THE EFFECTS OF FEEDING HIGH FREE FATTY ACID WHOLE COTTONSEED

ON THE PERFORMANCE OF HOLSTEIN CATTLE

by

HILARY M. SULLIVAN

(Under the Direction of Henry E. Amos)

ABSTRACT

Twenty-four multiparous cows were fed diets providing WCS with 3, 6, 9, and 12% FFA. There was no difference in DMI, milk yield, milk protein percentage, lactose, or SNF. Milk fat percentage was lower for the 6% FFA diet. Intake of CP, NDF, and apparent DMD were not affected by treatment. Apparent NDF digestibility was highest for the 3 and 6% FFA and CP digestibility was highest for the 3 and 9% FFA. Intake and digestibility of ADF increased linearly with increasing dietary FFA. Concentrations of milk C6:0 decreased and milk C16:1 increased with increasing dietary FFA. Concentrations of milk C8:0, C10:0, and C12:0 exhibited a cubic response to treatment. Differences in milk fatty acid concentration and nutrient digestibility suggest changes in rumen fermentation. However, these changes did not impact production at the FFA levels fed in this experiment. Diets containing WCS with 8, 11.3, 14.7, and 18% FFA were fed to four ruminally and abomasally cannulated Holstein steers in a 4 x 4 Latin square design. There was no difference in DM, OM, or ADF intake and flow of OM and DM to the abomasum. Ruminal DM digestibility was lower for the 11.3% FFA treatment. Intake of NDF increased linearly with increasing FFA level of the diet. Digestibility of NDF, kg of NDF digested, and ADF intake and digestibility were not affected by treatment. Intake of N, flow of microbial N, and efficiencies of microbial N production responded cubically to treatment. Flow of non-microbial N was lower and microbial N flow higher for the 14.7% FFA WCS treatment compared with the other treatments. Average pH decreased linearly with increasing levels of FFA from WCS. Molar percentages of acetate, butyrate, propionate, isovalerate, and valerate were not affected by treatment. Isobutyrate concentrations decreased linearly and A:P increased linearly with increasing dietary FFA. Flow of valine to the abomasum was higher for the 11.3% FFA WCS, and lower for the 18% FFA WCS. Total AA flow, and total NEAA were not different among treatments. Total EAA flow was higher for the 11.3 % FFA WCS than the 18% FFA WCS.

INDEX WORDS: Dairy, Free Fatty Acid, Ruminant Nutrition, Whole Cottonseed, Fat Feeding, Amino Acid Flow, Milk Fatty Acid Profile.

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DEDICATION

To Rick.

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CHAPTER 1

REVIEW OF THE LITERATURE

THE DIGESTIVE TRACT

Due to a unique digestive tract, ruminants have the ability to utilize feed on which monogastric animals could not survive. Unlike hindgut fermentors such as horses, ruminants ferment feedstuffs prior to the small intestine, allowing the host to take advantage of more end products of microbial metabolism. To achieve this unique method of digestion, ruminants have developed a four-compartment stomach.

The Reticulum

The reticulum is the first section of the ruminant forestomach, located between the end of the esophagus and the anterior of the omasum. The reticulum comprises about 5% of the digestive tract volume and secretes no enzymes. The primary function of the reticulum is to sort feed particles by size and density. Large feed particles are regurgitated as cud, while smaller particles are fermented in the rumen. Dense or fine feed particles from the rumen are allowed to pass into the omasum. By regulating the size of the feed particles that enter the rumen, a higher ratio of surface area to volume is achieved which allows for more effective microbial digestion.

The Rumen

The rumen is a large structure, making up more than 50% of the digestive tract volume, more than 60 liters in mature cattle, which allows the ruminant to utilize forage materials. The function of the rumen is two-fold: to provide a suitable environment to the microbial inhabitants and to provide a method by which the host can exploit the end

products of microbial metabolism. In order to maintain a stable microbiological population in the rumen, the environment must remain fairly constant. The maintenance of a suitable fermentation temperature and pH in the rumen, and removal of waste accumulation are highly important. In addition, because many of the microbial inhabitants are anaerobic or microaerophilic, a highly reduced environment must be maintained. The anaerobic environment is preserved by eructation of any oxygen that may enter the rumen with feed or water. Oxygen is prevented from entering the rumen liquor by the digesta mat, which provides a physical barrier and houses aerobic microorganisms that utilize any oxygen in the rumen.

