THE FEEDING VALUE OF WHOLE COTTONSEED IN THE DIETS OF LACTATING DAIRY CATTLE

by

KELLY MICHELLE COOKE

(Under Direction the of John K. Bernard)

ABSTRACT

Coating whole cottonseed with starch alters rumen fermentation and decreases fiber digestibility. The objective of this research is to investigate the effects of the addition of value added feeds to the starch coating on in vitro ruminal fermentation and performance of lactating dairy cows. In vitro fermentation results indicate that as starch increased, pH decreased while concentrations of ammonia increased. The addition of yeast culture decreased total VFA and acetate: propionate ratio due to an increase in molar proportions of propionate. Dry matter and fiber digestibility were improved with the addition of urea and sodium bicarbonate to the coating. Results of production performance indicate that addition of urea and yeast to the starch coating did not affect dry matter intake, nutrient digestibility, or milk yield. Efficiency of milk production was higher for the urea and yeast treatments compared with control. Results of these trials indicate that including urea, yeast culture, and sodium bicarbonate alters fermentation and dry matter and fiber digestibility in vitro. Production trial results indicate that inclusion of urea or yeast culture in the gelatinized starch coating does not change whole tract digestibility, but does improve milk production efficiency.

Delayed harvest of the cotton plant increases free fatty acid content in the seed which has been shown to alter rumen fermentation but not nutrient digestibility. The objective of this research is to examine the effects of feeding whole cottonseed with elevated concentrations of free fatty acids in the oil on intake and performance. Treatments included whole cottonseed with three concentrations of free fatty acids; 6.8 %, 24.1 %, and 22.3 % free fatty acids. Yield of milk and components was similar among treatments, but milk fat percentage was lower for elevated free fatty acid treatments compared with control. Molar proportions of butyrate and isobutyrate increased with elevated free fatty acids. Results indicate that changes in ruminal fermentation were not sufficient enough to decrease milk fat percentage and reduction probably occurs due to effects of reduced total fatty acid supply to the mammary gland for de novo synthesis of milk fat.

INDEX WORDS: cottonseed, starch, urea, yeast, sodium bicarbonate, free fatty acids

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DEDICATION

This is dedicated to the memory of my father, John Cooke.

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CHAPTER 1

INTRODUCTION

Whole cottonseed (WCS) is commonly used in the rations of lactating dairy cows as a source of energy, fiber, and protein. The average nutrient concentrations published by NRC (NRC, 2001) for WCS are 23.5 % crude protein (CP), 50.3 % neutral detergent fiber (NDF), 40.1 % acid detergent fiber (ADF), and 19.3 % ether extract (EE). The high oil content of WCS makes it an attractive energy dense feed for animals with high energy requirements, such as high producing lactating dairy cattle. The high fiber concentrations provided by the lint and the hull fractions are desirable for maintaining effective fiber levels in the diet. The ability to maintain energy while increasing effective fiber levels makes WCS a unique feedstuff.

The feeding value of WCS can vary depending on processing and environmental factors. The lint portion of WCS causes the seeds to stick together, or bridge, making it very difficult to handle in modern mechanized feeding systems. Recent research (Laird et al., 1997) has focused on coating WCS with gelatinized corn starch to improve handling characteristics. However, the addition of starch to the seed may reduce fiber digestibility by promoting amylolytic bacterial activity over cellulolytic bacterial activity. The addition of other nutrient supplements to the coating of WCS could maintain or even improve nutrient digestibility within the seed.

Because cellulolytic microorganisms use ammonia as their primary nitrogen source, the inclusion of urea in the starch coating of WCS may aid in improving fiber digestion. Supplemental yeast culture has been shown to increase cellulolytic bacterial numbers (Martin and Nisbet, 1992), which could potentially increase fiber digestion as well.

The presence of dietary starch may decrease ruminal pH through an accumulation of VFA and lactate in the rumen. The addition of a dietary buffer such as sodium bicarbonate ($NaHCO_3$) increases or stabilizes rumen pH (Thomas et al., 1984; Solorzano et al., 1989) and increases fiber digestibility (West et al., 1987; Solorzano et al., 1989). The addition of $NaHCO_3$ to the gelatinized corn starch coating of WCS may offset any detrimental effects associated with increased starch concentrations on pH and fiber digestibility.

Environmental factors also play a part in the feeding value of WCS. When harvest of cotton is delayed by warm, moist conditions such as tropical storms, the moisture level in the seed remains elevated and concentrations of free fatty acids (FFA) increase. Whole cottonseed with greater than 12% FFA is considered to be off-quality as defined by the National Cottonseed Products Association (1997) and are generally sold as livestock feed. Feeding supplemental fats with elevated concentrations of FFA to cows has been reported to reduce DMI and fiber digestibility (Eastridge and Firkins, 1991). Increased levels of unsaturated FFA in the rumen may have a toxic effect on the rumen micro flora and may lead to shifts in normal rumen fermentation patterns (Jenkins, 1993). When high levels of unsaturated FFA are fed in the diet of lactating cows, fats and oils can coat feed particles and prevent cellulolytic bacterial attachment, depressing fiber digestibility. The oil in WCS is 70% unsaturated (Keele et al., 1989) and increased levels of WCS in the diet of lactating dairy cows may negatively affect fiber digestion (DePeters and Cant., 1992). Previous research has demonstrated that feeding WCS with up to 18% FFA in the oil at 12.5% of the ration DM to Holstein steers increased the molar proportions of acetate and the acetate: propionate ratio, but did not negatively impact nutrient intake (Sullivan et al., 2005). Sullivan et al., (2004) noted that feeding WCS with up to 12%

FFA in the oil at 12.5% of the DM to lactating cows did not affect fiber digestibility or milk yield or composition.

Cottonseed quality can be affected either positively or negatively by processing or environmental factors. The addition of value added feeds to the gelatinized starch coating of WCS may improve nutrient digestibility, specifically fiber digestibility compared with fuzzy WCS. The first part of this research focuses on the addition of urea, yeast, and sodium bicarbonate to the starch coating to determine the effects on ruminal fermentation, nutrient digestibility and lactation performance.

When cotton harvest is delayed, the quality of WCS can be negatively affected. Previous research had examined feeding up to 18% FFA in the oil of WCS. However, the effects of feeding WCS with greater than 18% FFA in the oil have not been examined. The second objective of this work was to determine the effects of feeding WCS with elevated concentrations of FFA in the oil on nutrient intake, milk yield and composition, and ruminal fermentation of lactating dairy cows.

CHAPTER 2

LITERATURE REVIEW

Utilization of whole cottonseed as a feed ingredient

The production of cotton dates back over 7,000 years. While cotton is grown primarily for its fiber, the use of whole cottonseed (WCS), a byproduct of cotton production, is relatively new. The invention of the cotton gin by Eli Whitney in the late 1700's and the growth of the textile industry has made cotton production easier and more economically feasible. Approximately 5% of the harvested cottonseed is required for seed for planting. The majority of cottonseed is crushed to extract the oil for human consumption. Cottonseed oil has a long shelf life and is less prone to rancidity than other oils thereby providing quality oil for cooking and baking. However, WCS that is determined to be off quality and does not meet the requirements for human consumption is fed to livestock.

A grading system was established by the National Cottonseed Products Association (NCPA, 1997) to aid in the classification of WCS as prime (for human consumption) or off quality (for livestock consumption). The system is based on several factors including oil and ammonia content, moisture, foreign matter, and free fatty acids in the oil. If moisture content exceeds 12.5%, foreign matter is greater than 10.0%, or the percent of free fatty acids in the cottonseed oil is more than 20.0%, the cottonseed is considered off quality and is marketed to the livestock industry (NCPA, 1997).

Today, WCS is commonly used in the rations of lactating dairy cows as a source of energy, fiber, and protein. Average nutrient concentrations published by NRC (NRC, 2001), for WCS are 23.5 % crude protein (CP), 50.3 % neutral detergent fiber (NDF), 40.1 % acid detergent fiber (ADF), and 19.3 % ether extract (EE). The high oil content of WCS makes it an attractive energy dense feed for animals with high energy requirements, such as lactating dairy cattle. The high fiber concentrations provided by the lint and the hull fractions are desirable for maintaining effective fiber levels in the diet. The ability to maintain energy while increasing effective fiber levels makes WCS a unique feedstuff.

Gossypol levels and WCS

Gossypol is a yellow polyphenolic compound found in gossypol glands throughout the cotton plant and seed. It serves as a protective mechanism against common pests including bollworm, tobacco budworm, and pink bollworm (Smith and Cothran, 1999). Gossypol occurs in both the free and bound forms in the seed. Gossypol in the bound form is generally associated with a free amino group of an amino acid which renders it relatively inactive within the rumen. Free gossypol is not associated with any component of the WCS and generally has the greatest physiological effect on animals. Free gossypol is especially toxic to nonruminants (Morgan, 1989), while ruminants are able to detoxify the compound rendering it insoluble and unable to be absorbed by the animal (Reiser and Fu, 1962). There are two isomers of gossypol, (+) and (-), with the (-) gossypol isomer having greater biological activity (Wang et al., 1987). The ratio of (-) to (+) isomers is generally 60:40 in upland WCS (Calhoun et al., 1995b), but Pima WCS has a greater proportion of the (-) isomer.

Ruminants are able to detoxify gossypol by allowing free gossypol to bind to soluble proteins within the rumen. Specifically, the gossypol will bind to the ε-amino group of lysine and render it nontoxic in the rumen (Reiser and Fu, 1962). In vitro experiments (Reiser and Fu, 1962) examining the effects of free and bound gossypol on rumen fermentation parameters noted that for each mole of gossypol that disappeared from the rumen liquor, two moles of lysine ε-amino groups disappeared, regardless of temperature, presence of anaerobic or aerobic microorganisms, or addition of proteolytic enzymes to the rumen fluid. These results indicate that the binding of free gossypol to lysine in the rumen is most likely the mechanism by which ruminants detoxify free gossypol in the rumen. These researchers further observed that free gossypol binds to lysine in microbe free rumen liquor, indicating that the rumen microflora do not play a role in gossypol detoxification.

A similar mechanism for binding free gossypol occurs during the processing of CSM and WCS (Broderick and Craig, 1980; Calhoun et al, 1995a). Heat treatment of CSM reduced protein solubility and ε- reactive lysine residues and increased binding of free gossypol (Broderick, 1977). The two carbonyls per mol of gossypol will cross-link with protein molecules (Lyman et al., 1959) and may decrease the solubility of CSM and WCS protein or block lysine and arginine residues from enzyme hydrolysis in the rumen (Broderick and Craig, 1980).

Research (Lindsey et al., 1980; Hawkins et al., 1985) suggests that animals consuming about 24g/d of gossypol may exceed the animal's ability to detoxify the compound. Outward signs of gossypol toxicity include dyspnea, decreased or cessation of feed intake, decreased milk production, weakness, and in severe cases, sudden death (Lindsey et al., 1980). Dietary gossypol reduces iron absorption and retention thereby reducing hemoglobin concentrations in animals fed high levels of gossypol (Lindsey et al., 1980). Further, gossypol toxicity causes decreased packed cell volume and increased erythrocyte fragility (Lindsey et al., 1980; Mena et al., 2004). The effects of gossypol on erythrocytes and hemoglobin indicate that the compound may reduce the oxygen carrying or releasing capacity of the blood, thereby interfering with normal oxygen exchange in the animal (Lindsey et al., 1980).

In general, levels of free gossypol ingested have more affect on the animal than total gossypol levels in the feed. It has been suggested that feeding excess free gossypol may exceed the protective mechanism of binding to soluble proteins (Lindsey et al., 1980). Limited information is available on toxic feeding levels of gossypol in the diet of ruminants. Generally, total plasma gossypol concentrations should not exceed 5µg/dl (Calhoun et al., 1995b). Mena et al. (2001) fed up to 1050 mg/kg of free gossypol per day supplied either by WCS, CSM, or a combination of the two and reported no signs of gossypol toxicity. While plasma gossypol concentrations increased, lactation performance was relatively unaffected. The authors of that study noted that after 4 to 5 wk of increased gossypol intake, plasma gossypol levels reached a plateau and may not have been indicative of the possible accumulation of gossypol over an extended period of time. A follow-up study (Mena et al., 2004) examined the effects of feeding up to 960 mg/kg of free gossypol per day over an 84 d period and observed no signs of gossypol toxicity. Again, plasma gossypol concentrations reached a plateau after 28 d of treatment. During this study, there was a small increase in erythrocyte fragility in animals consuming greater amount of free gossypol. However, there was no effect on lactation performance in these cows.

There is some evidence that when animals ingest bound gossypol, the digestion processes may release the compound from its bound protein, rendering it free and absorbable by the animal (Noftsger et al., 2000). Noftsger et al. (2000) compared diets containing WCS with diets containing different levels of expanded-expelled WCS and noted that expanded-expelled WCS contained 0.11% free gossypol compared with 0.76% free gossypol in unprocessed WCS diets.

While animals consuming the processed WCS had higher intakes of bound gossypol than cows consuming unprocessed seed, plasma gossypol concentrations were higher in these animals as well. These researchers indicated that the binding of gossypol to soluble protein during the heating process was weak enough to allow for absorption of bound gossypol in these animals. These researchers concluded that bound gossypol contributes to plasma gossypol in a similar manner as free gossypol and therefore free gossypol should not be used as the sole measurement when considering amounts of WCS to feed to avoid toxicity. Rather, the combined effect of bound and free gossypol availability should be considered and plasma gossypol concentrations are a better indicator of these effects.

There is some evidence that CSM is more likely to cause gossypol toxicity than WCS. Cottonseed meal is a byproduct of the oil extraction process and is often fed as a protein supplement in the diets of lactating dairy cattle. Compared with WCS, CSM has no linted outer hull fraction to protect it and slow its digestion in the rumen. Because it generally passes through the rumen at a higher passage rate than WCS, feeding CSM may increase the chance of gossypol toxicity, possible due to decreased retention time in the rumen to allow for binding of free gossypol to soluble proteins (Mena et al., 2001). Mena et al. (2001) fed diets differing in concentrations of total and free gossypol with WCS, CSM, or a combination of the two as gossypol sources and noted no signs of gossypol toxicity. Dietary treatments included a gossypol free control diet (diet A), 1020 mg/kg total gossypol with 989 mg/kg free gossypol from WCS (diet B), 900 mg/kg total gossypol with 64 mg/kg free gossypol from CSM (diet C), 960 mg/kg total gossypol with 531 mg/kg free gossypol from a combination of WCS and CSM (diet D), and 1922 mg/kg total gossypol with 1050 mg/kg free gossypol from a combination of WCS and CSM (diet E). However, concentrations of free plasma gossypol were highest when

CSM was fed compared with WCS. Others (Blackwelder et al., 1998) have observed similar increases in plasma gossypol concentration when feeding CSM compared with WCS.

Lysine availability in cows fed diets containing gossypol has been variable. Blackwelder et al. (1998) compared diets containing CSM or soybean meal as protein sources with or without a rumen undegradable protein (RUP) supplement and noted that cows fed diets containing CSM had lower concentrations of plasma lysine than cows fed soybean meal, regardless of RUP supplementation. These results indicate that availability of lysine in CSM diets was limiting because it was bound to free gossypol in an effort to detoxify the compound. Blauwiekel et al. (1997) examined the effects of feeding glanded or glandless cotton with or without rumen protected lysine supplementation and reported that lysine supplementation had no effect on plasma gossypol concentrations, presumably because the lysine was unavailable in the rumen for binding to gossypol. These researchers observed that there were no interactions between the gossypol content of the diets and animal response to rumen protected lysine supplementation which indicates that gossypol did not affect lysine utilization for milk or protein yields.

Milk production response is generally unaffected by normal levels of gossypol intake. As previously reported (Blauwiekel et al., 1997), gossypol did not affect lysine utilization for milk synthesis or milk protein yield. Most likely, any effects on milk yield or yield~~s~~ of milk components observed when feeding WCS are associated with the high fat content of WCS and not due to any relationship with gossypol intake.

Reproductive performance may be affected by the intake of gossypol in ruminants. In males, fertility may be decreased with feeding increased levels of free gossypol. A review of the literature by Randel et al. (1992) reported that gossypol decreased the germinal epithelium wall thickness and lowered the number of germ cell layers in the seminiferous tubules of bulls. The integrity of sperm cell membranes may be compromised and puberty may be delayed in young male ruminants when feeding WCS.

Reproductive performance in females is also negatively affected by feeding high levels of gossypol. Santos et al (2003) observed that feeding increased levels of gossypol reduced subsequent conception rates after the first postpartum artificial insemination service and lowered pregnancy rates. Increasing the level of gossypol in the diet increased the number of abortions and resulting in increased days open in dairy cows.

Whole cottonseed and heat increment

The addition of dietary fat generally reduces body temperature during heat stress (Moody, 1962) and decreases heat increment in ruminants. Coppock et al. (1985b) observed that with increasing levels of WCS in the diet, respiration rates were reduced. Although body temperatures were not different among diets, researchers hypothesized that the decrease in respiration rates may have been due to the lower heat increment of WCS and the resulting reduction in metabolic heat load that the animals needed to dissipate. Holter et al. (1992) reported that cows fed WCS or WCS with protected fats reduced heat production by 6.7 and 9.7 % respectively, and reduced total heat loss by 4.9 and 7.0 % respectively compared with diets containing no WCS.

Whole cottonseed and DMI

A review by Coppock et al. (1987) noted that in 18 trials where fuzzy WCS was fed at up to 25% of the dry matter (DM) of the ration, there was no effect of WCS feeding on DMI. In a study by Coppock et al. (1985b) in which WCS was fed at 0, 15, and 30% (Experiment 1), DMI decreased linearly as percentage of WCS in the diet increased. The same study (Experiment 2) examined the possible toxicity effects of feeding WCS up to 55% of the complete ration and noted a significant decrease in DMI as the percentage of WCS in the diet increased. Others (Mohamed et al., 1988) noted a decrease in DMI (23.0 vs., 21.4 kg/d) when feeding WCS at approximately 16% in the diet of lactating dairy cattle compared with no WCS. The decrease in DMI of cows fed high amounts of WCS may possibly be related to the rumen fill effects of the high fat and fiber content of WCS. However, Coppock et al. (1985b) noted that because of the high energy density of WCS, calculated net energy of lactation (NE_L) intake was not depressed with decreased DMI. Dietary treatments for that study included WCS at 0, 15, or 30 % of the DM of the ration. While DMI decreased as concentration of WCS in the diet increased (3.91, 3.63, and 3.49 kg/100 kg BW for 0, 15, and 30% WCS, respectively), calculated NE_L intakes did not differ (6.79, 6.59, and 6.60 Mcal/ 100 kg BW for 0, 15, and 30 % WCS, respectively). Similar results have been reported by Hawkins et al. (1985) when feeding 18.5% WCS.

