminology used to describe sampling site in the rumen varies widely (McGavin and Morrill, 1976, Stobo et al., 1956, Harrison et al., 1960). Often more than one section is sampled from the rumen. Results from this study indicate that procedure may not be the most accurate method. By sampling from one section a great amount of sampling error can be eliminated. For example, sampling primarily from the cranio-ventral region of the rumen may provide the most useful data simply because this is where most of the ruminal contents are contained. Researchers are most likely to find the best papillae development in this region and the best indicators of potential nutrient absorption ability.

The dissection and tissue analysis methods used in this study provide an efficient and accurate way to determine morphological and histological information about rumen papillae. The use of histology slides and scanning electron microscopy allow for exact determination of morphological measurements, which are difficult to determine accurately by hand techniques.

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Figure 2. Effects of Additive on Body Weight in Jersey Calves