Rumen pH is maintained by a buffering system provided by the host. Mineral components of saliva along with rumen volatile fatty acids (VFA) help minimize variation in rumen pH. The primary buffering systems are comprised of sodium phosphate (pKa 7.21) and bicarbonate (pKa 6.7), which are provided by copious amounts of saliva, up to 150 liters per day in adult cattle (Church, 1982). In addition, VFA are removed from the rumen ecosystem by diffusion across the rumen wall. However, diet dependent variations in rumen pH still range from between 5 and 8.

Digestion in the rumen

Composition of the feed consumed by ruminants is more highly varied than those of monogastric livestock species. In addition to concentrate feeds, which are high in digestible energy and often rapidly fermented, ruminants consume structural carbohydrates and protein, as well as a small amount of fat. Many factors contribute to the ruminal digestibility of a feedstuff including level of feed intake, nutrient deficiencies, associative effects of the diet, and feed processing (Church, 1982).

The rumen produces no mammalian enzymes; its primary function is to retain feed, delay passage, and allow for microbial digestion. Digestion in the rumen is performed by a wide variety of bacteria, fungi, and protozoa. Bacteria are by far the most numerous ruminal organisms, present at concentrations of 10^{10} to 10^{12} cell/mL. Over 200 species of rumen bacteria have been identified, of these, 30 are considered prominent (present at greater than 10^{7}cell/mL) (Ogimoto and Imai, 1981). Protozoa exist in the rumen at approximately 10^{6} cells/ml and over 20 species have been identified. Rumen protozoa are difficult to study because they require co-culturing with bacteria; however, they play a key role in rumen nitrogen recycling because they predate bacteria and other protozoa. Rumen methanogens live in association with rumen ciliated protozoa; the protozoa provide a stimulatory effect to these bacteria. Removal of protozoa decreases methane production in the rumen by about 13% (Hegarty, 1999). Relatively little is known about rumen fungi. They have only been recognized in the rumen for about 20 years. Fungi are usually attached to feed particles or found as zoospores, which are associated with the liquid fraction (Gordan and Phillips, 1998). Rumen fungi contain cellulases, hemicellulases, pectinases, and phenolic acid esterases, allowing them to colonize and digest lignocellulose and play an important role in animals on a high forage diet (Ho and Abdullah, 1999).

Non-Structural Carbohydrate Digestion

Carbohydrates make up the majority of the ruminant diet and are the primary source of energy. Carbohydrates are extremely diverse, from simple sugars to complex polysaccharides. The mammalian GI tract can only absorb monosaccharides; therefore complex polysaccharides in plant material must be broken down before absorption by the

host (Church, 1982). The rumen microorganisms also require monosaccharides for fermentation since polysaccharides cannot enter the glycolytic pathway, the complex carbohydrates in the ruminant diet must be hydrolyzed to simple sugars before fermentation. Polysaccharide degradation has a variety of mechanisms and substrates in the rumen because the ruminant liquor contains simple, rapidly fermentable, non-structural carbohydrates (sugars) and more complex non-structural carbohydrates (starches). Starch is comprised of amylose and amylopectin. Amylose is a repeating chain of glucose in an α 1, 4 conformation with a molecular weight of 150,000 to 600,000. Amylopectin is composed of many amylose subunits linked by α 1,6 branches every 20-24 glucoses and a molecular weight of 1 to 6 million. Amylolytic bacteria are the primary utilizers of starch in the rumen, although many rumen bacteria can utilize simple sugars. Addition of non-structural carbohydrates to the diet tends to shift rumen fermentation towards propionate and lactate and away from acetate. This tends to lower rumen pH and increase the rate of fermentation in the rumen.