Harrison et al. (1995) compared DM and NE_L intakes in two herds of lactating dairy cattle fed WCS or control diets without WCS. In herd 1, diets containing 12% WCS were formulated to contain 1.49 or 1.47 Mcal/kg for WCS and control diets, respectively. These researchers observed an increase in DMI (22.1 vs. 23.0 kg/d; control vs. WCS) and NE_L (32.5 vs.34.2 Mcal/d; control vs. WCS) intakes for animals fed WCS compared with control diets for weeks 3 through 44 of the lactation. In herd 2, treatments included a control diet without WCS and a diet that contained WCS at 12% of the DM. Rations were balanced to be isonitrogenous rather than based on NE_L values (1.54 vs. 1.60 Mcal/kg, control vs. WCS). There was no difference in DMI or NE_L intakes for primiparous cows but average DMI (26.0 vs. 23.3 kg/d) and NE_L intakes (40.1 vs. 37.2 Mcal/d) decreased in multiparous cows fed WCS over the 41 week treatment period.

Dry matter digestibility is generally unaffected by WCS intake (Sullivan et al., 1993a; Zinn, 1995). Moreira et al. (2004) did not observe any differences in DM digestibility for cows fed a control diet without cottonseed with that of cows fed diets containing either whole linted cottonseed or acid delinted cottonseed at 13% of the DM. Smith et al. (1981) noted an average DM digestibility of 65.6 % for cows fed diets containing 0, 5, 10, or 15% WCS of the DM.

NDF digestibility and WCS

Fiber is defined as the slowly digestible or indigestible fraction of feeds that occupies space in the gastrointestinal tract of animals (Mertens, 1997). Adequate fiber intake is essential for optimal animal health and production and to maintain a healthy and stable rumen environment. According to the NRC (2001) the recommended dietary neutral detergent fiber (NDF) requirement ranges from a minimum of 25 to 33%, with 75% of NDF supplied by forages. However, these recommendations do not take into account the physical effectiveness of the fiber fraction generally associated with the particle size and density of the feedstuff. Effective fiber is generally characterized by its ability to stimulate chewing and therefore buffering capacity in the rumen (Allen, 2001). Effectiveness of fiber influences the animal's ability to maintain milk fat percentage, ruminal fermentation, and overall animal health and metabolism (Mertens, 1997). Two general terms have been used to discuss fiber effectiveness. Physically effective fiber (peNDF) is related mainly to the particle size of the fiber source. This physical characteristic influences chewing activity and ruminal mat consistency in the animal. Effective NDF (eNDF) refers to the ability of a feed to replace a forage source in the ration to maintain milk fat percentage (Mertens, 1997).

In general, forage sources of NDF contain more physically effective fiber and maintain milk fat percentage much better than do nonforage NDF sources (Clark and Armentano, 1993;

Swain and Armentano, 1994). Mertens (1997) noted that based on chewing activity, NDF from byproducts high in NDF was 0.4 times as effective as that of forage sources of NDF. The NRC (2001) estimates of the peNDF of nonforage NDF sources were 50% of that of forage NDF. The lone exception to this rule is linted whole cottonseed. Whole cottonseed, while of a small particle size, still maintains milk fat percentage provided it does not drastically reduce DMI (Mooney and Allen, 1997).

Mooney and Allen (1997) examined the peNDF of WCS as compared with alfalfa silage cut at two chop lengths, 4.8 mm (short) and 9.5 mm (long). Results indicated that the peNDF of WCS was 50% that of long chop alfalfa silage and 127% that of short chop silage. Clark and Armentano (1993) compared the effectiveness of NDF in diets with 19% forage either supplied completely by alfalfa haylage or by 13% alfalfa haylage and 6% WCS and noted that WCS was just as effective in increasing eating and rumination activity compared with alfalfa haylage. Time spent chewing similar for among treatments-(286 and 302 min/d for WCS plus alfalfa haylage and alfalfa haylage, respectively). These researchers concluded that WCS had a peNDF ratio 1.3 times that of alfalfa haylage.

It is recommended that diets be formulated using ingredient NDF concentrations because of the positive relationships between NDF and rumen fill and the negative relationships between NDF and energy density (Mertens, 1997; Tjardes et al., 2002). In general, NDF is less digestible than nonstructural carbohydrates (NSC) and as the concentration of NDF increases in a feedstuff, the energy content decreases. However, WCS is unique in that it maintains digestible NDF levels without sacrificing energy density. The lint and hull fractions of the seed provide adequate effective fiber to stimulate chewing and rumination in the animal. Whole cottonseed is composed of 10 to 15 % lint, or linters, and these linters are almost 100% cellulose. This provides an excellent source of highly digestible fiber to the animal (Palmquist, 1995). Palmquist (1995) noted that cottonseed linters were greater than 90% digestible when incubated in sacco for 72 to 96 hours or in vitro for 120 hours.

Coppock et al. (1985a) compared whole linted cottonseed to acid delinted cottonseed in the diets of lactating dairy cattle and observed that the percentage of intact seeds excreted as a percentage of seed eaten was 0.4% for linted cottonseed compared with 11.3% for acid delinted cottonseed. Chemical composition of the cottonseed retrieved from the feces was similar to the cottonseed in the ration, implying very little digestion of the seed. The authors noted that linted cottonseed was most likely stratified in the rumen contents to allow for regurgitation with the forage and subsequent mastication of the seed, thereby increasing digestibility of the seed. The acid delinted seed had a greater passage rate and was not as thoroughly digested, leading to decreased fiber digestibility. Others (Harvatine et al., 2002b; Moreira et al., 2004) have observed similar responses when comparing linted and acid delinted WCS.

Smith et al. (1981) noted that crude fiber digestibility was not different among animals fed 0, 5, 15, or 25 % WCS. Neutral detergent fiber digestibility averaged 40.5 % of DM for all treatments in their study. Others have observed a reduction in NDF digestibility when feeding WCS. Coppock et al., (1985b) noted a quadratic response in NDF digestibility when feeding WCS at 0, 15, or 30 % of the dietary DM to lactating dairy cows. Apparent digestibility of NDF for the 0, 15, and 30% treatments was 30.5, 19.3, and 31.5% of the DM, respectively. However, the researchers noted that the use of chromic oxide as a marker in the digestibility trial may have skewed results due to incomplete recovery of the marker. Harvatine et al. (2002a) did not observe any change in ruminal NDF digestibility when feeding 0, 5, 10, or 15% WCS to replace alfalfa silage NDF in the ration. However, postruminal NDF digestibility decreased as WCS levels increased, indicating possible negative associative affects associated with decreasing forage content in the ration on apparent intestinal NDF digestibility.

WCS as a supplemental fat source

Dietary fat is generally included in lactating dairy cattle rations to increase energy density and possibly the energy intake of the diet, especially in periods of decreased DMI (Allen, 2000). General feeding recommendations for feeding fat in the rations of dairy cattle are to feed supplemental fat at 5 to 7% of the DM of the ration for optimal production response (NRC, 2001). It is generally recommended that ruminally active fats, which include oilseeds and animal and animal-vegetable blends, provide 3 to 5% of the total fat in the ration and any additional supplemental fat should be provided by ruminally inert fat sources such as calcium salts of fatty acids or partially hydrogenated fatty acids (Palmquist and Jenkins, 1980; Coppock and Wilks, 1991).

Effects of supplemental dietary fat on digestion and nutrient availability are variable depending on the source of supplemental fat. Dietary sources high in unsaturated fatty acids tend to inhibit ruminal microbial fermentation, specifically of structural carbohydrates (Palmquist and Jenkins, 1980). Jenkins (1993) suggested that including high levels of polyunsaturated fatty acids in the diet could physically coat feed particles with a lipid layer preventing cellulolytic bacterial attachment or enzyme contact with the feed particulate. Physical attachment of microbes to feed particles is essential for cellulose digestion in the rumen (Church, 1988) and interference of attachment by a lipid covering reduces fiber digestibility (Eastridge and Firkins, 1991).

The addition of calcium to diets high in unsaturated fatty acids may offset any detrimental effects in the rumen by forming ruminally inert calcium soaps which pass through the rumen relatively unchanged and are digested and absorbed in the intestines. A free carboxyl group is necessary for the initiation of biohydrogenation by bacterial isomerases (Jenkins, 1993). Calcium soaps form when a calcium ion binds to a free carboxyl group. The calcium soaps are insoluble in the rumen and have little to no effect on cellulolytic bacteria and consequently, no effect on fiber digestion. The calcium soaps pass through the rumen relatively unchanged and are digested and absorbed in the intestines.

Research into the inhibitory effects of fats and fatty acids on rumen microbial metabolism has also focused on the toxic effects of fats on rumen microorganisms. Dietary fatty acids in the rumen may alter biological membrane functions by attaching to the lipid bilayers of membranes associated with rumen microbes. Long chain fatty acids are hydrophobic and amphiphilic in nature and therefore readily associate with the lipid bilayers of biological membranes (Jenkins, 1993). Attachment of fatty acids to the lipid bilayer may result in disruption of membrane function (Gruber and Low, 1988) and uncoupling of oxidative phosphorylation (Borst et al., 1962), among other negative effects which may be deadly to the rumen microorganisms.

The oil fraction of WCS provides a good source of energy and maintains milk production without compromising fiber digestibility. In general, the release of the oil from the seed is slow and subsequent extensive biohydrogenation occurs, resulting in minimal effects of fatty acids on cellulolytic microbial activity and therefore fiber digestion (Moore et al., 1986). Zheng et al. (2005) observed that when feeding varying sources of vegetable oils, milk fat percentages were decreased compared with control diets containing WCS. The addition of oils may coat feed particulate matter and hinder the attachment of microbes to feed for fermentation. High levels of oil in the rumen may also be toxic to the micro flora. The toxic effects of increased amounts of oil within the rumen will be discussed later.

Feeding WCS increases ether extract digestibility in lactating cows (Coppock et al., 1985b; Smith et al., 1981). Harvatine et al. (2002a) reported a linear increase in apparent total tract fatty acid digestibility when feeding 0,5,10, or 15% WCS to lactating dairy cattle. Others (Wu et al., 1994) noted no change in fatty acid digestibility when feeding 12% WCS.

Biohydrogenation of the fatty acids from the oil of WCS occurs extensively in the rumen, which helps to offset any detrimental effects of polyunsaturated fatty acids on rumen microbial function. Zinn and Plascencia (1993) noted that supplementation with 20 % WCS to cannulated Holstein steers increased the proportion of C 16:0 entering the small intestines by 10.6% and C 18:0 by 17.6% while subsequently decreasing the proportions of C 18:1 by 37.2% and C 18:2 by 40.6%. Due to the slow release of the oil from the WCS, extensive biohydrogenation occurs when feeding WCS (Moore et al., 1986).

Protein digestibility of WCS

The relationship between crude protein (CP) and DMI in the diets of lactating cows is often positive, primarily because of the positive effect of rumen degradable protein (RDP) on digestibility of feeds (Oldham, 1984). The availability of nitrogen sources to rumen microbes provides for increased fiber and DM digestibility and decreased distension of the rumen to allow for more DMI. Presumably, increasing DMI will also increase production in these animals.

According to NRC (2001) values, WCS is 23.5% crude protein which is highly degradable in the rumen. The soluble protein fraction of WCS is three times greater than the insoluble fraction (Wadhwa et al., 1993) and contributes 50% of the rumen degradable protein fraction (Stutts et al., 1988). Estimates of rumen CP degradability of WCS fall within the range of 70-77%, (Arieli et al., 1989; Zinn and Plascencia, 1993; Pires et al., 1997). Harvatine et al. (2002a) observed a decrease in nonmicrobial nonammonia N flow to the duodenum when cows were fed up to 15% WCS. This decrease is indicative of greater ruminal digestibility of the protein fraction of WCS with less dietary protein available to the small intestines. Others (Zinn and Plascencia, 1993) have observed similar results. Smith et al. (1981) noted an increase in total tract CP digestibility when feeding up to 25% WCS. However, others (Holter et al., 1992; Wu et al., 1994) have noted no difference in CP digestibility in animals fed WCS compared with control animals.

Ruminal fermentation parameters

Energy for milk production and general metabolism is provided primarily by volatile fatty acid (VFA) produced during microbial fermentation in the rumen. Volatile fatty acids account for 60 to 70% of the available metabolizable energy in ruminants (Armentano, 1992). Acetic and butyric acids are the precursors for milk fat synthesis and are the substrates for oxidation for energy production in the ruminant (Church, 1988). Propionic acid is glucogenic and provides 65 to 80% of the net glucose supply in dairy cows (Reynolds, 2003).

A review by Seymour et al. (2005) of 20 different research studies that included 96 treatments representing the general feeding practices of lactating dairy cattle noted that the average concentration of acetate in the rumen was 71.6 mmol while propionate concentrations averaged 29.5 mmol and butyrate concentrations averaged 15.0 mmol. Of these three principle VFA, propionate and butyrate correlated most closely with dry matter intake, NE_L, and milk yield while acetate correlated most closely with milk fat production.

The effect of WCS on rumen fermentation is somewhat variable. Generally, feeding supplemental fat decreases molar proportions of acetate and methane production whereas molar proportions of propionate are increased (Czerkawski et al., 1966; Zinn, 1988, 1989). Harvatine et al. (2002a) observed that as the level of WCS substituted for alfalfa forage in the diet increased from 0 to 15%, the average ruminal pH decreased linearly from 6.28 to 5.93. These researchers attributed the decrease in pH with increased proportions of WCS feeding to increased intake of organic matter (OM) and nonstructural carbohydrate (NSC), which increased ruminally fermentable substrates. Zinn and Plascencia (1993) reported that pH increased 6.43 to 6.92 with addition of 20% WCS in the diet. Others (Anderson et al., 1979; Clark and Armentano, 1993) have not observed any difference on pH when WCS were fed to lactating dairy cows.

Feeding WCS tends to increase molar concentrations of propionate thereby reducing the ratio of acetate to propionate (Horner et al., 1988; Mohamed et al., 1988; Harvatine et al., 2002a). Mohamed et al. (1998) compared rumen fermentation patterns associated with feeding diets containing no WCS with diets containing cottonseed oil and WCS and reported that propionate levels increased from 21.5 mM (control) to 24.5m M when WCS were fed. This resulted in a decrease ratio of acetate: propionate for cows fed WCS compared with control cows. These researchers attributed this shift in VFA patterns to reduced fiber digestibility and decreased numbers of methanogenic bacteria with lipid addition. As previously noted, whole cottonseed oil is 70% unsaturated (Keele et al., 1989) which may have contributed to the shift in acetate: propionate.

Protozoa populations in the rumen decrease when WCS is fed (Horner et al., 1988). Mohamed et al. (1988) observed a decrease in ruminal protozoa from 7.5×10^5 when animals were fed a control diet without WCS to 3.35×10^5 when WCS was included in the diet. Rumen protozoa primarily produce acetate and butyrate with only traces of propionate which would account for the increase in molar concentrations of propionate and reduced acetate: propionate ratio.

Holter et al (1992) observed a decrease in methane production with the addition of WCS to the ration of lactating dairy cattle from 4.1 to 3.6% of the gross energy of the ration in control compared with cows fed WCS. Methane is positively correlated with intake of digestible cellulose, hemicellulose, and nonfiber carbohydrates whereas fat reduces methane production (Wilkerson et al., 1995). Because WCS is an excellent source of both fiber and fat, the reduction in methane production implies that the effect of the fat in WCS in greater than the effect of the fermentable carbohydrates in WCS on methane production.

Milk yield and composition of cows fed WCS

The high fiber and energy content of WCS has the potential to alter milk composition and yield when fed to lactating dairy cows. Milk yield increased in lactating cows fed WCS compared with those fed none (Mooney and Allen, 1997). Anderson et al.(1979) noted that milk yield increased from 24.1 to 26.9 kg/d when cows were fed WCS compared with cows fed no WCS. Others (Clark and Armentano, 1993; DePeters et al., 1985) have not observed any change in milk yield when WCS were included in the diet.

Feeding WCS to lactating dairy cattle increases milk fat yield. Clark and Armentano (1993) observed an increase in milk fat yield when WCS replaced alfalfa haylage (1.04 versus 1.00 kg/d). Smith et al., (1981) also reported that when lactating dairy cow were fed increasing concentrations of WCS (0, 5, 15, and 20% of the dietary DM) milk fat yield increased linearly as concentrations of WCS in the diet increased (0.82, 0.76, 0.93, and 0.96 kg/d, respectively). An increase in milk fat percentage has also been observed when animals are fed WCS (Anderson et al., 1979; Clark and Armentano, 1993; DePeters et al., 1985).

The increased milk fat yield associated with feeding WCS can be attributed to several factors. A comparison of normal bovine milk fatty acid composition versus milk fatty acid

Table 1.1. Fatty acid composition of milk fat for cows fed alfalfa based diets with or without whole cottonseed (WCS)[1].

	Whole cottonseed in diet			
Fatty acid	0	10	15	20
	-------------------------------- % of total fatty acids ---------------------------			
4:0	3.8	4.0	4.1	4.0
6:0	2.4	2.1	2.1	1.8
8:0	1.4	1.2	1.1	0.9
10:0	3.1	2.3	2.1	1.6
12:0	3.7	2.5	2.4	1.8
14:0	11.3	9.0	8.7	7.5
16:0	28.9	26.0	25.4	25.3
16:1	4.1	3.6	3.4	3.3
18:0	9.3	13.3	13.3	14.9
18:1	23.5	28.8	29.7	32.0
18:2	5.5	4.9	5.2	4.8
18:3	3.0	2.3	2.5	2.1

[1] DePeters et al., 1985.

composition of cows fed WCS (DePeters et al., 1985) is presented in Table 1.1. In general, bovine milk is composed of almost 30% palmitic acid ($C_{16:0}$) followed by oleic acid ($C_{18:1}$) at 22%. The fatty acid composition of milk changes with the addition of WCS to the ration. The long chain fatty acids (LCFA) concentrations in the milk increase while the short (SCFA) and medium (MCFA) chain fatty acids decrease when feeding WCS. Long chain fatty acids are supplied to the mammary gland from circulating lipids, either from dietary sources or from non esterified fatty acids released from body reserves while milk MCFA and SCFA are generally derived from de novo synthesis in the mammary gland. Whole cottonseed is high in LCFA with linoleic acid ($C_{18:2}$) composing 51.5% of total fatty acids, palmitic acid ($C_{16:0}$) at 22.7%, and oleic acid ($C_{18:1}$) at 17.0% (NRC, 2001). The increase in dietary LCFA serves to increase uptake of LCFA by the mammary gland, thereby inhibiting de novo synthesis of MCFA and SCFA. Alternatively, the influx of LCFA from the WCS into the milk via circulating lipids may serve to dilute the concentrations of SCFA and MCFA and therefore may be responsible for the lower proportions of SCFA and MCFA (Bitman et al., 1996). Wu et al. (1994) noted that cows fed 12% WCS had significantly greater amounts of all C18 isomer fatty acids in the milk compared with animals fed no WCS, indicating considerable transfer of dietary lipid to the milk fat.

Regardless of which mechanism is responsible for the change in milk fat composition, the increase in LCFA in milk of cows fed WCS results in a change in mean molecular mass of the milk fat (Bitman et al., 1996). Feeding 10% WCS resulted in an increase in mean molecular mass of the milk fat from 230.5 to 239.9 mg/dl, an increase of 4.1%. This translates into a greater milk fat yield when expressed as kg/d.

Researchers (Anderson et al., 1979; Bitman et al., 1996) have observed a decrease in milk protein percentage and yield when feeding up to 10% WCS. Smith et al. (1981) noted that the inclusion of WCS at up to 20% of the diet decreased milk protein by approximately 0.1 percentage units. Wu et al. (1994) reported a decrease in milk protein percentage for cows fed 12%WCS compared with cows fed no WCS (3.03 versus 3.20%). The reduction in milk protein percentage has been attributed to a dilution effect due to the increase in milk yield normally associated with feeding supplemental fat in the diet (DePeters and Cant, 1992). However, this theory has been questioned and others have offered alternative explanations of how increasing supplemental fat affects milk protein synthesis.

A study examining the effect of supplemental dietary fat on milk protein yield (Cant et al., 1993) reported that reduction may be related to a 7% reduction in blood flow to the mammary gland. The researchers hypothesized that the decreased blood flow decreased uptake of critical AA, resulting in depressed milk protein synthesis. Palmquist and Moser (1981) hypothesized that dietary fat may induce insulin resistance in animals. Insulin is important for AA transport and uptake in muscle tissue and any reduction or inhibition of insulin may reduce AA uptake in the mammary gland, thereby reducing milk protein synthesis.

Blood serum albumin, immunoglobulins, and γ- casein are all precursors of casein, β-lactalbumin, and α-lactalbumin, the major constituents of milk protein (Schmidt, 1971). These precursors are not synthesized in the mammary gland and therefore must be acquired through circulating pools. Coppock et al. (1985b) reported a tendency for a linear decrease in serum albumin with increasing levels of WCS in the diet. Smith et al. (1981) noted decreased milk casein levels when feeding increasing amounts of WCS. The reduction in circulating precursors may lead to decreased casein levels and may explain the tendency for decreased milk protein yield in animals fed WCS.

Bertrand et al. (1998) fed ruminally protected AA to lactating Jerseys consuming WCS compared with WCS alone or without WCS or AA supplementation. Cows consuming WCS without supplemental AA had decreased percentages of milk protein, total nitrogen, and casein nitrogen. When supplemental ruminally protected AA were fed along with WCS , these milk components increased, thereby alleviating the issues associated with feeding WCS on milk protein.

With the decrease in milk protein percentage, a corresponding decrease in solids-not-fat (SNF) is observed. Bitman et al. (1996) noted a decrease from 8.87 to 9.10 % SNF when cows were fed 10% WCS compared with cows fed no WCS. Smith et al., (1981) also reported that a linearly decrease in SNF percentage as WCS increased from 0 to 20% of the dietary DM. The reduction in percent SNF is most likely attributed to the reduction in milk protein percentage. Feeding WCS generally has no effect on lactose yield~~s~~ or percentage (Mooney and Allen, 1997; Mena et al., 2004).

Effects of free fatty acids in the diets of lactating cows

Lipid digestion within the rumen begins with lipolysis which causes the release of free fatty acids (FFA) from esterified plant lipids. Microbial lipases and esterases hydrolyze triglycerides to fatty acids and glycerol. Glycerol concentrations are generally very low in the rumen due to the compound’s rapid fermentation to yield propionic acid (Jenkins, 1993).

Lipolysis creates a free carboxyl end on the FFA which is critical for the next step in lipid digestion, biohydrogenation. Biohydrogenation is a protective mechanism that converts unsaturated fatty acids (UNFA) into more saturated fatty acids (SFA). Increased levels of UNFA in the rumen may have a toxic effect on the rumen micro flora and may lead to shifts in normal rumen fermentation patterns (Jenkins, 1993). When high levels of UNFA are fed in the diet of lactating cows, fats and oils can coat feed particles and prevent cellulolytic bacterial attachment and enzyme contact, depressing fiber digestibility. Also, the attachment of long chain fatty acids (LCFA) to the lipid bilayer of biological membranes of rumen microbes may negatively alter the function of the biological membrane and be toxic to the microorganism. Biohydrogenation converts UNFA to more SFA by decreasing double bonds with the addition of hydrogen. Initially, an isomerase converts the cis-12 double bond of the UNFA to a trans-11 isomer. If the UNFA is polyunsaturated, the hydrogenation of other double bonds is carried out by a reductase (Jenkins, 1993). Complete biohydrogenation of fatty acids in the rumen is dependent on conditions in the rumen and the concentrations of UNFA (Jenkins, 1993).

There is also a minor amount of microbial fatty acid synthesis in the rumen. Microbes may use dietary fats as a source for fatty acid formation or may use byproducts of fermentation for de novo synthesis. Microorganisms use acetate and glucose as precursors for even number chained fatty acids, propionate and valerate for odd numbered chains, and isobutyrate, isovalerate, and 2-methyl butyrate for branched chain fatty acids (Jenkins, 1993). Minimal amounts of fatty acids are metabolized to VFA and CO_2 fermentation or to absorption across the rumen epithelium. Dietary and synthesized fatty acids are absorbed into the intestinal epithelium where they are re-esterified and packaged as chylomicrons for transport through the lymph system to the liver or mammary gland (Church, 1988).

Oilseeds high in FFA are generally unsuitable for commercial oil production and are sold to the livestock industry for feed. The addition of fat to the diet of lactating cows may disrupt rumen fermentation and reduce nutrient digestibility, specifically structural carbohydrates if fed in excess (Jenkins and Palmquist, 1984). Zinn and Plascencia (1993) observed that supplementation with yellow grease (15% FFA) decreased molar proportions of acetate and increased molar proportions of propionate in Holstein steers thereby reducing acetate: propionate ratios. Plascencia et al. (1999) observed a quadratic response for molar proportions of acetate when steers were supplemented with yellow grease (15 % FFA), griddle grease (42% FFA) or a combination of the two (28.5 % FFA). Acetate proportions for steers consuming the 15 % FFA diet (44.26 mol/100mol) decreased compared with control (50.61 mol/100mol) and increased with 28.5 % FFA to 58.27 mol/100mol. Molar proportions of acetate decreased as FFA levels increased to 42 % (50.14 mol/100mol). A similar quadratic response was noted for molar proportions of propionate with the 28.5 % FFA diet reporting the lowest concentrations of propionate. These results are in contrast to research by Avila et al. (2000) in which supplemental fat in the diet from yellow grease or tallow tended to increase molar proportions of acetate and acetate: propionate ratio. Bock et al. (1991) observed no differences in VFA production when feeding soybean oil soapstocks with 50% FFA and tallow with 15% FFA at 3.5% of the diet. Rumen pH is generally unaffected by FFA supplementation (Bock et al., 1991; Zinn and Plascencia, 1993).

Feeding higher levels of FFA generally has little effect on nutrient digestibility provided dietary fat levels do not exceed the recommended 5-7 % of DM. Zinn and Plascencia (1993) reported a tendency for total tract organic matter digestibility to decrease with increased FFA intake. This decrease was attributed to a decrease in OM digestibility in the rumen. Efficiency of microbial nitrogen synthesis increased 10.2 % and rumen nitrogen efficiency (nonammonia N entering the small intestine/ N intake) increased by 6.2 %with the addition of yellow grease to the diet. Plascencia et al. (1999) observed no differences in total tract digestibility when steers were fed increasing levels of FFA from yellow grease and griddle grease.

Feeding WCS with increased levels of FFA

When harvest of cotton is delayed by warm, moist conditions such as tropical storms, the moisture level in the seed remains elevated and concentrations of free fatty acids (FFA) increase. Previous research by Sullivan et al. (2004, 2005) has examined the effects of feeding WCS with elevated concentrations of FFA in the oil on rumen fermentation in Holstein steers and performance of lactating cattle. Whole cottonseed with FFA concentrations of 8.0, 11.3, 14.7, and 18.0 % were fed to ruminally and abomasally cannulated Holstein steers at 12.5 % of the ration DM (Sullivan et al., 2005). There were no differences in DMI among treatments indicating that increasing levels of FFA in WCS did not affect the acceptability of the seed in the diet. Digestibility of ADF (kg/d) decreased linearly as amount of FFA increased while ADF digestibility (%) was similar among treatments. Average pH decreased linearly while molar proportions of acetate and acetate: propionate ratio increased with increasing levels of FFA in WCS. Molar proportions of propionate responded cubically with increasing FFA levels with higher concentrations for 8 and 14.7 % compared with 11.3 and 18.0%. There was also a cubic response to total VFA with higher concentrations for 11.3 and 18 % FFA compared with 8 and 14.7 %. The authors noted that the effects of FFA in WCS for this trial were not indicative of major shifts in microbial fermentation or fiber digestion.

Research examining the effects of elevated FFA in WCS on lactation performance and nutrient digestibility (Sullivan et al., 2004) indicated that WCS with 3, 6, 9, or 12 % FFA fed at 12.5 % of the ration DM did not alter DMI or milk yield in lactating Holstein cows. A cubic response for milk fat percentage indicated higher milk fat percent at 9 % FFA with the lowest milk fat percent associated with the 6 % FFA diet. Researchers noted that milk yield was numerically lower for the 9 % diet which may indicate a dilution effect for milk fat percentage. All other milk components were similar among treatments.

Milk fatty acid composition was slightly altered with increasing levels of FFA in WCS (Sullivan et al., 2004). Concentrations of C6:0 decreased linearly as FFA levels increased. Concentrations of C16:0, C16:1, and total medium chain fatty acids increased linearly with increasing levels of FFA in WCS. The authors noted that increasing FFA in WCS does not appear to alter MCFA or LCFA which are generally associated with off-flavors in the milk.

Apparent digestibility of CP and NDF indicated a cubic response to increasing levels of FFA in WCS. The CP digestibility was highest for the 3 and 9 % FFA and lowest for 6 and 12 % FFA. Digestibility of NDF was highest for WCS with 6 % FFA and lowest for the 9 % FFA diet. Apparent intake and digestibility of ADF increased linearly with increasing levels of FFA in WCS.

Effects of dietary starch in ruminants

Starch is the major energy component of cereal grains and represents 70 to 80% of the grains composition (Nocek and Tamminga, 1991). The starch granule is composed of two major compounds, amylose and amylopectin. Amylose is a compound composed of linear α-1-4 glucose molecules while amylopectin is a branched chain compound composed of α-1-4 and α-1-6 glucose molecules. Most cereal grain starches contain 15 to 30% amylose (Church, 1988). While all cereal grains contain some starch, the histological features and chemical heterogeneity differ among grain sources. These differences give each starch source its own digestibility and characteristics within the ruminant digestive system.

In general, starch is rapidly and totally degradable in the rumen. Up to 15 different strains of amylolytic bacteria have been identified with 8 amylolytic enzymes produced in the rumen (Kotarski et al., 1992). Starch is quickly hydrolyzed by bacterial fermentation into maltose then broken down into glucose by saccharolytic bacteria (Church, 1988). Glucose then enters the glycolysis pathway and is converted to ATP for use as an energy source for bacteria. Microorganisms depend on the carbon skeletons and ATP produced from the fermentation of readily available carbohydrates for microbial protein synthesis.

The production of VFA associated with feeding starch is variable depending on forage to concentrate ratio of the ration. In general, as concentrate levels increase in the diet, molar proportions of propionate increase and subsequently acetate: propionate ratios decrease (Church, 1988). Propionate is a glucogenic fatty acid that allows for increased glucose production in the animal, providing increased energy available for milk production. However, decreased acetate production signals a possible decrease in milk fat production in animals with increased starch intake.

Excess fermentation of starch can be associated with reduced buffering capacity and ruminal pH (Krause et al., 2003). Feeding starch can increase molar concentrations of propionate and lactate concentrations in the rumen (Slyter, 1976). The accumulation of VFA and lactate causes a decrease in rumen pH which may surpass the buffering capacity of ruminal fluid.

While small grains such as barley and sorghum are about 94% fermentable in the rumen, corn starch is only about 74% digestible in the rumen (Waldo, 1973). Five to 20% of starch consumed is digested postruminally, primarily in the small intestine (Streeter et al., 1989; Zinn, 1991; Huntington, 1997). Bypass of starch into the small intestine allows for enzymatic digestion and absorption of starch into glucose which is more energetically efficient than fermentation of starch and absorption of VFA in the rumen (Owens et al., 1986).

Dry matter intake and digestibility response to corn starch is variable depending on several factors associated with feeding readily fermentable starch including grain processing and forage to concentrate ratio. As previously stated feeding high levels of readily fermentable starch may overwhelm the buffering capacity of the rumen and cause a decrease rumen pH. A decrease in ruminal pH can decrease appetite (Britton and Stock, 1987) thereby leading to decreased intake. Others (McCarthy et al., 1989; Aldrich et al., 1993) have noted a decrease in DMI when feeding corn as a source of energy. Others (Joy et al., 1997; Crocker et al., 1998) have demonstrated no change in DMI as level of starch increased in the diet.

Callison et al. (2001) compared the digestibility of fine, medium, and coarse ground corn and noted that as particle size decreased, apparent total tract digestibility of OM and NCS increased in lactating cows fed a diet based on alfalfa silage. Ruminal digestibility of NDF tended to decrease linearly as corn particle size decreased. Krause et al. (2003) observed no effects of increasing levels of corn starch in the diet on DM or NDF digestibility but noted an increase in starch digestibility with increasing levels of corn starch. These authors indicate that starch from other feeds in the ration had a total tract digestibility of 85.1% while the total tract digestibility of corn starch was 98 %. Smaller particle size increases the surface area of the corn and allows for greater microbial attachment in the rumen. This allows for increased digestibility of the smaller particulate matter.

Urea as a non-protein nitrogen source

Urea is the most widely used non-protein nitrogen (NPN) source in ruminant diets. Urea consists of approximately 46 % nitrogen and is found naturally in many plants. The use of urea as a protein source is especially cost effective when plant sources such as soybean meal are more expensive (Stanton, 2004). Urea is considered to be completely degraded in the rumen and is quickly converted to ammonia for use in microbial protein synthesis. The use of urea in ruminant diets can be traced back to the 1920's and 1930's in Europe and was officially approved in the United States as a feed ingredient in ruminant diets in 1940 by the Association of American Feed Control Officials (National Research Council, 1976).

There are four general guidelines concerning the feeding of urea in the ruminant diet (Jurgens, 1976). First, no more than one-third of the total N in the ration should be fed as urea. Second, urea should constitute no more than 1 % of the diet or 3% of the concentrate mix. Third, no more than 10 to 15 % of a typical protein supplement should be fed as urea. Lastly, no more than 5 % of a urea supplement should be fed in rations composed of low quality roughage.

There are several factors that influence the use of urea in ruminant diets. First, the amount and kind of carbohydrates fed affects the level at which urea is fed in the diet. Huber and Kung (1981) noted that the major factor limiting utilization of NPN is a readily available energy source. In general, cattle fed diets high in digestible energy, such as high grain diets, utilize urea more efficiently than those fed high forage diets (Stangel, 1963; Casper and Schingoethe, 1989). Johnson (1976) theorized that ruminal availability of carbohydrate and ammonia source (urea) is necessary for optimal microbial response to urea in the diet. Urea is quickly degraded in the rumen, as are grains. As the carbohydrate source is digested, urea is simultaneously being degraded. The presence of NH_3 and energy levels are optimal for microbial uptake and protein synthesis. In contrast, high forage diets are more slowly degraded in the rumen. Because urea is more quickly degraded, N availability may be limited for protein synthesis with these diets.

Urea should be fed at least daily to obtain the maximum benefits. Feeding urea continuously or at a constant level will improve its utilization compared with periodic or abrupt intake (NRC, 1976). Studies have noted that after a single administration of dietary urea, rumen ammonia levels peak at 60 to 90 minutes and then decline to initial concentrations within 4 to 5 hours (NRC, 1976). This creates an erratic ammonia pool for the microflora that can be balanced by feeding urea over a period of time.

Also, the level of feeding of urea is important. Low levels of urea are utilized more efficiently and with fewer problems to the animal than high levels. Polan et al. (1976) fed diets containing 0, 10, 20, 30, and 40 % of the dietary CP supplied by urea. Results indicated that as urea level in the diet increased, ruminal NH_3 concentrations increased from 20 to 50 mg/100ml of rumen fluid. These researchers also noted a tendency for increased ruminal pH in animals consuming the greatest amount of urea. Elevated ruminal pH adversely affects ruminant digestion. As previously noted by Jurgens (1972), no more than one-third of the total N in the ration should be fed as urea.

When substituting urea for a plant protein supplement such as soybean meal, the quality and quantity of minerals available is also decreased. Specifically, the addition of a readily available sulfur supplement is needed when feeding urea in the diet (NRC, 1976). This additional sulfur may come from an organic supplement or from other feedstuffs in the ration.

Dietary protein solubility further influences the feeding of urea to ruminants. Wohlt and Clark (1978) compared a highly soluble natural CP source (soybean meal) with urea in a 310 d lactation trial. A corn silage based ration was fed with CP levels ranging from 9 to 14.5 % of DM. Protein source did not affect total DMI or milk yield at lower protein levels. However, as CP levels increased, milk and protein yield was greatest for animals fed diets supplemented with soybean meal. Broderick et al. (1993) compared urea with SBM and meat and bone meal as CP supplements and observed decreased blood urea nitrogen (BUN) levels in diets containing meat and bone meal. Lower BUN concentrations imply that ruminally degraded protein may have been lower on these diets.

Research concerning the effect of urea on DMI is somewhat variable but overall demonstrates no significant effect. The variability among studies involving urea is generally related to carbohydrate source and level of urea fed as previously stated. While some researchers (Wohlt and Clark, 1978; Cameron et al., 1991; Broderick et al., 1993- trial 1) did not observe any differences in DMI when feeding urea compared with other NPN supplements, others (Broderick et al., 1993- trial 2; Broderick et al., 2000) have observed decreased DMI with urea supplementation. The rations fed in the trials conducted by Broderick consisted of high proportions of alfalfa silage, which may have reduced DMI for these animals.

Production response to urea is variable. Increased milk yield has been observed with the addition of urea to the diet compared with no supplemental NPN source in the diet (Polan et al., 1976; Cameron et al., 1991). However, when compared to SBM as NPN source in the ration, addition of urea yielded no change (Wohlt et al., 1978) or decreased milk yield (Broderick et al., 1993)

Yield of fat corrected milk and milk fat were not affected by the addition of urea to the diet (Casper et al., 1990; Cameron et al., 1991). Milk protein percentage and yield may increase with the addition of urea to the diet (Casper and Schingoethe, 1989; Broderick et al., 1993; Broderick et al., 2000). It is assumed that the increased protein in the milk is an increase in the NPN fraction of the milk (Casper et al., 1990). Solids-not-fat may also increase (Casper et al., 1990; Broderick et al., 2000) as a result of the increase in milk protein. Milk urea nitrogen also increases with the addition of dietary urea (Broderick et al., 1993; Broderick et al., 2000).