Structural Carbohydrate Digestion

The ability of rumen microbes to digest structural carbohydrates is what enables the ruminant to survive on poor quality forages. Structural carbohydrates are composed of cellulose, hemicellulose, and lignin. Cellulose is structurally quite similar to starch. It is a polymer of glucose linked in a β 1, 4 configuration with of a molecular weight of 150,000 to 1 million. The β 1, 4 bond of cellulose, unlike the α 1, 4 bond of starch, cannot be hydrolyzed by mammalian enzymes. Rumen cellulolytic microorganisms can hydrolyze the β 1, 4 bond by using a cellulase, often cell associated, to hydrolyze

cellulose to cellubiose and glucose. Cellubiose can be utilized by a wide variety of rumen microbes, in addition to ones containing a cellulase.

Hemicellulose is a complex polymer with both α and β bonds comprised of arabinans, mannans, galactans, and xylans. Hemicellulose is up to 30% xylose arranged in a β1,4 conformation. Hemicellulose requires a number of enzymes for complete degradation, including xylanases, mannases and galactanases. Hemicellulose is amorphous in structure and easily degraded, therefore many cellulolytic and non-cellulolytic rumen microorganisms can utilize it.

Lignin is a complex phenolic polymer of widely varying molecular weight. It is often associated with cellulose in the plant cell wall and tends to increase with plant maturity. Lignin contains many random phenolic rings, which few rumen microorganisms can ferment. Therefore lignin passes relatively undigested through the tract.

Lipid Digestion

The addition of fat to dairy cattle diets has been investigated in recent years as a method to increase energy density of the diet and help alleviate the loss of milk production attributed to heat stress. Much of the fat in the unsupplemented ruminant diet is comprised of galactosyl diglycerides typically found in plants (Church, 1982). Addition of other fat sources can disrupt rumen fermentation, reducing the digestibility of the diet.

The rumen microorganisms are not tolerant of high levels of unprotected lipid; typically the maximum added fat is 5-7% of the diet (Church, 1982). Composition of dietary lipid is important in predicting effects on rumen fermentation. Lipids that are

partially soluble in the rumen liquor are particularly problematic; these include C8 to C14 fatty acids (**FA**) and unsaturated long chain fatty acids (**LCFA**) (Coppock and Wilks, 1991). Some evidence suggests that the LCFA and saturated fatty acids (**SFA**) are more likely to form insoluble salts with soluble cations, allowing them to pass through the rumen as protected lipids (Jenkins and Palmquist, 1982). This has led to the practice of adding additional calcium to fat-supplemented diets in an attempt to bind lipids forming rumen insoluble FA salts (Palmquist and Jenkins, 1980).

Upon entering the rumen, dietary unprotected fats undergo microbial lipolysis releasing free fatty acids (**FFA**) from esterified lipids (Jenkins, 1993). Unprotected FA are then biohydrogenated by rumen microorganisms. Microbes in the rumen also often change the location of double bonds, or modify the bonds from *cis* to *trans* (Church, 1982). The polyunsaturated FA typically found in the ruminant diet are 60-90% saturated in the rumen and the rate of saturation is not drastically affected by the amount of fat supplementation (Grummer, 1991). The rumen has a high capacity for lipolysis and biohydrogenation. Production of FFA in the rumen is catalyzed by both esterases and lipases. There are 74 identified strains of rumen bacteria that can hydrolyze ester bonds, however it is unclear if these bacteria are capable of hydrolyzing lipid esters (Fay et al., 1990; Jenkins, 1993). Hespell and O'Bryan-Shah (1988) noted that, while a wide variety of rumen bacteria examined had esterase activity, only a few of these bacteria could hydrolyze triglycerides of LCFA (Jenkins, 1993). This suggests that there are many organisms with esterase activity in the rumen but there are far fewer lipolytic organisms. Lipases found in some bacteria are extracellular; these enzymes are released in vesicles and are capable of hydrolyzing acylglycerols completely to FFA and glycerol

(Jenkins, 1993). Glycerol rarely accumulates in the rumen because it is rapidly fermented, yielding primarily propionic acid (Garton et al, 1961, Jenkins, 1993).