Ruminal NH_3 concentrations are greatly increased when diets are supplemented with urea (Broderick et al, 1993; Rihani et al., 1993; Broderick et al., 2000). Cameron et al. (1991) noted a two-fold increase in NH_3 concentrations in animals fed urea compared with animals with no NPN supplement (15.3 vs. 7.3 mg/dl). Ruminal pH also tends to increase with the addition of urea to the diet (Cameron et al., 1991; Broderick et al, 1993). Rumen volatile fatty acid profiles are not generally affected by urea supplementation.

The variability of animal response to urea can most often be explained by the variation in rations and amount of urea fed to the animals in each study. As previously noted, carbohydrate availability, protein solubility, and frequency and level of feeding are all factors to consider when feeding urea in the ruminant diet.

Use of yeast as a feed additive

With the growing concern over the use of antibiotics and other growth stimulants in the animal industry, there is increased interest in the use of direct-fed microbials such as yeast culture to improve animal performance. Yeast culture is a feed additive commonly used by commercial dairies. Direct-fed microbials containing yeast primarily consist of *Saccharomyces cerevisiae* cultures. These products contain viable yeast cells plus a starch-rich growth medium and are generally fed at a range of 3 to 110 g/d per animal (Martin and Nisbet, 1992). The addition of yeast culture to the diet of dairy cattle has been shown to increase DMI (Gomez-Alarcon et al., 1990; Williams et al., 1991; Erasmus et al., 1992) and milk yield (Williams et al., 1991; Wohlt et al., 1991). Ruminal fermentation characteristics can also be altered with the addition of yeast to the diet. Total VFA concentrations (Malcolm and Kiesling, 1990; Miller-Webster et al., 2002), pH (Harrison et al., 1988), and cellulolytic bacterial counts (Martin and Nisbet, 1992) are improved whereas rumen NH_3 concentrations are decreased (Erasmus et al., 1992; Yoon and Stern, 1996). However, both production and ruminal fermentation responses are variable within the literature. Specifically, the type of diet and stage of lactation are important variables to consider when supplementing dairy cattle diets with yeast.

An increase in DMI was observed in animals fed diets containing yeast cultures (Williams et al., 1991; Wohlt et al., 1991; Wohlt et al., 1998). Erasmus et al. (1992) observed an increase of 1.4 kg/d in DMI in cows fed diets containing yeast culture. Dann et al. (2000) noted improved DMI in Jersey cows fed 60 g/d of yeast culture compared with control (13.7 vs. 11.9 kg/d). Others (Arambel and Kent, 1990; Piva et al., 1993) have not observed any difference in DMI when supplemental yeast culture was fed.

Milk yield response of cows fed diets containing yeast culture is also variable. Wohlt et al. (1998) observed increased 3.5 % fat corrected milk yield when Jersey cows were fed diets supplemented with 0, 10, or 20 g/d of yeast culture (37.7, 40.7, and 41.4 kg/d respectively). These results are in agreement with other studies (Williams et al., 1991; Wohlt et al., 1991). However, others (Swartz et al., 1994) have reported no differences in milk yield with the addition of yeast culture to the diet.

Ruminal fermentation characteristics are also altered with the addition of yeast culture to the diets of lactating dairy cattle. The addition of yeast culture to the diet tends to reduce the acetate: propionate (A: P) ratio (Erasmus et al., 1992; Miller-Webster et al., 2002). The decrease in A: P ratio is generally associated with increased propionate concentrations rather than a decrease in molar proportions of acetate. Furthermore, yeast supplementation decreases the mean concentration of lactic acid and lowers the peak lactic acid concentration (Williams et al., 1991; Erasmus et al., 1992). This change in lactic acid may be due to the ability of yeast cultures to stimulate the activities of *Selenomonas ruminantium*, which utilizes lactic acid (Erasmus et al., 1992; Callaway and Martin, 1997). With the decrease in lactic acid production a corresponding increase in ruminal pH is expected. However, a consistent change in ruminal pH by yeast supplementation has not been observed (Dawson et al., 1990; Yoon and Stern, 1996; Miller-Webster et al., 2002). While individual molar proportions of VFA may change with the addition of yeast culture, total VFA concentration is not altered with yeast supplementation (Piva et al., 1993; Yoon and Stern, 1996).

Reduced ruminal ammonia concentrations have been observed in animals fed yeast culture (Harrison et al., 1988). Erasmus et al. (1992) noted a 10% decrease in ruminal ammonia concentrations when diets contained 10 g/d yeast culture. Lower ruminal ammonia concentrations may indicate a possible stimulatory effect of yeast culture on microbial protein synthesis where ammonia N is incorporated in microbial protein. An increase in numbers of rumen proteolytic microorganisms has been observed in animals supplemented with yeast culture (Yoon and Stern, 1996).

Examination of bacterial populations in yeast supplemented animals indicates that there is an increase in the numbers of cellulolytic bacteria in the rumen of animals supplemented with yeast culture. Dawson et al. (1990) examined the effects of the addition of yeast culture to continuous culture and to the rumens of steers fed fescue- hay based roughage diet and found that cellulolytic bacterial numbers were 5 to 40 times greater than those observed in control cultures and steers. However, this increase in cellulolytic bacteria numbers may not be biologically significant. While population numbers are increased, a consistent increase in fiber digestion has not been observed (Martin and Nisbet, 1992).

The variability associated with feeding yeast cultures to lactating dairy cattle can be attributed to several factors. Stage of lactation, type of diet fed, and forage: concentrate ratios all have an effect on the response of animals to yeast supplementation. As previously stated, there are several reports where both DMI and milk yield increase in response to supplemental yeast culture. However, the majority of these studies included animals in early lactation (wk 1 to 18). Studies involving animals in the later stages of lactation are variable and show less production response to the addition of yeast to the diet (Arambel and Kent, 1990; Robinson and Garrett, 1999). Also, diets with greater proportions of concentrates tend to elicit a greater response to yeast supplementation, possibly because of increased lactic acid utilizing bacteria and resulting improvement in ruminal pH (Williams and Nebold, 1990; Williams et al., 1991).

Animal response to yeast culture supplementation is generally associated with high stress periods- parturition, early lactation, heat stress, and diet changes. However, on commercial dairies where a group TMR is commonly fed, the ability to target only stressed animals is not economically feasible. Therefore, it is recommended that yeast culture be added to the TMR for the entire lactation period to be economically feasible (Shaver and Garrett, 1997).

Dietary buffers and sodium bicarbonate

Maintaining ruminal pH in an optimal range is critical to animal health. Rumen pH generally ranges from 5.5 to 7.0 (Church, 1988). High producing cows that consume large amounts of energy in the form of concentrates may exceed the rumen's natural buffering capacity, which decreases pH causing reduced acetate production and consequently a depression in milk fat. Low ruminal pH leads to health problems, including laminitis, acidosis, hepatic abscesses, and in severe cases, death. Ruminal pH is inversely related with VFA production and absorption, water flux across the rumen wall, salivary buffers and the flow of saliva to the rumen, feed acidity, and water outflow to the omasum (Erdman, 1988). Regulation of pH is dependent on these factors and when the animal is unable to maintain physiological pH in the rumen, the use of dietary buffers is warranted.

A buffer is defined as “a material that when present in aqueous solution, causes an effective resistance to change in pH of that solution when a strong acid or base is added” (Erdman, 1988). A buffer must meet three specific criteria. First, it must be water soluble. Second, it should be a weak acid or base or a salt. Finally, a buffer should have a pKa near the physiological pH of the system it is to buffer.

The first line of defense in maintaining rumen pH is the buffering capacity of saliva. Saliva composes the majority of the liquid phase in the rumen to help maintain a suitable environment for microbial growth and fermentation. Saliva contains 125 meq/L of bicarbonate with 26 meq/L of phosphate and has a pH of about 8.4 in cattle (Church, 1988; Erdman, 1988). On average, dairy cattle produce about 171 L of saliva each day, depending on the composition and intake of the feed (Erdman, 1988).

The buffering capacity of ingested feed also serves to maintain rumen pH. Forages have a greater buffering capacity than concentrates and legumes having greater acid buffering capacity than grasses or whole plant corn (Playne and McDonald, 1966). Concentrates and cereal grains have a very low buffering capacity while protein has a higher capacity (Jasaitis et al., 1987). Fermented feeds, including silages, have two to three times the buffering capacity of fresh forages most likely due to the presence of lactic acid in the silage (Jasaitis et al., 1987). While the buffering capacity of forages serves to maintain ruminal pH, forage intake also stimulates chewing and saliva production which further acts to buffer the rumen environment (Church, 1988).

High producing dairy cows need an energy dense diet to maintain high milk yield. To maintain the energy density of the ration, many producers are forced to feed greater proportions of concentrate in the diet. As previously stated, concentrates have a lower buffering capacity than forage and heavy concentrate feeding leads to decreased pH, reduced fiber digestibility, and an altered rumen microbial population (Church, 1988; Rogers et al., 1982). To overcome the reduced pH of the rumen, the addition of dietary buffers is warranted. Dietary buffers, which include sodium bicarbonate ($NaHCO_3$) and potassium carbonate, are commonly added to the rations of lactating dairy cows to aid in maintaining rumen pH.

Sodium bicarbonate is by far the most common dietary buffer added to the diets of lactating cows. The pKa of $NaHCO_3$ is 6.25, which is very similar to the normal pH of the rumen. As previously stated, a good buffer must have a pKa that is close to the physiological pH of the biological system that it buffers. The theoretical acid consuming capacity of $NaHCO_3$ is 12.2 meq/g and its water solubility is 6.9 g/100ml (Erdman, 1988). There may be issues with the palatability of $NaHCO_3$ when fed as part of a concentrate mix that is fed separately from forage. Erdman et al. (1982) noted that palatability issues could be overcome if the buffer were gradually added to the diet to allow the animal to adapt. Palatability is generally not an issue when $NaHCO_3$ is added to a total mixed ration or to the forage portion of the ration if forage and concentrate are to be fed separately (Erdman et al., 1980).

Research including $NaHCO_3$ in the diets of lactating dairy cattle indicates that feeding $NaHCO_3$ may increase DMI (West et al., 1987; Solorzano et al., 1989). Vicini et al. (1988) fed lactating dairy cows rations with or without $NaHCO_3$ and observed that at addition of 1% $NaHCO_3$, DMI increased from 23.2 to 26.0 kg/d. Others (Ghorbani et al., 1989; Kennelly et al., 1999) have reported no effect of added $NaHCO_3$ on DMI.

The addition of dietary $NaHCO_3$ increases or stabilizes ruminal pH (Thomas et al., 1984; Solorzano et al., 1989). In a review of the effects of dietary buffers, Erdman (1988) reported that the greatest effect of supplemental dietary buffers on ruminal pH occurred 4 to 8 h post feeding when pH tends to be low. These authors noted that rumen pH was greater at 6 to 8 h post feeding when diets were supplemented with $NaHCO_3$ and magnesium oxide. Tucker et al. (1992) observed that addition of 1.5% $NaHCO_3$ to the diet reduced ruminal hydrogen ion concentrations for up to 6 h post feeding, indicating an increase in ruminal pH.

Molar proportions of propionate decrease (Snyder et al., 1983; Solorzano et al., 1989) while molar proportions of acetate and butyrate and acetate: propionate ratio increases (Erdman et al., 1982; Rogers et al., 1982, Clayton et al., 1999) when feeding $NaHCO_3$ in the diets of lactating dairy cattle. The increased acetate and butyrate concentrations aid in maintaining or increasing milk fat production in cows consuming dietary buffers, specifically in diets high in concentrates where milk fat depression may occur. Dietary buffers increase rumen fluid turnover which leads to faster passage rates of soluble carbohydrates. As fluid turnover increases, soluble carbohydrates and proteins may escape rumen degradation completely. As rumen liquid dilution rate increases, molar proportions of acetate increase while propionate decreases (Harrison et al., 1975) which aids in maintaining rumen pH.

The increase in the molar proportions of acetate and butyrate in response to supplemental $NaHCO_3$ leads to increased milk yield (Thomas et al., 1984; Rogers et al., 1985) and milk fat production (Solorzano et al. 1989; Kennelly et al., 1999) compared with animals consuming no added dietary buffer. Vicini et al., (1988) noted that with the inclusion of 1.0 % $NaHCO_3$ in the diet of lactating Holsteins, milk fat percentage increased from 0.99 to 1.07 kg/d. Others (West et al., 1987, Ghorbani et al., 1989) have not observed any difference in milk fat yield or percent with feeding $NaHCO_3$. No other differences in milk fat components are associated with feeding $NaHCO_3$ (West et al., 1987; Clayton et al., 1999).

West et al. (1987) noted increased apparent digestibility of DM, ADF, and NDF in response to the addition of 1.5 % $NaHCO_3$ to the diet compared with no added buffer. Others (Solorzano et al., 1989) have reported similar responses in nutrient digestibility with the addition of $NaHCO_3$ to the diet. The increase in fiber digestibility may explain the increase in DMI in animals supplemented with buffers. Increasing fiber digestibility allows for faster rate of passage of digesta out of the rumen which allows for greater DMI (Erdman et al., 1982). Increased fiber digestibility also results in greater acetate production which would support greater milk fat production in the animal.

Erdman (1988) analyzed data from 82 experiments with a total of 3,065 cows to examine the effects of dietary buffers when fed at low, medium, and high roughage: concentrate ratios. When diets contained less than 30% forage as a percent of the DM, DMI decreased numerically from 13.2 to 12.1 kg/d in response to supplemental $NaHCO_3$; however 4% FCM increased from 13.8 to 14.5 kg/d. For animals consuming less than 30% forage, rumen pH increased from 6.31 to 6.53 with supplemental $NaHCO_3$.

Overall, $NaHCO_3$ had little effect on intake and milk production when animals consumed moderate to high levels of forage (greater than 30%). Dry matter intake and milk yield did not differ with the addition of $NaHCO_3$ to the diet. Milk fat percentage increased from 3.54 to 3.64 % most likely due to a slight increase in acetate: propionate ratio in these animals (2.45 vs. 2.65). These researchers concluded that if the dietary forage source stimulated rumination and salivation, the need for dietary buffer addition was negligible. Others (Kennelly et al., 1999) have reported similar results when feeding high concentrate diets with or with out the addition of dietary buffers.

The effects of feeding dietary buffers to alleviate milk fat depression have generally been attributed to the increased ruminal buffering capacity of the buffer. However, research by Russell and Chow (1993) disputes this explanation. According to these researchers, for buffering capacity to increase in the rumen, the concentrations of bicarbonate, dissolved CO_2, and sodium must increase with the addition of the buffer. These researchers submit that ruminal fluid is saturated with CO_2 and that the sodium balance is highly controlled in the rumen to prevent hemoconcentration or hemodilution. Alternatively, researchers propose that the addition of bicarbonates as dietary buffers increases water intake which increases ruminal fluid dilution rate. As previously stated, increased ruminal fluid dilution rate allows for increased flow of soluble carbohydrates from the rumen. Because the soluble nutrients are not degraded in the rumen, the subsequent increase in propionate production does not occur and pH is maintained.

The coating process

The high oil content of WCS makes it an attractive energy dense feed for animals with high energy requirements, such as lactating dairy cattle. The high fiber concentrations provided by the lint and the hulls are desirable for maintaining effective fiber levels in the diet. However, the lint makes WCS difficult to handle in mechanized feeding systems and limits its use in many commercial feed mills and dairy farms. The lint causes bridging, which occurs when the lint portion causes the WCS to clump together and clog the typical grain handling equipment used at feed mills and dairy farms. Recently a process has been developed in which WCS are coated with gelatinized corn starch to produce a free flowing product that can be handled with the typical machinery already in place at the mill or farm (Laird et al., 1997). The addition of a starch coating to WCS binds the linters to the hull to create a flowable product.

To begin the coating process, WCS are run through a gin stand to remove any excess linters. Next, feed grade corn starch is mixed with cool water at a ratio of 0.089 kg of starch per L of water (Laird et al., 1998). In general, a 3 to 5% add-on starch level (dry weight basis) was determined to adequately cover the WCS (Laird et al., 1997, 1998). However, as starch concentrations increase in the coating, moisture content also increases by 33% with 3.5% add-on starch (Laird et al., 1998) and 36% with 3.5% starch (Laird et al., 1997). The greatest cost associated with coating WCS is the cost of drying the product. Therefore the level of starch added should be closely evaluated to minimize added moisture and costs associated with the drying process.

Adequate water temperatures and levels are critical for adequately mixing the feed grade starch into solution with the water for coating the WCS. Water temperatures below 60° C cause the corn starch to lump and not mix well. Once the starch is dissolved in the water, the temperature of the mixture is increased to 77° C to cause the starch to gelatinize. If water levels are inadequate to fully penetrate and wet the linter fraction of the seed, the coating will become spongy when applied to the seed and dried, causing the seed to stick together resulting in bridging in machinery (Laird et al., 1998).

Once the corn starch mixture has gelatinized, WCS that has been processed through a modified gin stand to remove tags and loose lint is loaded onto a mixing conveyor and the hot gelatinized starch solution is sprayed over it to completely cover the WCS. Stirring reels mix the seed at about 3.7 m intervals on the mixing conveyor to allow for greater air flow to the seed and further speed drying time. This mixing also keeps WCS from sticking together until the seeds dry. Once coated, the coated WCS is dropped into a single cross conveyor that moves the WCS to the drying belt. The coated WCS is loaded onto the belt so that it is in an even layer for faster drying. Coated WCS must be dried to less than 10% moisture. Generally WCS are dried at around 149° C for approximately 10 min and are then cooled before storage. Drying at this temperature for a short amount of time does not affect acid detergent insoluble nitrogen levels in the seed (Bernard, 1999).

Previous coating work

Previous research on coated WCS has focused on addition of starch to improve handling characteristics and to create a free flowing product that can be handled in mechanized feeding systems (Laird et al., 1997). Early work (Bernard et al., 1999) compared the effects of coating WCS with 5% gelatinized corn starch or 5% gelatinized corn starch with 10% maltodextrin sugar with fuzzy WCS. Researchers observed no effect on DMI or milk yield when feeding coated WCS compared with fuzzy WCS. Furthermore, there were no observed differences in percentage of milk protein or lactose, or yield of milk components among treatments. However, milk fat percentage decreased for cows fed diets containing WCS coated with 5% corn starch and 10% maltodextrin compared with fuzzy WCS (4.49 vs. 4.99 %, respectively). In vitro ruminal fermentation analysis demonstrated that the addition of starch and maltodextrin increased total VFA production compared with fuzzy WCS. Specifically, propionate production increased from 26.36 to 33.42 mol/100mol with the addition of starch and maltodextrin. The increased production of propionate would provide additional energy substrates for milk synthesis. This would allow for reduced mobilization of body tissues as evidenced by decreased non-esterified fatty acid concentrations in cows fed WCS coated with starch and sugar. Furthermore, acetate production was reduced in animals consuming WCS coated with starch and maltodextrin compared with fuzzy WCS. Because acetate is the precursor for milk fat synthesis, reductions in acetate concentrations may explain the reduced milk fat percentage. Researchers reported no difference in body weight among treatments.

As previously stated, the addition of starch to the diet may alter ruminal fermentation and nutrient digestibility. In this study, Bernard et al. (1999) observed a decrease in the apparent digestibility of DM for WCS coated with starch and maltodextrin and a decrease in ADF and NDF digestibility with both coated WCS treatments compared with fuzzy WCS. Increasing starch concentrations in the diet reduces ruminal digestion of ADF and NDF (Cameron et al.,1991). In the presence of increased amounts of readily fermentable carbohydrates, cellulolytic bacterial activity is reduced (Hoover 1986). The inhibition of cellulolytic activity leads to decreased fiber digestion and a general reduction in acetate production. The reduction in fiber digestibility and acetate production may explain the reduced milk fat percentage in animals fed WCS coated with starch or starch and maltodextrin.

While the addition of 5% corn starch to the coating of WCS maintained DMI, reduction of the amount of starch added to the coating could reduce drying time of the seed, thereby reducing costs. As previously discussed, the energy associated with drying coated WCS is the most expensive cost of the process. Less starch in the coating would require less water and hence, less drying time for the seed. A follow-up study (Bernard, 1999) was conducted to examine the effects of coating WCS with 2.5% starch on performance of lactating dairy cows. Dry matter intake was similar between treatments, indicating that coating WCS does not alter the palatability of the seed. Furthermore, milk yield and concentration and yield of milk components were similar among treatments. Energy corrected milk per unit of DMI and BW gain were greater for the 2.5% starch group compared with animals consuming fuzzy WCS. The authors concluded that these differences would suggest improved energy utilization for animals consuming the 2.5% starch coated WCS.

As previously noted, the addition of readily fermentable carbohydrates to the diet alters ruminal fermentation and depresses milk fat percentage (Poore et al., 1993). Cellulolytic bacterial activity is inhibited in the rumen thereby reducing fiber digestion. Cellulolytic bacteria use ammonia as their primary nitrogen source, which may be limiting when feeding rapidly fermentable carbohydrates. The addition of urea to the coating of starch coated WCS may provide a readily available nitrogen source to cellulolytic bacteria, offsetting any effects associated with feeding fermentable carbohydrates. Research examining ruminal fermentation parameters (Bernard et al., 2001, 2003) compared WCS coated with combinations of starch and urea at varying concentrations with fuzzy WCS. In the first in vitro ruminal fermentation study (Bernard et al.,2001), treatments were arranged in a 3 x 4 factorial to provide three concentrations of starch (0.0, 2.5, and 5.0%) and four concentrations of urea (0.0, 0.25, 0.5, and 1.0%). Researchers reported a linear decrease in pH, molar proportions of acetate and butyrate, and acetate to propionate ratio as starch concentrations increased. A linear increase in hydrogen, methane, and total VFA concentrations, and molar proportions of propionate was observed as starch content of the coating increased. As urea concentration increased, there was a linear increase in pH, methane and ammonia concentrations, and molar proportions of butyrate while hydrogen concentrations decreased. There was no effect of urea on molar proportions of acetate, propionate, acetate: propionate, or total VFA. An increase in hydrogen has been associated with decreased NDF digestibility (Piwonka and Firkins, 1996).

There was an interaction between starch and urea for hydrogen, methane, ammonia, and L-lactate concentrations. Hydrogen concentrations decreased as urea increased at 0 and 2.5 %

starch, but increased with 0.5% urea and 5.0% starch. Methane concentrations were relatively constant at 0 and 2.5% starch as urea increased. However, as starch increased to 5.0 %, methane levels increased with increasing levels of urea. Researchers noted that these results are consistent with increases in methane and hydrogen associated with feeding increased levels of rapidly fermentable carbohydrate. The lack of response at the 2.5 % level of starch indicates that urea was effective at maintaining cellulolytic activity or that the amount of starch was not sufficient to significantly alter hydrogen and methane production.

A tendency for increased ammonia concentrations was observed as urea increased for the 2.5 % starch WCS, but no increase was observed at the 5.0 % starch level until urea levels reached 1.0 %. When starch to urea concentrations were maintained at a 10:1 ratio, ammonia concentrations remained steady. However, as urea increased to 1.0 %, ammonia concentrations began to increase and were in excess of acceptable levels. Researchers suggested that addition of 1 % urea to the starch coating was excessive to maintain cellulolytic activity.

In a second in situ rumen fermentation trial (Bernard et al., 2003), treatments were arranged in a 4 x 5 incomplete Latin square study to determine the effects of including two concentrations of starch (2.5 and 5.0%) and two concentrations of feed grade urea (0.25 and 0.5%) in the coatings of WCS. Coating WCS decreased pH and molar proportions of isobutyrate compared with fuzzy WCS while concentrations of ammonia nitrogen increased with coated WCS. Researchers noted the reduction in molar proportions of isobutyrate indicates reduced lysine fermentation for cows fed coated WCS.

There were no reported effects on nutrient intake or apparent digestibility of DM, OM, and ether extract in animals consuming coated WCS compared with fuzzy WCS in vivo (Bernard et al., 2003). However, there was an observed interaction between starch and urea levels for

DMI. At the 0.5% urea level, DMI decreased as starch level increased. Also, there was a tendency for ruminal NDF digestibility to increase at the 0.5 % urea level over each of the two starch levels. These researchers concluded that while the addition of starch and urea to the coating of WCS may slightly alter ruminal fermentation, these effects are relatively minor and most likely would not have a great effect on digestion in the animal.

The addition of up to 5% starch in the coating of WCS would increase dietary starch concentrations by 0.75 percentage units when fed at 15% of the ration DM. This small amount of starch is most likely not adequate to alter rumen pH or total VFA production. However, the immediate area surrounding the coated WCS may be affected. If the immediate surrounding area were considered its own microenvironment, pH changes within that environment may favor amylolytic bacteria over cellulolytic bacteria in the presence of starch. Manipulation of the ratio of starch to urea or the addition of other value added feeds may improve the digestibility of coated WCS to surpass uncoated seed.

Conclusions

The feeding value of WCS can be negatively or positively affected. Delayed harvest of cotton allows for germination of the seed and increases in moisture and FFA levels. This off quality seed is generally sold to the livestock industry as feed for cattle. Previous research has demonstrated that feeding WCS with up to 18% FFA in the oil to Holstein steers increased the molar proportions of acetate and the acetate: propionate ratio, but did not negatively impact nutrient intake (Sullivan et al., 2005). Sullivan et al., (2004) noted that feeding WCS with up to 12% FFA in the oil at 12.5% of the DM to lactating cows did not affect fiber digestibility or milk yield or composition. The effects of feeding WCS with even higher concentrations of FFA have not been examined. The objectives of this study were to determine the effects of feeding WCS with elevated concentrations of FFA in the oil on nutrient intake, milk yield and composition, and ruminal fermentation of lactating dairy cows.

Recent research efforts have focused on coating WCS with value added feeds to improve flowability of the seed in commercial feed mills and add nutritive value to the seed. Coating WCS with gelatinized corn starch binds the lint producing a free flowing product that improves handling characteristics (Laird et al., 1997). However, the addition of starch may decrease fiber digestibility of the seed. The addition of value added feeds such as urea, yeast culture, and $NaHCO_3$ to the gelatinized corn starch coating may improve fiber digestibility and animal performance. The objectives of these trials were to determine the effects of the addition of urea, yeast or $NaHCO_3$ in the gelatinized corn starch coating applied to WCS on in vitro mixed ruminal microorganism fermentation and on nutrient intake and digestibility and on milk yield and composition in lactating dairy cows.

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CHAPTER 3

EFFECT OF INCLUSION OF UREA, YEAST CULTURE OR SODIUM BICARBONATE IN THE STARCH COATING OF WHOLE COTTONSEED ON IN VITRO RUMINAL FERMENTATION[1]

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ABSTRACT

Two in vitro mixed ruminal microorganism fermentation studies were conducted to determine the effect of coating whole cottonseed with value added feeds. In experiment 1, treatments were arranged in a 2 x 2 x 2 factorial and included two concentrations of starch (0, 2.5 %), two concentrations of urea (0, 0.25 %) and two concentrations of yeast (0, 2 %). As starch increased, pH and molar proportions of valerate decreased while concentrations of ammonia increased. The addition of yeast to the coating decreased total VFA and acetate: propionate ratio due to an increase in molar proportions of propionate. Urea alone had very little effect on rumen fermentation when added to the coating. There was an interaction for starch and urea levels for molar proportions of valerate, isovalerate, and pH. There was also an interaction observed between starch and yeast concentration in the coating for pH and molar proportions of valerate. A urea by yeast interaction was reported for molar proportions of valerate and a tendency for interaction for ammonia, butyrate, and acetate: propionate ratio. A starch by urea by yeast interaction was noted for pH, ammonia, total VFA, and acetate: propionate ratio.

In experiment 2, treatments included 2.5% gelatinized corn starch coated WCS with three concentrations of urea (0, 0.5, 0.75%) and four concentrations of sodium bicarbonate (0, 0.06, 0.08, and 0.10%) in a 3 X 4 factorial arrangement. As concentration of urea in starch coated WCS increased, ammonia concentrations and molar proportions of butyrate increased while molar proportions of propionate decreased. The addition of sodium bicarbonate to the starch coating decreased ammonia concentrations, and molar proportions of butyrate, isobutyrate, and valerate.

As the level of urea in the coating increased, total VFA concentrations increased with increasing concentrations of sodium bicarbonate. There was also an observed interaction between urea and sodium bicarbonate level for molar proportions of propionate, butyrate, valerate, and acetate: propionate ratio. The addition of urea and sodium bicarbonate increased NDF digestibility. Results from these experiments indicate that including urea, yeast, and sodium bicarbonate to the gelatinized corn starch coating of WCS may alter in vitro ruminal fermentation and provide for increased cellulolytic bacterial activity which would increase NDF digestibility of the starch coated WCS.

(**Keywords** : cottonseed, starch, urea, yeast culture, sodium bicarbonate)

INTRODUCTION

Whole cottonseed (WCS) is commonly used in rations fed to lactating dairy cows as a source of energy, fiber, and protein. According to NRC values (NRC, 2001), WCS contains 23.5 % crude protein (CP), 50.3 % neutral detergent fiber (NDF), 40.1 % acid detergent fiber (ADF), and 19.3 % ether extract (EE). The high oil content of WCS makes it an attractive energy dense feed for animals with high energy requirements, such as lactating dairy cattle. The high fiber concentrations provided by the lint and the hulls are desirable for maintaining effective fiber levels in the diet. The ability to maintain energy while increasing effective fiber levels makes WCS a unique feedstuff.

While the lint and hull portions of WCS provide effective fiber, the lint portion of the seed makes WCS difficult to handle in mechanized feeding systems and limits its use in many commercial feed mills and dairy farms. Recent research efforts have focused on coating WCS with value added feeds to improve flowability of the seed in commercial feed mills and add nutritive value to the seed. Coating WCS with gelatinized corn starch binds the lint producing a free flowing product that improves handling characteristics (Laird et al., 1997). Coating WCS with 5% corn starch was observed to reduce fiber digestibility and milk fat percentage in lactating dairy cows (Bernard et al., 1999). However, when the coating was reduced to 2.5%, no depression in milk fat percentage occurred (Bernard, 1999). When WCS coated with gelatinized corn starch were subjected to mixed ruminal microbial fermentation, total VFA concentration and molar proportion of propionate increased while molar proportion of acetate decreased (Bernard et al., 1999; 2001).

Starch stimulates amylolytic bacteria activity and reduces cellulolytic activity within the rumen. One possible explanation for the altered ruminal fermentation and fiber digestibility associated with feeding starch coated WCS is that reduced cellulolytic activity in the area surrounding the starch coated WCS may be responsible for the subsequent reduction in molar proportion of acetate and increase in total VFA concentration and molar proportion of propionate. Because cellulolytic microorganisms use ammonia as their primary nitrogen source, the inclusion of urea in the starch coating of WCS may aid in improving fiber digestion. Supplemental yeast culture has also been shown to increase cellulolytic bacterial numbers (Martin and Nisbet, 1992), which could potentially increase fiber digestion as well.

The addition of dietary starch has been reported to decrease ruminal pH (Krause et al., 2003) through an accumulation of VFA and lactate in the rumen (Mackey and Gilchrist, 1979). A decrease in ruminal pH is generally associated with decreased cellulolytic activity and fiber digestion. The addition of a dietary buffer such as sodium bicarbonate ($NaHCO_3$) increases or stabilizes rumen pH (Thomas et al., 1984; Solorzano et al., 1989) and increases fiber digestibility (West et al., 1987; Solorzano et al., 1989). The addition of $NaHCO_3$ to the gelatinized corn starch coating of WCS may offset any detrimental effects associated with increased starch concentrations on pH and fiber digestibility. The objectives of these trials were to determine the effects of the addition of urea, yeast or $NaHCO_3$ in the gelatinized corn starch coating applied to WCS on in vitro mixed ruminal microorganism fermentation.

MATERIALS AND METHODS

Experiment 1. Whole cottonseed that had been processed through a modified gin stand to remove tags and loose lint was obtained from a commercial gin for use in a replicated completely randomized design experiment. Treatments were arranged in a 2 x 2 x 2 factorial and included 2 concentrations of starch (0 [0S], 2.5 [2S] %), two concentrations of urea (0 [0U], 0.25 [2U] %) and two concentrations of yeast (0 [0Y], 2 [2Y] %). Treatments without starch were prepared by mixing approximately 100 g of WCS plus the desired amount of urea or yeast before grinding to pass through a 6-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). For the starch coated treatments, the appropriate amount of urea and/or yeast was dissolved into 300 g of water and then the appropriate amount of corn starch was added to the solution. The mixture was heated to 85° C and held at that temperature until the starch gelatinized. The heated solution was thoroughly mixed with 1000 g of cottonseed and dried in a forced-air oven at 120° C for 30 min. The coated cottonseed was periodically stirred during drying. After drying, the coated cottonseed was allowed to cool to room temperature and then stored in open plastic bags for 3 d for further equilibration to ambient temperature and moisture. The starch coated treatments were ground to pass through a 6-mm screen using a Wiley Mill before conducting the in vitro fermentations.

In vitro fermentations were performed on duplicate days with two replicated per day (n=4) according to the techniques of Tilley and Terry (1963). Ruminal fluid from a ruminally cannulated steer was collected once daily and strained through 3 layers of cheesecloth. Particle-free fluid was anaerobically transferred (20% vol/vol) to a medium (pH 6.7) containing 292 mg of K_2HPO_4, 240 mg of KH_2PO_4, 480 mg of $(NH_4)_2$ SO_4, 480 mg of NaCl, 100 mg of Mg $SO_4·7H_2O$, 64 mg of $CaCl_2·2H_2O$, 4000mg of Na_2CO_3, and 600 mg of cysteine hydrochloride per liter (Russell and Martin, 1984; Russell and Strobel, 1988). Particle-free fluid and medium were mixed, and 40 ml was transferred anaerobically to 100 ml serum tubes that contained 0 or 1.2 g of treated WCS. The tubes were sealed and placed in a 39° C water bath and periodically mixed. After 24 h of incubation, tubes were uncapped and pH measured immediately. Tubes were then emptied into centrifuge tubes and centrifuged (10,000 x g, 4° C, 15 min) and the cell free supernatant was removed and stored at 4° C. Volatile fatty acids in the supernatant were measured by HPLC (make and model). Supernatant was analyzed for ammonia levels (make and model).

Experiment 2. Treatments were prepared as in experiment 1. Treatments were arranged in a 3 x 4 factorial and included one concentration of gelatinized corn starch (2.5%), three concentrations of urea (0 [0U], 0.5 [5U] and 0.75 [7U] %), and four concentrations of sodium bicarbonate (0 [0BC], 0.06 [6BC], 0.08 [8BC], and 0.10 [10BC] %).

Treated WCS was ground to pass through a 6-mm screen using a Wiley Mill before in vitro mixed ruminal fermentation analysis was performed using the same methods as experiment 1. Chemical analysis of pH, NH_3, and total VFA were conducted as described for experiment 1. Analysis of DM (AOAC, 1990) and NDF (ANKOM^{200} Fiber Analyzer, Ankom Technology Corp., Fairport, NY) were conducted on the treatments and residues after fermentation to determine DM and NDF digestibility.

Statistical analysis. In vitro ruminal fermentation and nutrient digestibility data were subjected to analysis of variance using PROC GLM procedures of SAS (2001). The model included replication, day, treatment, and error. Least square means across all levels of WCS are reported.

RESULTS AND DISCUSSION

Experiment 1. Results of the in vitro fermentation of WCS are presented in Table 3.1. Coating WCS with gelatinized corn starch decreased pH ($P < 0.001$), increased NH_3 concentrations ($P = 0.02$), and had a tendency to decrease molar proportions of valerate ($P = 0.06$). Bernard et al. (1999) observed that the addition of 5 % gelatinized corn starch or 5 % gelatinized corn starch with 10% maltodextrin to the coatings of WCS decreased pH, and increased total VFA and propionate compared with fuzzy WCS. These researchers (Bernard et al., 2001) also reported a linear decrease in pH at 0, 2.5, and 5.0 % levels of starch addition to the coating of WCS. Total VFA and molar proportions of propionate increased linearly with increasing levels of starch in the coating and L- lactate levels increased quadratically as starch increased. The resulting decline in pH was attributed to the increased levels of total VFA, specifically propionate, and L- lactate in that study. While the results of our trial do not indicate any major shifts in VFA production associated with starch level in the coating, pH did decrease with added starch from 6.51 to 6.36. The increase in NH_3 concentrations is in contrast to previous observations when 5 % starch coated WCS was subjected to in vitro ruminal fermentation (Bernard et al., 1999; Bernard et al., 2001). Others (Cameron et al., 1991; Stern et al., 1978) observed a decrease in NH_3 concentrations with the addition of dietary starch.

Table 3.1. In vitro fermentation analysis of whole cottonseed (WCS) coated with combinations of gelatinized corn starch, urea, and yeast culture.

Starch, %	0				2.5											
Urea, %	0		0.25		0		0.25			Treatments			Contrasts			
Yeast, %	0	2	0	2	0	2	0	2	SEM	S	U	Y	S*U	S*Y	U*Y	S*U*Y
pH	6.53	6.54	6.53	6.42	6.35	6.35	6.35	6.38	0.03	<0.001	0.2	0.21	0.08	0.05	0.15	0.03
NH_3, mg/dl	6.28	5.95	4.98	5.60	6.83	7.83	8.40	4.93	0.70	0.02	0.15	0.28	0.87	0.17	0.09	0.01
Total VFA, mM	45.5	37.8	40.2	40.3	41.5	40.3	45.9	28.9	3.41	0.47	0.32	0.01	0.66	0.29	0.42	0.03
	--- molar proportion % --															
Acetate	61.0	64.1	60.3	59.0	60.8	59.3	59.1	60.2	0.02	0.38	0.25	0.82	0.38	0.70	0.75	0.22
Propionate	20.5	25.1	21.0	24.4	20.4	24.2	21.8	23.0	0.01	0.56	0.98	<0.001	0.89	0.25	0.13	0.58
Butyrate	14.0	15.2	14.4	13.1	13.9	13.0	14.2	12.9	0.01	0.10	0.28	0.16	0.24	0.17	0.06	0.16
Isovalerate	3.3	3.5	3.0	2.5	2.8	2.4	3.5	3.0	0.01	0.28	0.95	0.17	0.01	0.53	0.37	0.63
Valerate	1.2	1.6	1.3	1.2	1.1	1.1	1.4	1.2	0.01	0.06	0.89	0.54	0.01	0.04	0.004	0.23
A: P	2.98	2.58	2.85	2.40	3.00	2.45	2.70	2.62	0.08	0.91	0.06	<0.001	0.42	0.30	0.06	0.02

The addition of readily fermentable carbohydrates to the ruminant diet decreases available ammonia for microbial protein synthesis.

When increased amounts of readily fermentable carbohydrates are included in the diet, DM digestibility may decrease (Soofi et al., 1982) due to increased lactate and propionate production which causes a subsequent decrease in pH. The addition of starch was not adequate to reduce pH levels enough to decrease DM digestibility. Dry matter digestibility averaged 85.9 % at 0 % starch and 85.0 % at 2.5 % added starch.

The addition of urea to the starch coating had minimal effects on ruminal fermentation. There was a tendency ($P = 0.06$) for decreased acetate: propionate ratio with increased levels of urea. There were no other effects of the addition of urea to the coating of WCS.

Yeast culture supplementation decreased total VFA concentration ($P = 0.01$) and acetate: propionate ratios ($P < 0.001$) and increased molar concentrations of propionate ($P < 0.001$). Others (Erasmus et al., 1992; Miller-Webster et al., 2002) have reported similar responses to the addition of yeast culture to the diet of lactating cows. These authors noted that the decrease in acetate: propionate ratio was not due to any increase in molar proportions of acetate but due to increased proportions of propionate. Propionate is a glucogenic fatty acid that allows for increased glucose production in the animal, providing increased energy available for milk production (Church, 1988).

There was an interaction between starch and urea levels for molar proportions of valerate ($P = 0.01$) and isovalerate ($P = 0.01$) and a tendency ($P = 0.08$) for starch and urea interaction for pH. Valerate and isovalerate concentrations were highest at 0S0U but decreased with the addition of 2.5 % starch at 0% urea. The addition of 0.25 % urea to the coating yielded similar valerate concentrations regardless of starch level. Cameron et al. (1991) observed no difference in molar percentages of VFA with the addition of starch and urea to the diet of lactating Holstein cows. Bernard et al. (2003) observed that as starch level increased in the coating of WCS from 2.5 to 5.0 %, valerate levels increased, regardless of urea addition. An interaction between starch and urea in the coating for molar proportions of isovalerate was also reported due to higher levels for the 5 % starch 0.5 % urea treatment compared with the 5 % starch 0.25 % urea treatment. Isovalerate is the precursor to the branched-chain amino acid leucine and reduced levels of isovalerate production may reduce leucine availability in the animal.

The tendency for an interaction of starch and urea for pH was most likely attributed to the 0S2U diet. At all other levels of 0 % starch WCS, pH averaged 6.54 while the 0S2U diet decreased to an average of 6.47. The pH across the 2.5 % starch level was similar regardless of level of urea. Cameron et al. (1991) reported a decrease in pH in animals supplemented with starch and urea compared with supplemented with only starch or urea and attributed the decrease to decreased ruminal pH associated with urea. The decrease in pH associated with urea is in contrast to previous research (Broderick et al., 1993; Bernard et al., 2003). The addition of urea should provide a readily available source of nitrogen which would increase the pH and stimulate cellulolytic activity (Polan et al., 1976; Archimede et al., 1999).

There was an observed interaction for starch and yeast levels in the coating of WCS for pH (*P*= 0.05) and molar proportions of valerate (*P*= 0.04). The pH levels were relatively constant at 2.5 % starch level regardless of yeast supplementation. However, the addition of yeast to the 0% starch diet decreased pH. This effect was again contributed to the 0S2U2Y WCS which was considerably lower (6.42) than the other treatments at 0 % starch. The effect of the addition of yeast to rumen pH has been variable. Yeast supplementation decreases the mean concentration of lactic acid and lowers the peak lactic acid concentration in the rumen (Williams et al., 1991;

Erasmus et al., 1992), resulting in a decrease in pH. However, the results have been variable with some reporting a decrease (Williams et al.,1991) while others report an increase (Dawson et al., 1990) or no change (Yoon and Stern, 1996) in rumen pH with yeast culture supplementation.

There was an interaction for urea and yeast for molar proportions of valerate ($P= 0.004$) and a tendency for an interaction for NH_3 ($P= 0.09$), butyrate ($P= 0.06$), and acetate: propionate ratio ($P= 0.06$). The difference in molar proportions of valerate is most likely due to the decreased valerate levels associated with the 0U0Y diet compared with the 0U2Y WCS treatment at the 0 % starch level.

There was a noted interaction among starch, urea, and yeast levels for pH ($P= 0.03$), NH_3 ($P= 0.01$), total VFA ($P= 0.03$), and acetate: propionate ($P= 0.02$). In vitro pH was relatively similar within starch treatments except for the 0S2U2Y WCS. For this treatment, pH decreased to levels similar to the 2.5 % starch WCS. Concentrations of NH_3 and total VFA were lowest for the 2S2U2Y treatment compared with all other treatments and may have accounted for the interaction effect of these measurements.

Experiment 2. The results of the in vitro fermentation of WCS coated with combinations of urea and $NaHCO_3$ are presented in Table 3.2. Coating WCS with 2.5 % gelatinized corn starch and urea increased concentrations of NH_3 ($P< 0.001$) in vitro. This is in agreement with previous research (Bernard et al., 2001, 2003) in which WCS was coated with combinations of starch and urea. The inclusion of urea in the gelatinized starch coating of WCS also reduced molar proportions of propionate ($P= 0.03$) and increased molar proportions of butyrate ($P= 0.007$) and isovalerate ($P= 0.06$). Bernard et al. (2001) observed an increase in molar proportions of butyrate with increasing levels of urea in coated WCS but reported no other differences in molar proportions of individual VFA. Cameron et al. (1991) noted that addition of

Table 3.2. In vitro fermentation analysis of whole cottonseed (WCS) coated with 2.5% gelatinized corn starch and different concentrations of urea or sodium bicarbonate.

Urea, %	0				0.5				0.75							
Bicarb, %	0	0.06	0.08	0.1	0	0.06	0.08	0.1	0	0.06	0.08	0.1	SEM	Urea	Bicarb	Urea* Bicarb
pH	7.39	7.29	7.38	7.32	7.41	7.36	7.38	7.34	7.36	7.36	7.41	7.35	0.03	0.41	0.04	0.71
NH_3, mol/ 100ml	25.6	16.1	19.1	22.4	26.8	19.0	20.4	20.6	30.6	31.6	33.6	30.7	2.0	<0.001	0.02	0.10
Total VFA, mM	37.1	34.2	37.6	31.4	36.6	35.9	34.1	34.7	36.6	32.3	37.3	37.0	1.5	0.79	0.08	0.03
	-- % molar proportion --															
Acetate	63.2	64.7	64.3	65.7	64.4	63.6	62.0	62.4	60.8	62.9	64.0	62.4	0.01	0.14	0.86	0.40
Propionate	17.7	18.2	18.3	16.7	16.4	17.0	16.6	17.8	17.1	16.9	16.4	18.1	0.004	0.03	0.27	<0.001
Butyrate	14.4	12.4	12.0	12.1	13.1	14.6	16.3	12.8	15.5	15.3	12.8	13.5	0.006	0.007	0.03	0.001
Isobutyrate	2.1	1.3	1.7	1.4	1.8	1.4	1.9	2.0	2.0	1.1	2.2	2.0	0.002	0.32	0.005	0.23
Isovalerate	2.2	1.9	1.9	2.1	2.1	2.0	2.6	2.2	2.7	2.0	2.7	2.2	0.02	0.06	0.06	0.33
Valerate	2.0	1.5	1.8	1.8	2.2	1.8	1.9	1.6	1.9	1.9	2.0	1.7	0.0008	0.34	0.01	0.014
A:P	3.57	3.55	3.51	3.93	3.92	3.74	3.73	3.50	3.55	3.72	3.90	3.45	0.12	0.64	0.33	0.006

supplemental urea to the diet of lactating cows had no effect on total VFA concentrations or molar proportions of individual VFA.

Rapidly fermentable carbohydrates decrease cellulose digestion in lactating dairy cattle by reducing pH and promoting amylolytic bacterial activity over cellulolytic activity. The addition of urea in the starch coating of WCS would provide a readily available source of NH_3 which could stimulate cellulolytic activity (Archimede et al., 1999). Increased acetate and butyrate production and decreased propionate proportions are generally associated with increase cellulolytic activity. The addition of urea to the coating of WCS may improve cellulolytic activity within the rumen thereby improving fiber digestibility and possibly milk fat production.

The addition of $NaHCO_3$ to the starch coating of WCS decreased pH at the 0.06 and 0.10 % (average 7.33 for each concentration) levels but was similar for control and 0.08 % (both averaged 7.38) $NaHCO_3$ treatments. The decrease in pH at 0.06 and 0.10% $NaHCO_3$ were unexpected since generally the addition of $NaHCO_3$ should increase pH. Addition of $NaHCO_3$ to the starch coating decreased NH_3 concentrations ($P = 0.02$), molar proportions of butyrate ($P = 0.03$), isobutyrate ($P = 0.005$), and valerate ($P = 0.01$), and tended to increase molar proportions of isovalerate ($P = 0.06$) and total VFA concentrations.

As the level of urea in the coating increased, total VFA concentrations increased with increasing concentrations of $NaHCO_3$ ($P = 0.03$). There was also an observed interaction between urea and $NaHCO_3$ level for molar proportions of propionate ($P < 0.001$), butyrate ($P = 0.001$), valerate ($P = 0.01$), and acetate: propionate ($P = 0.006$). As the level of $NaHCO_3$ increased, molar proportions of propionate increased at the 0.5 and 0.75% level of urea but decreased with increasing $NaHCO_3$ levels at the 0% urea level. Molar proportions of butyrate tended to decrease with increasing levels of $NaHCO_3$ at the 0 and 0.75% urea level.

Table 3.3. In vitro nutrient digestibility analysis of whole cottonseed (WCS) coated with 2.5% gelatinized corn starch and different concentrations of urea or sodium bicarbonate.

Urea, %	0				0.5				0.75							
Bicarb, %	0	0.06	0.08	0.01	0	0.06	0.08	0.1	0	0.06	0.08	0.1	SEM	Urea	Bicarb	Urea* Bicarb
IVDMD	83.0	81.1	82.1	84.8	86.9	82.1	81.4	81.3	79.0	82.7	89.3	80.6	1.02	0.95	0.05	<0.001
IVNDFD	53.8	53.1	52.5	55.6	48.2	55.8	50.4	53.3	58.4	62.2	49.4	53.3	1.04	<0.001	<0.001	<0.001

Dry matter and NDF in vitro digestibility data are presented in Table 3.3. The addition of urea increased in vitro NDF digestibility ($P< 0.001$). The addition of urea provides a NH_3-N source for cellulolytic bacteria that increases their activity and provides for greater NDF digestibility. In vitro data supports the increased NDF digestibility as indicated by increased NH_3-N concentrations and increased molar proportions of butyrate. The addition of $NaHCO_3$ to the starch coating also increased NDF digestibility ($P< 0.001$). Sodium bicarbonate can increase pH of rumen fluid thereby favoring cellulolytic bacterial activity compared with amylolytic activity which may improve NDF digestibility.

There was an interaction between urea and $NaHCO_3$ for DM ($P< 0.001$) and NDF ($P< 0.001$) digestibility. Dry matter digestibility was highest for the 7U8BC WCS while NDF digestibility was greatest for the 7U6BC WCS.

CONCLUSIONS

Coating WCS with combinations of starch, urea, yeast and sodium bicarbonate slightly alters in vitro fermentation. In experiment 1, increasing levels of starch decreased pH and increased NH_3 concentrations while addition of urea had little effect on in vitro fermentation. Yeast culture supplementation decreased total VFA concentration and acetate: propionate ratios and increased molar concentrations of propionate. There was an interaction between starch and urea levels for molar proportions of valerate and isovalerate and a tendency for starch and urea interaction for pH. There was an interaction of starch level and yeast culture supplementation for pH and molar proportions of valerate and an interaction of urea and yeast for molar proportions of valerate. A three way interaction for starch, urea, and yeast was observed for pH, NH_3 concentrations, total VFA, and acetate: propionate ratio.

In experiment 2, the addition of urea to starch coated WCS increased concentrations of NH_3, molar proportions of butyrate and isovalerate, and reduced molar proportions of propionate. Addition of $NaHCO_3$ to the starch coating decreased NH_3 concentrations, molar proportions of butyrate, isobutyrate and valerate and tended to increase molar proportions of isovalerate and total VFA concentrations. The addition of urea and $NaHCO_3$ to the starch coating of WCS increased in vitro NDF digestibility. Results from these experiments indicate that including urea, yeast, and $NaHCO_3$ to the starch coating of WCS may alter in vitro ruminal fermentation and provide for increased cellulolytic bacterial activity which would increase NDF digestibility of the starch coated WCS.

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CHAPTER 4

EFFECT OF INCLUSION OF UREA OR YEAST CULTURE IN THE STARCH COATING OF WHOLE COTTONSEED ON NUTRIENT DIGESTIBILITY AND PERFORMANCE OF LACTATING DAIRY COWS[2]

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[2] Cooke, K.M., J.K. Bernard, and J.W. West. Accepted by Journal of Dairy Science. Reprinted here with permission of the publisher.

ABSTRACT

Thirty lactating Holstein cows were used in an 8-wk completely randomized design trial to test the viability of select additives included in the gelatinized cornstarch coating applied to whole cottonseed (WCS) on nutrient intake and digestibility and milk yield and composition. Treatments included WCS coated with 2.5% gelatinized cornstarch (control); control plus 0.5% urea; or control plus 2.0% yeast culture. The treated WCS represented 12.6% of the dietary dry matter. Cellulose intake was lower for the control coating compared with either the urea or yeast coating because of slightly lower cellulose concentrations in the control treatment. Intake of all other nutrients was similar for all treatments. Whole-tract nutrient apparent digestibility was not altered by treatment. Dry matter intake and milk yield were similar among treatments. Percentage solids-not-fat was lower for the yeast treatment compared with control, but no other differences were observed in milk composition among treatments. Efficiency of milk production (energy-corrected milk yield per unit of dry matter intake) was higher for the urea and yeast treatments compared with control because of slightly higher yield of milk fat and energy-corrected milk. No differences were observed in body weight change during the trial between treatments. Results of this trial indicate that including urea or yeast culture in the gelatinized starch coating does not change whole tract digestibility, but does improve milk production efficiency.

Key words: cottonseed, starch, urea, yeast

INTRODUCTION

Whole cottonseed (WCS) is commonly used in rations fed to lactating dairy cows as a source of energy, fiber, and protein. The high oil content of WCS makes it an attractive, energy-dense feed for animals with high energy requirements, such as lactating dairy cattle. The high fiber concentrations provided by the lint and hulls are desirable for maintaining effective fiber levels in the diet. However, the lint makes WCS difficult to handle in mechanized feeding systems and limits its use in many commercial feed mills and dairy farms.

Coating WCS with gelatinized cornstarch binds the lint and results in a free-flowing product with improved handling characteristics (Laird et al., 1997). Coating WCS with 5% cornstarch was observed to reduce fiber digestibility and milk fat percentage in lactating dairy cows (Bernard et al., 1999). However, when the coating was reduced to 2.5% cornstarch, no depression in milk fat percentage occurred (Bernard, 1999). When WCS coated with gelatinized cornstarch were subjected to mixed ruminal microbial fermentation, total VFA concentration and molar proportion of propionate increased whereas molar proportion of acetate decreased (Bernard et al., 1999, 2001).

One possible explanation for the slightly altered ruminal fermentation in cows fed starch-coated WCS is that the gelatinized starch coating stimulates amylolytic bacteria activity over the cellulolytic microorganisms surrounding the WCS. Reduction of cellulolytic activity could account for the decreased fiber digestion associated with feeding starch-coated WCS. Because cellulolytic microorganisms use ammonia as their primary nitrogen source, the inclusion of urea in the coating may aid in improving fiber digestion. Previous research by Bernard et al. (2003) noted that the addition of 0.5% urea in the gelatinized starch coating of WCS tended to improve ruminal NDF digestibility compared with uncoated WCS. Supplemental yeast culture has been shown to increase cellulolytic bacterial numbers (Martin and Nisbet, 1992), which could also increase fiber digestion. The objectives of this trial were to determine the effects of the addition of urea or yeast in the gelatinized cornstarch coating applied to WCS on nutrient intake and digestibility and on milk yield and composition in lactating dairy cows.

MATERIALS AND METHODS

One lot of WCS was processed through a modified gin stand to remove tags and loose lint before application of the experimental coatings (Ellis Brothers Gin, Inc., Center, AL). Treatments included WCS coated with 2.5% gelatinized cornstarch (WCS-C); control plus 0.5% feed-grade urea (284% CP, DM basis) included in the coating (WCS-U); or control plus 2.0% yeast culture (Diamond V Mills XP Yeast Culture, Cedar Rapids, IA; WCS-Y). The 0.5% urea coating was based on previous research (Bernard et al., 2003) and the yeast culture rate was based on the manufacturer’s recommendation to provide 56 g/d based on the intended feeding rate. The components of the coating represented approximately 0.3% starch, 0.06% urea, and 0.25% yeast culture of the total dietary DM in the experimental diets. For each treatment, the starch was heated to 85°C using forced air and held at that temperature until it gelatinized. The urea or yeast culture was weighed and mixed into the heated solution before pouring over a known quantity of WCS. The treated WCS was thoroughly mixed and then dried at 120°C for 30 min. After drying, WCS were allowed to cool to room temperature and then stored in tote bags for further equilibration to ambient temperature and moisture. Treated WCS were transported to the Tifton Dairy Research Center (Tifton, GA) for use in a lactation trial.

Thirty lactating Holstein cows were used in an 8-wk completely randomized design trial during the spring of 2004. All protocols were reviewed and approved by the University of

Georgia Institute of Animal Care and Use Committee. The trial consisted of a 2-wk standardization period followed by a 6-wk experimental period. Cows were housed in a free-stall barn and averaged 182 ± 40 DIM, 38.0 ± 7.7 kg/d of milk yield, and 3.2 ± 1.7 lactations at the beginning of the trial. All cows were trained to eat behind Calan doors (American Calan Inc., Northwood, NH) before beginning the trial. During the standardization period, all cows were fed the control diet containing WCS. Cows were individually fed once daily (0800 h) in amounts to provide approximately 10% orts for ad libitum consumption. At the end of the standardization period, cows were assigned randomly to 1 of the 3 experimental diets (WCS-C, WCS-U, or WCS-Y). Experimental diets contained 12.6% WCS, 38.4% corn silage, 5.9% alfalfa hay, and 43.1% concentrate (steam-flaked corn, soybean meal, plus mineral-vitamin premix; DM basis, Table 4.1) and fed as a TMR. Amounts of TMR offered and refused were recorded daily. Cows were milked twice daily at approximately 0400 and 1500 h and milk yield was recorded electronically (Alpro, DeLaval, Kansas City, MO) at each milking and summed each day.

Sample Collection and Analysis

Milk samples were collected from 2 consecutive p.m. and a.m. milkings each week. Samples were shipped to Dairy Farmers of America (Knoxville, TN) for analyses of fat, protein, lactose, SNF, and MUN concentrations.

Samples of treated WCS, experimental diets, and orts were collected 3 times each week and dried in a forced air oven at 55°C for 48 h. Orts were collected from all cows within each treatment and mixed before sampling. Samples were composited by week and ground to pass through a 1-mm screen using a Wiley mill (Arthur Thomas, Philadelphia, PA). Samples were analyzed for DM, CP, ash, ether extract, (AOAC, 1990), ADF, NDF, and acid detergent lignin (Ankom200 Fiber Analyzer, Ankom Technology Corp., Fairport, NY). During wk 5 of the

Table 4.1. Ingredient composition of experimental diets containing whole cottonseed coated with starch or starch plus urea or yeast culture.

Ingredient	% of DM
Alfalfa hay	5.93
Corn silage	38.41
Cottonseed	12.60
Brewers grains, wet	11.08
Steam-flaked corn	20.31
Soybean meal	7.70
Mineral and vitamin premix[1]	3.97

[1] Mineral and vitamin premix provided (DM basis): 25.8% CP, 55.2% ash, 8.59% Ca, 1.65% P, 3.58% Mg, 6.99% K, 6.51% Na, 4.04% Cl, 0.22% S, 5.88 ppm Co, 339 ppm Cu, 1,800 ppm Fe, 30 ppm I, 1,038 ppm Mn, 7.5 ppm Se, 990 ppm Zn, 67,640 IU of Vitamin A, 27,050 IU of Vitamin D, and 425 IU of Vitamin D.

experimental period, fecal grab samples were collected on 4 consecutive days at 12-h intervals with a 2-h advancing schedule each day. Collection times were at 0500, 1700 h; 0700, 1900 h; 0900, 2100 h; and 1000, 2300 h, respectively, on the 4 d. Samples were composited by cow and dried in a forced-air oven at 60°C for 72 h. All samples were ground to pass through a 1-mm screen using a Wiley mill and analyzed for DM, CP, ash, ether extract, ADF, NDF, and acid detergent lignin as described previously. Fecal and corresponding diet and ort samples were also analyzed for indigestible ADF as an internal marker using the techniques described by Cochran et al. (1986) using an Ankom DaisyII Incubator (Ankom Technology, Macedon, NY).

Body weights were recorded on 2 consecutive days during the standardization period and wk 6 of the experimental period and once during wk 2 and 4. To minimize variation, BW was recorded immediately after the p.m. milking and before animals had access to feed or water.

Statistical Analysis

Nutrient intake and digestibility measured during wk 5 and BW and BW change data were subjected to ANOVA using PROC GLM procedures of SAS (SAS Institute, 2001). The model included cow, treatment, and error. Weekly DMI, milk yield, and composition data were subjected to analysis of covariance using the PROC MIXED procedures of SAS (SAS Institute, 2001). The model included covariate, treatment, week, treatment by week, and error. For each variable the corresponding value from the preliminary period and initial DIM were used as covariates. Cow within treatment was included as a random effect and week as a repeated effect. Contrast of control vs. urea and control vs. yeast were included in the analysis of all data.

RESULTS AND DISCUSSION

Chemical Composition of WCS and TMR.

The chemical composition of the WCS treatments is presented in Table 4.2. The composition of the coated WCS was similar except that the CP content of WCSU was slightly higher (19.3%) compared with WCSC and WCS-Y (17.5 and 17.7%, respectively). This is consistent with planned differences associated with addition of urea to the coating. Experimental diets contained similar concentrations of nutrients (Table 4.3). The supplemental urea was calculated to increase dietary CP concentrations by approximately 0.2% percentage units. Based on the measured values, it would appear that the control diet contained slightly more CP than planned. Average concentration of DM, CP, and NDF for the experimental diets was 48.4, 17.9, and 38.6% of DM, respectively.

Nutrient Intake and Digestibility

Nutrient intake, corrected for orts, and apparent digestibility data measured during wk 5 are presented in Table 4.4. No differences were observed among treatments for DMI, which averaged 25.9 kg/d. Crude protein intake was lower ($P = 0.04$) for WCS-Y (4.5 kg/d) compared with WCS-C (5.0 kg/d). The lower CP intake is related to numerically lower DMI and slightly lower CP concentration in WCS-Y (25.2 kg/d and 17.8%) compared with WCS-U (26.5 kg/d and 18.0%). Cellulose intake (1.84, 2.50, and 2.47 kg/d, respectively) was lower for WCS-C compared with WCS-U ($P = 0.01$) and WCS-Y ($P = 0.01$) primarily because of slightly lower dietary concentrations in WCS-C compared with WCSU and WCS-Y (7.8, 9.3, and 8.7%, respectively).

Nutrient digestibility was similar among treatments (Table 4.4). These results are in agreement with Bernard et al. (2003) who noted no difference in total-tract nutrient digestibility

Table 4.2. Chemical composition (mean ± SD) of whole cottonseed (WCS) coated with 2.5% gelatinized corn starch (WCS-C), 2.5% gelatinized corn starch plus 0.5% urea (WCS-U) or 2.5 gelatinized corn starch plus 2.0% yeast culture (WCS-Y).

	WCS-C (control)	WCS-U	WCS-Y
	---------------------------------- %	----------------------------------	
DM	94.1 ± 1.3	94.3 ± 1.3	94.1 ± 1.4
	----------------------------- % of DM	-----------------------------	
CP	17.5 ± 0.7	19.3 ± 0.6	17.7 ± 0.9
NDF	50.3 ± 1.8	48.7 ± 2.1	49.4 ± 1.3
Hemicellulose	14.6 ± 1.4	14.1 ± 1.7	14.2 ± 2.6
ADF	35.7 ± 1.2	34.6 ± 0.9	35.2 ± 2.2
Cellulose	22.5 ± 2.5	20.8 ± 2.4	20.1 ± 3.5
Acid detergent lignin	13.2 ± 2.3	13.8 ± 2.5	15.2 ± 2.9
Ether extract	18.4 ± 0.7	18.4 ± 1.1	18.1 ± 1.4
Ash	3.6 ± 0.2	3.3 ± 1.0	3.7 ± 0.2

Table 4.3. Chemical composition (mean ± SD) of experimental diets containing whole cottonseed (WCS) coated with starch (WCS-C), starch plus urea (WCS-U) or starch plus yeast culture (WCS-Y).

	WCS-C (control)	WCS-U	WCS-Y
	---------- % ----------		
DM	48.7 ± 1.6	48.3 ± 1.3	48.0 ± 1.7
	---------- % of DM ----------		
CP	18.0 ± 0.8	18.0 ± 0.8	17.8 ± 0.9
NDF	38.9 ± 1.4	39.1 ± 2.5	38.3 ± 1.6
Hemicellulose	21.4 ± 0.9	21.1 ± 1.3	20.5 ± 1.3
ADF	17.5 ± 1.2	18.0 ± 1.7	17.8 ± 1.0
Cellulose	7.8 ± 3.1	9.3 ± 2.8	8.7 ± 2.8
ADL	10.2 ± 2.8	8.3 ± 1.9	9.2 ± 2.6
EE[2]	4.5 ± 0.1	4.7 ± 0.6	4.7 ± 0.4
Ash	5.9 ± 0.3	5.9 ± 0.1	5.9 ± 0.4

[1] Values represent mean ± standard deviation.

[2] Ether extract

Table 4.4. Nutrient intake and apparent digestibility of lactating dairy cows fed diets containing whole cottonseed coated with gelatinized corn starch (WCS-C), starch plus urea (WCS-U) or starch plus yeast culture (WCS-Y).[1]

					Contrasts[2]	
Item	WCS-C (control)	WCS-U	WCS-Y	SEM	1	2
Intake, kg/d					*P* <	
DM	26.49	25.62	25.16	0.88	0.49	0.30
CP	5.04	4.73	4.54	0.16	0.19	0.04
NDF	10.05	10.24	10.01	0.35	0.71	0.95
Hemicellulose	5.50	5.64	5.20	0.19	0.63	0.26
ADF	4.54	4.60	4.82	0.16	0.81	0.24
Cellulose	1.84	2.50	2.47	0.08	0.01	0.01
Ether extract	1.25	1.22	1.20	0.04	0.67	0.43
Apparent digestibility, %						
DM	62.36	59.59	60.86	1.25	0.13	0.41
CP	67.51	63.12	63.44	1.81	0.10	0.13
NDF	40.50	38.61	40.71	1.35	0.33	0.91
Hemicellulose	53.26	50.97	50.82	1.94	0.42	0.38
ADF	26.57	24.55	29.82	1.27	0.29	0.08
Cellulose	25.39	32.33	33.19	2.99	0.19	0.14
Ether extract	62.00	72.54	65.93	4.76	0.13	0.57

[1] Data were collected during wk 5 of the trial.

[2] 1 = control versus urea; 2 = control versus yeast culture.

in animals fed WCS coated with gelatinized starch or gelatinized starch plus urea. Ruminal fermentation characteristics in the same study noted a reduction in molar proportions of isobutyrate in diets containing urea-coated WCS, suggesting reduced fermentation of lysine.

Cellulose digestion has been shown to decrease in the presence of a rapidly fermentable carbohydrate (Stern et al., 1978) due to competition between amylolytic and cellulolytic bacteria (Martin and Nisbet, 1992). Urea provides an NH3-N source for cellulolytic microorganisms that promotes their activity to counteract the effect of starch in the coating. Although the diets fed would not be expected to be limiting in N, the addition of urea has consistently improved the ruminal environment based on in vitro results (Bernard et al., 2001). The CP content of WCS-U was in agreement with our estimated concentration; concentrations in the coating may have been lower than that required to stimulate or optimize cellulolytic bacterial populations based on whole-tract digestibility of fiber fractions. Digestibility of ADF ($P = 0.08$) tended to be higher when cows were fed diets containing WCS-Y compared with WCS-C (Table 4). This is in agreement with previous studies (Weidmeier et al., 1987; Erasmus et al., 1992) that noted increased ADF and cellulose digestion with supplemental yeast culture. Others (Williams et al., 1991; Wohlt et al., 1991) have reported little or no effect of yeast culture on diet digestibility. No other differences were observed in the apparent digestibility of other nutrients with WCS-Y.

Production Response

Dry matter intake, milk yield, and milk composition were similar among treatments (Table 4.5). There was a tendency for slightly higher yields of milk fat ($P = 0.06$) and ECM ($P = 0.06$) increasing the efficiency of milk production ($P = 0.04$) for cows fed WCS-U compared with WCS-C. Previous research has not demonstrated a positive response in milk fat yield when supplemental urea was included in the diet (Casper et al., 1990; Cameron et al., 1991). In vitro mixed ruminal microorganism fermentation of WCS coated with combinations of starch and urea increased butyric acid concentrations (Bernard et al., 2001), which may partially account for the slight increase in milk fat yield with WCS-U.

Concentrations of milk protein (P = 0.06) and SNF (P = 0.03) were lower for cows fed WCS-Y compared with WCS-C. This decrease is due to numerically higher milk yield with WCS-Y because yield of milk protein and SNF was not different among treatments. Wohlt et al. (1991) reported lower milk protein percentage when supplemental yeast culture was included in diets fed to lactating dairy cows. Efficiency of milk production was higher (P = 0.02) for cows fed WCS-Y compared with WCS-C. The increase was because of a tendency for slightly higher (P = 0.10) yield of milk fat and ECM yield with similar DMI. Increased FCM was observed for cows supplemented with 0, 10, or 20 g/d of yeast culture resulting in FCM yield of 37.7, 40.7, and 41.4 kg/d, respectively (Wohlt et al., 1998). The results from the current trial are consistent with previous studies (Williams et al., 1991; Wohlt et al., 1991). No other differences were observed for milk yield or milk components in animals fed WCS-Y.

CONCLUSIONS

Coating WCS with gelatinized starch or gelatinized starch plus urea or yeast did not alter nutrient intake or whole tract nutrient digestibility. Milk production efficiency was improved for both WCS-U and WCS-Y because of a tendency for improved yield of fat and ECM without any difference in DMI. These results suggest that the inclusion of urea or yeast culture in the gelatinized cornstarch coating applied to WCS could improve nutrient utilization in support of milk production.

Table 4.5. Dry matter intake, milk yield and composition of cows fed diets containing cottonseed coated with starch (WCS-C), starch plus urea (WCS-U) or starch plus yeast culture (WCS-Y).

Item	WCS-C (control)	WCS-U	WCS-Y	SEM	Contrasts[1] 1	Contrasts[1] 2
					$P <$	
DMI, kg/d	26.2	26.5	25.6	0.56	0.62	0.48
Milk, kg/d	37.4	38.4	38.2	1.05	0.50	0.60
Fat, %	3.87	4.06	4.11	0.11	0.32	0.20
Fat, kg/d	1.45	1.56	1.57	0.13	0.06	0.10
Protein, %	3.19	3.20	3.11	0.03	0.87	0.06
Protein, kg/d	1.19	1.23	1.19	0.10	0.44	0.95
Lactose, %	4.89	4.83	4.84	0.04	0.30	0.40
Lactose, kg/d	1.75	1.80	1.78	0.14	0.48	0.60
SNF, %	8.99	8.92	8.84	0.04	0.31	0.03
SNF, kg/d	3.20	3.31	3.24	0.29	0.44	0.69
ECM, kg/d	39.2	41.2	41.0	1.02	0.06	0.10
MUN, mg/dl	9.22	8.74	8.19	0.72	0.63	0.35
Efficiency[2]	1.50	1.56	1.60	0.04	0.04	0.02
SCC	268.6	365.2	311.8	124.0	0.59	0.81
Initial BW, kg	646.2	637.6	645.7	17.7	0.74	0.99
BW gain, kg	26.0	21.8	27.0	7.01	0.68	0.91

[1] Contrasts- 1= control versus urea; 2= control versus yeast

[2] Defined as ECM per unit of DMI

Data represents least square means for entire experimental period.

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CHAPTER 5

PERFORMANCE AND RUMINAL FERMENTATION OF DAIRY COWS FED WHOLE COTTONSEED WITH ELEVATED CONCENTRATIONS OF FREE FATTY ACIDS IN THE OIL.[3]

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ABSTRACT

Twenty-four lactating Holstein cows were used in an 8 wk completely randomized design trial to examine the effects of feeding whole cottonseed (**WCS**) with elevated concentrations of free fatty acids in the oil (**FFA**) on intake and performance. Treatments included WCS with normal concentrations of FFA (6.8%, **CONTROL**) and two lots of WCS with elevated FFA; **HFFA1** (24.1%) or **HFFA2** (22.3%). Compared with CONTROL and HFFA1, the HFFA2 contained slightly more moisture and less oil and were visibly discolored. Concentrations of ADF, NDF, or minerals were similar among WCS treatments. The WCS comprised 14% of the total DM of the ration. Dry matter intake tended to be slightly higher for cows fed HFFA2 compared with CONTROL and HFFA1. Yield of milk and components was similar among treatments, but milk fat percentage was lower for HFFA1 and HFFA2 compared with CONTROL. Percentages of milk protein, lactose, and SNF were similar among treatments. In a concurrent 3 x 3 Latin square trial with six ruminally cannulated Holstein cows, molar proportions of butyrate and isobutyrate were higher for HFFA1 and HFFA2 compared with CONTROL, but no differences were observed in concentrations of acetate or propionate. Results of these trials indicate that feeding WCS with high concentrations of FFA does not alter yield of milk or components, but milk fat percentage is decreased. Although minor changes in ruminal fermentation were observed, they were not sufficient to account for the decrease in milk fat percentage. The decreased milk fat percentage was probably related to reduced total fatty acid supply for incorporation into milk fat during de novo synthesis by the mammary gland for high FFA WCS.

(**Keywords**: cottonseed, free fatty acids)

INTRODUCTION

Whole cottonseed (**WCS**) is commonly used in rations for lactating dairy cows as a source of energy, fiber, and protein. The high oil content of WCS makes it an attractive energy dense feed for animals with high energy requirements, such as lactating dairy cattle. The high fiber concentrations provided by the lint and the hulls are desirable for maintaining effective fiber levels in the diet.

The warm, humid conditions that occur when tropical storms delay harvest of cotton frequently result in higher concentrations of free fatty acids in the oil (**FFA**) of WCS. As defined by National Cottonseed Products Association (1997), WCS with greater than 12% FFA are considered to be off-quality and are typically sold as livestock feed. Sullivan et al., (2004) noted that feeding WCS with up to 12% FFA in the oil at 12.5% of the DM to lactating cows did not alter fiber digestibility, milk yield or composition. When diets containing WCS with up to 18% FFA in the oil were fed to Holstein steers, increases in the molar proportion of acetate and acetate to propionate ratio were observed, but no negative impact on nutrient intake was observed (Sullivan et al., 2005). The potential effects of feeding diets containing WCS with even higher concentrations of FFA have not been examined. Feeding supplemental fats with elevated concentrations of FFA to cows was reported to reduce DMI and fiber digestibility (Eastridge and Firkins, 1991). Increased levels of unsaturated FFA in the rumen may have a toxic effect on the rumen micro flora resulting in changes in normal rumen fermentation patterns (Jenkins, 1993). This author also suggested that including high levels of polyunsaturated fatty acids in the diet could also physically coat feed particles preventing cellulolytic bacterial attachment which would also depress fiber digestibility. The oil in WCS is 70% unsaturated (Keele et al., 1989) and inclusion of high levels of WCS in the diet of lactating dairy cows may negatively affect fiber digestion (DePeters and Cant, 1992). The objective of this study was to determine the effect of feeding WCS with elevated concentrations of FFA on nutrient intake, milk yield and composition, and ruminal fermentation of lactating dairy cows.

MATERIALS AND METHODS

Three lots of WCS differing in FFA concentrations were obtained from a warehouse in South Georgia and transported to the University of Georgia Dairy Research Center in Tifton, GA. One lot represented normal WCS with an average FFA content of 6.8% in the oil (**CONTROL**). The two additional lots of WCS had elevated concentrations of FFA in the oil; 24.1% (**HFFA1**) or 22.3% (**HFFA2**). One lot was normal in appearance (HFFA1) but the other lot was discolored suggesting that some heating may have occurred during storage (HFFA2). However, subsequent analysis for unavailable protein did not indicate any differences.

Production Trial

Twenty-four lactating Holstein cows were used in an 8 week completely randomized block trial at the Dairy Research Center in Tifton, GA. All protocols were reviewed and approved by the University of Georgia Institute of Animal Care and Use Committee. The trial consisted of a 2-wk standardization period followed by a 6-wk experimental period. Cows were housed in a freestall barn and averaged 159 ± 97 DIM and 33.1 ± 4.8 kg/d of milk at the beginning of the trial. Cows were trained to eat behind Calan doors (American Calan Inc., Northwood, NH) before the beginning of the trial. All cows received the control diet (Table 5.1)

Table 5.1. Ingredient composition of experimental diets containing whole cottonseed (WCS) with increasing concentrations of free fatty acids in the oil (FFA).

Ingredient	% of DM
Corn silage	39.87
Alfalfa hay	5.48
Wet brewers grains	12.38
Steam-flaked corn	12.96
Whole cottonseed	13.96
Concentrate[1]	15.35

[1] Concentrate contained (DM basis) 52.8% soybean meal, 48% CP; 13.2% calcium salts of long chain fatty acids; 13.2% Prolak (H. J. Baker & Bro., Inc., Stamford, CT); 4.8% limestone; 2.6% dicalcium phosphate; 1.1% magnesium oxide; 1.8% salt; 4.0% sodium bicarbonate; 0.8% potassium-magnesium-sulfate; 1.6 % yeast culture; and 1.6% trace mineral-vitamin premix.

during the 2-wk standardization period. Cows were individually fed a TMR once daily (0800) in amounts to provide approximately 10% orts for ad libitum consumption. At the end of the standardization period, cows were assigned randomly to one of the three treatments. Treatments included TMR containing CONTROL, HFFA1, or HFFA2 at approximately 14% of the ration DM (Table 5.1). Amount of feed offered and refused were recorded daily. Cows were milked twice daily at approximately 0400 and 1500 h. Individual milk yield was recorded electronically (Alpro, DeLaval, Kansas City, MO) at each milking and summed for each day.

Sample Collection and Analysis

Milk samples were collected from two consecutive p.m. and a.m. milkings each week. Samples were shipped to Dairy Farmers of America (Knoxville, TN) for analyses of fat, protein, lactose, solids-not-fat, and MUN concentrations (AOAC, 1990).

Samples of WCS, experimental diets, and orts were collected three times each week and dried in a forced air oven at 55 °C for 48 h. Samples were composited each week of the trial and ground to pass through a 1-mm screen using a Wiley mill (Arthur Thomas, Philadelphia, PA). Samples were shipped to Cumberland Valley Analytical Laboratories (Hagerstown, MD) for chemical analysis of DM (forages according to Goering and Van Soest (1970) and grains, mixed feeds, concentrates, and byproducts according to AOAC (1990)), CP (Leco FP-528 Nitrogen Analyzer, St. Joseph, MI), ADF (AOAC, 1990), NDF and lignin (Goering and Van Soest, 1970), ash, and minerals (AOAC, 1990). An additional set of composite samples of WCS were shipped to Mid-Continent Laboratories, Inc. (Jackson, MS) for analysis of moisture, oil, FFA, and protein according to the procedures outlined by NCPA (1997).

Body weights were recorded on two consecutive days during the standardization period and wk 6 of the experimental period. To minimize variation, all BW were recorded after the PM milking and before animals had access to feed or water.

Ruminal fermentation trial

Six lactating Holstein cows previously fitted with ruminal cannulae were used in a concurrent replicated 3 X 3 Latin square design trial with 2 wk periods to evaluate the effect of FFA in WCS on ruminal fermentation. Cows were housed in a freestall barn and trained to eat behind Calan doors (American Calan Inc., Northwood, NH) before beginning the trial. Cows averaged 21.7 ± 7.7 kg/d of milk at the beginning of the trial. Treatments and experimental diets were the same as those used in the production trial. Feeding schedules and management were the same as that described for the production trial.

On the last day of the experimental period, ruminal fluid samples were collected at 0, 2, 4, 6, and 8 h post feeding. Approximately 50 ml of ruminal fluid was collected and strained through three layers of cheesecloth. A 40 ml sub-sample was strained through three layers of cheesecloth and immediately mixed with 10 ml of metaphosphoric acid (25% w/v). The sample was frozen for later analyses of VFA (Erwin et al., 1961). These samples were later thawed and centrifuged at 10,000 x g for 10 min and the supernatant collected for VFA analysis using a Hewlett Packard 2890A gas chromatograph (Hewlett Packard Company, Avondale, PA). The remaining sample was analyzed for pH.

Statistical analysis

Data from the production trial were subjected to analysis of covariance using PROC MIXED procedures of SAS (2001). The model included covariate, treatment, week, treatment by week, and error. The corresponding data from standardization period was used as a covariate. Cow within treatment was included as a random effect and week as a repeated effect. Ruminal pH and VFA data were subjected to analysis of variance using PROC MIXED procedures of SAS (2001). The model included cow, hour, period, treatment, hour by treatment, and error. Hour was included as a repeated effect. When a significant F test was determined ($P < 0.05$), the PDIFF option was used for treatment mean separation.

RESULTS AND DISCUSSION

Chemical Composition of WCS and TMR

The chemical composition of WCS treatments is presented in Table 5.2. The DM content averaged 89.5% for all WCS treatments. The FFA content of the WCS averaged 6.8, 24.1, and 22.3% for CONTROL, HFFA1 and HFFA2. Both lots of WCS with elevated FFA had slightly higher concentrations of moisture and CP, but lower concentrations of oil and ADF compared with CONTROL. The experimental diets were similar in nutrient content (Table 5.3).

Production trial

Cows fed diets containing HFFA2 tended to have higher ($P = 0.06$) DMI compared with CONTROL and HFFA1 (Table 4). These results are in agreement with previous work in which lactating cows or steers fed diets containing WCS with FFA up to 18% consumed similar amounts of DM as controls (Sullivan et al., 2004 and 2005). However, Plascencia et al. (1999) noted an increase in DMI when feeding Holstein steers yellow grease with up to 42% FFA. Bock et al. (1991) also noted increased DMI in steers supplemented with soybean oil soap stocks with 50% FFA and tallow with 15% FFA at 3.5% of the diet.

Yield of milk fat, and components was similar among treatments (Table 5.4). However, milk fat percentage was lower ($P = 0.007$) for cows fed diets containing either HFFA1 or HFFA2

Table 5.2. Chemical composition of whole cottonseed (WCS) differing in concentrations of free fatty acids in the oil (FFA) [1].

Item	CONTROL	HFFA1[2]	HFFA2
Moisture, %[3]	9.4 ± 0.4	10.6 ± 0.5	11.9 ± 0.7
	------------------------	% of DM	------------------------
Oil, %[3]	18.4 ± 0.6	17.1 ± 0.3	15.9 ± 0.8
FFA, % of oil[3]	6.8 ± 1.0	24.1 ±2.0	22.3 ± 3.9
Protein, %[3]	19.6 ± 0.4	20.7 ± 0.3	21.0 ± 0.4
	----------------------------	%	----------------------------
DM	90.7 ± 0.7	89.4 ± 0.4	88.6 ± 0.9
	------------------------	% of DM	------------------------
CP	20.2 ± 0.6	21.9 ± 0.4	22.5 ± 0.8
NDF	51.3 ± 2.4	50.1 ± 1.4	50.1 ± 1.9
ADF	42.1 ± 1.6	39.8 ± 0.6	39.6 ± 1.2
Ash	4.0 ± 0.4	4.2 ± 0.2	4.1 ± 0.4
NFC	23.6 ± 2.8	22.3 ± 2.4	22.3 ± 2.8
Ca	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
P	0.57 ± 0.03	0.56 ± 0.01	0.57 ± 0.04
Mg	0.35 ± 0.01	0.36 ± 0.01	0.37 ± 0.02
K	1.14 ± 0.04	1.14 ± 0.04	1.16 ± 0.04
	------------------------	ppm of DM	------------------------
Mn	16.8 ± 1.0	19.0 ± 0.09	17.5 ± 1.8
Zn	28.0 ± 2.8	30.8 ± 1.2	36.5 ± 2.3
Cu	5.7 ± 2.0	6.7 ± 2.3	7.7 ± 2.0

[1] All data are presented as mean ± SD.

[2] HFFA1 = WCS with 24.1 % FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

[3] Results of NCPA (1997) analysis.

Table 5.3. Chemical composition of experimental diets containing whole cottonseed (WCS) with increasing concentrations of free fatty acids (FFA) in the oil [1].

Item	CONTROL	HFFA1[2]	HFFA2
DM, %	44.6 ± 0.8	43.9 ± 0.9	44.4 ± 0.8
	------------------------	% of DM	------------------------
CP	17.2 ± 0.6	18.8 ± 1.0	17.7 ± 1.2
NDF	32.6 ± 3.3	31.2 ± 3.3	32.1 ± 4.1
ADF	22.6 ± 2.8	21.6 ± 2.5	22.4 ± 3.1
Ash	7.1 ± 0.7	7.0 ± 1.2	6.7 ± 0.8
NFC	40.1 ± 2.4	40.5 ± 3.8	40.5 ± 3.2

[1] All data are presented as mean ± SD.

[2] HFFA1 = WCS with 24.1 % FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

Table 5.4. Dry matter intake, milk yield and composition of cows fed diets containing whole cottonseed (WCS) with increasing concentrations of free fatty acids (FFA) in the oil.

Item	CONTROL	HFFA1[1]	HFFA2	SE	*P*
DMI, kg/d	21.6^{d}	22.0^{d}	23.5^{c}	0.6	0.06
Milk, kg/d	35.0	34.0	35.1	1.0	0.68
Fat, %	4.22^{a}	3.64^{b}	3.58^{b}	0.14	0.007
Fat, kg/d	1.38	1.25	1.29	0.05	0.19
Protein, %	3.15	3.08	3.06	0.05	0.44
Protein, kg/d	1.07	1.05	1.05	0.03	0.90
Lactose, %	4.71	4.80	4.73	0.04	0.35
Lactose, kg/d	1.67	1.64	1.65	0.04	0.89
SNF, %	8.74	8.67	8.64	0.07	0.62
SNF, kg/d	3.05	2.97	3.00	0.08	0.82
ECM, kg/d	36.5	34.5	35.7	1.0	0.36
MUN, mg/dl	8.86	11.11	9.39	0.78	0.15
Efficiency					
Milk/DMI	1.58	1.58	1.50	0.08	0.76
ECM/DMI	1.66	1.57	1.52	0.07	0.36

^{ab}Means in the same row with superscripts differ ($P < 0.01$).

^{cd}Means in the same row with superscripts differ ($P < 0.10$).

[1] HFFA1 = WCS with 24.1 % FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

compared with CONTROL; 4.22, 3.64, and 3.58% for CONTROL, HFFA1 and HFFA2, respectively. Concentrations of protein, lactose, and SNF were not affected by FFA in WCS.Because DMI and ECM were similar among treatments, efficiency of milk production was similar among all treatments.

These results are consistent with those reported by Bernard et al. (2006) in which cows fed diets containing WCS with either 23.1, or 35.5% FFA produced similar quantities of milk with reduced milk fat percentage compared with those fed diets containing WCS with 10.7% FFA. In contrast with the results of the current trial, Sullivan et al. (2004) did not observe any change in milk fat percentage when cows were fed diets containing either low FFA WCS or WCS with 12% FFA (Sullivan et al., 2004).

Ruminal fermentation analysis

No interaction of treatment and sampling time was observed, treatment means across all sampling times are reported in Table 5.5. Ruminal pH was similar among all treatments of WCS. Total VFA concentrations tended to be higher (*P* = 0.09) when WCS with elevated FFA were fed compared with CONTROL. There were no differences among treatments for molar proportions of acetate or propionate, but molar proportions of butyrate tended to be higher (*P* = 0.08) and molar proportions of isobutyrate were higher (*P* = 0.0004) for HFFA1 and HFFA2 compared with CONTROL.

Sullivan et al. (2005) noted a cubic response in total ruminal VFA concentrations when WCS containing 8, 11.3, 14.7, or 18% FFA were fed to Holstein steers. The highest concentration of total VFA was associated with the 11.3% FFA treatment, intermediate for 8 and 18% FFA intermediate, and lowest for 14.7% FFA. These authors also reported a linear decrease in molar proportions of isobutyrate as FFA concentrations in WCS increases. Decreased molar

Table 5.5. Effect of increasing levels of free fatty acids (FFA) in the oil of whole cottonseed (WCS) on rumen VFA concentrations[1].

Item	CONTROL	HFFA1[2]	HFFA2	SE	*P*
pH	6.07	6.14	6.21	0.06	0.28
Total VFA, mM	84.77	85.88	89.13	1.44	0.09
	---------------- % of total VFA -------------------				
Acetate (A)	60.51	59.82	59.56	0.50	0.38
Propionate (P)	24.82	24.96	24.62	0.50	0.89
Butyrate	10.16	10.06	10.60	0.17	0.08
Isobutyrate	0.47	0.52	1.08	0.11	0.0004
Isovalerate	2.06	2.16	2.05	0.05	0.22
Valerate	1.99	1.89	2.10	0.07	0.14
A:P	2.49	2.44	2.46	0.07	0.87

[1] Data represent LSMeans across all sampling time. No interaction of treatment and sampling time ($P > 0.10$) was observed.

[1] HFFA1 = WCS with 24.1 % FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

proportions of butyrate have been reported as dietary concentrations of FFA increase (Avila et al., 2000; Keele et al., 1989). The changes in rumen VFA concentrations observed in this trial do not indicate major changes in rumen microbial fermentation patterns that would explain the reduced milk fat percentage.

In general, oils and oilseeds with a high degree of unsaturation disturb rumen fermentation and fiber digestibility leading to lower acetate production and milk fat synthesis (Coppock and Wilks, 1991). The oil in WCS is 70% unsaturated and is completely hydrolyzed to FA in the rumen (Keele et al., 1989). A shift in VFA production from acetate to propionate is often associated with decreased milk fat percentage (Schmidt, 1971). However, ruminal fermentation analysis for this trial does not indicate a shift in VFA production that would account for the decreased milk fat percentage observed with HFFA1 and HFFA2. Sullivan et al. (2005) did not observe a change in FA profile of WCS oil containing 8 or 18% FFA, so reduced milk fat percentage is not likely due to changes in dietary fatty acids that would alter the transfer of FA into the mammary gland.

One possible explanation for the decrease in milk fat percentage in cows fed HFFA1 and HFFA2 compared with CONTROL may involve the effects of FFA on de novo synthesis of milk fat in the mammary gland. Negligible amounts of FFA are incorporated into milk fat and most FFA that are absorbed by the mammary gland are released into the venous blood (Schmidt, 1971). It is probable that as FFA in WCS increases, less FA or triglycerides would be available for incorporation into milk fat accounting for the decrease in milk fat percentage. Increasing supplemental dietary fat has generally been reported to decrease de novo synthesis of fatty acids in the mammary gland (Grummer, 1991; Palmquist et al., 1993) as FA from the supplemental dietary fat is incorporated directly into milk fat. Litherland et al. (2005) recently reported a decrease in milk fat percentage and short and medium chain milk fatty acid concentrations as the amount of highly unsaturated FA from soy oil infused into the abomasum increased from 0 to 600g/d.

CONCLUSIONS

Feeding diets containing WCS with elevated FFA did not alter DMI, milk yield, or yield of milk components, but does reduce milk fat percentage. The decline in milk fat percentage appears to be related to changes in FA available for incorporation into milk fat by the mammary gland as ruminal fermentation is not greatly altered.

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CHAPTER 6

CONCLUSIONS

The feeding value of whole cottonseed can be improved with the addition of value added feeds to the starch coating. Coating WCS with combinations of starch, urea, yeast and sodium bicarbonate slightly alters in vitro fermentation. In experiment 1, increasing levels of starch decreased pH and increased NH_3 concentrations while addition of urea had little effect on in vitro fermentation. Yeast culture supplementation decreased total VFA concentration and acetate: propionate ratios and increased molar concentrations of propionate. There was an interaction between starch and urea levels for molar proportions of valerate and isovalerate and a tendency for starch and urea interaction for pH. There was an interaction of starch level and yeast culture supplementation for pH and molar proportions of valerate and an interaction of urea and yeast for molar proportions of valerate. A three way interaction for starch, urea, and yeast was observed for pH, NH_3 concentrations, total VFA, and acetate: propionate ratio.

In experiment 2, the addition of urea to starch coated WCS increased concentrations of $NH_{3,}$ molar proportions of butyrate and isovalerate, and reduced molar proportions of propionate. Addition of $NaHCO_3$ to the starch coating decreased NH_3 concentrations, molar proportions of butyrate, isobutyrate and valerate and tended to increase molar proportions of isovalerate and total VFA concentrations. The addition of urea and $NaHCO_3$ to the starch coating of WCS increased in vitro NDF digestibility. Results from these experiments indicate that including urea, yeast, and $NaHCO_3$ to the starch coating of WCS may alter in vitro ruminal fermentation and provide for increased cellulolytic bacterial activity which would increase NDF digestibility of the starch coated WCS.

Coating WCS with gelatinized starch or gelatinized starch plus urea or yeast did not alter nutrient intake or whole tract nutrient digestibility. Milk production efficiency was improved for both WCS-U and WCS-Y because of a tendency for improved yield of fat and ECM without any difference in DMI. These results suggest that the inclusion of urea or yeast culture in the gelatinized cornstarch coating applied to WCS could improve nutrient utilization in support of milk production.

Feeding diets containing WCS with elevated FFA does not alter DMI, milk yield, or yield of milk components, but does reduce milk fat percentage. The decline in milk fat percentage appears to be related to changes in FA available for incorporation into milk fat by the mammary gland as ruminal fermentation is not greatly altered.