Biohydrogenation in the rumen may serve to protect rumen microbes from the toxic effects of unsaturated FA. Lipolysis of fats is necessary before biohydrogenation can occur because *cis*-12 isomerization requires a free carboxyl group. Biohydrogenation of polyunsaturated FFA begins with the isomerization of the *cis*-12 double bond to a *trans*-11 configuration. A reductase then catalyzes the conversion of the *cis*-9 double bond to a single bond. The last step involves the action of another reductase on the *trans*-11 bond to convert it to a single bond (Jenkins, 1993).

Vegetable and seed oils, unlike the lipids in forages or fats from animal sources, are high in C18:2 and C18:3 FA; cottonseed oil 52.7% C18:2, and soybean oil 55% C18:2 and 7% C18:3 (Glouwacki, 1969; Messina, 1994). The addition of C18:1, C18:2, or C18:3 to *in vitro* ruminal fermentation has been shown to yield 20% C18:0 and 17, 48, and 67% *trans*-C18:1 respectively (Shorland et al., 1957). Biohydrogenation of C18:1, C18:2, and C18:3 FA in the rumen forms *trans*-11-C18:1 as a metabolic intermediate, because the rate limiting step of biohydrogenation in the rumen is conversion of *trans*-C18:1 to C18:0, *trans*-C18:1 isomers tend to accumulate (Grummer, 1991).

Addition of lipid to the ruminant diet tends to decrease DMI. A 50% reduction in structural carbohydrate digestion has been observed with 10% added fat (Chalupa, 1984; Boggs et al., 1987; Jenkins, 1993). Reduction in fiber digestibility may be at least partially responsible for reduced intake; however, results are varied and tend to be complicated by hormonal influences on intake. Pantoja et al. (1994) suggested that the

degree of saturation of the FA might affect DMI, with FA iodine values between 18-62 optimal for both FA digestibility and DMI (Tackett, et al., 1996).

Addition of lipid to the diet has been shown to reduce acetate:propionate and reduce methane and hydrogen production (Jenkins, 1993; Jenkins et al., 1996). Selner and Schultz (1980) reported that addition of 500 mL of unprotected C18:1 to the diet of lactating dairy cattle reduced rumen acetate:propionate ratios. Henderson (1973) observed that addition of C18:1 to *in vitro* pure culture fermentations did not inhibit the rumen amylolytic and lactolytic organisms that produced propionate and succinate; however, fiber digesters, acetate producers, and methane producers, such as *Butyrivibrio fibrisolvens, Rumincoccus sp.,* and *Methanobrevibacter ruminantium*, were inhibited. Chalupa et al. (1984) reported that the melting point of lipid added to *in vitro* fermentations accounted for 95% of variation in VFA production. Added lipid has also been shown to decrease rumen protein digestibility, increase N flow, and decrease rumen ammonia concentration (Ikwuegbu et al., 1982). It has been postulated that these changes are due to decreased rumen protozoa and reduced N recycling (Ikwuegbu et al., 1982; Jenkins and Palmquist, 1984; Jenkins, 1993).

The inhibition of rumen fermentation by fat feeding has been the subject of a number of theories. One is that the lipids coat feed particles, which prohibits microbial attachment and digestion of cellulose (Chen et al., 1991; Jenkins, 1993). Due to the non-polar nature of fats they are generally associated with feed particles instead of solublized in the rumen liquor (Pantoja et al., 1994). Many cellulolytic bacteria contain cell surface cellulases. The location of these enzymes requires close contact between bacteria and substrate (Stewart and Flint, 1989; Felix and Ljungdahl, 1993). Barsuhn et al. (1988)

showed that addition of lipid to feed particles not only detached bacteria from the cellulose fraction, but also caused permanent microbial cellular damage that inhibited cellulose attachment even after the lipid was removed (Jenkins, 1993). Another theory suggests that FFA inhibit the attachment of cellulase to cellulose (Immig et al., 1991; Jenkins, 1993). Still another theory is that FFA act as an uncoupler, by attaching to the membrane and altering microbial membrane function (Jenkins, 1993).

Evidence suggests that little dietary fat is lost from the rumen by catabolism to VFA or absorption across the rumen epithelium (Jenkins, 1993). Microbial FA acids are synthesized using carbohydrate precursors, resulting in straight chain, branched chain, even numbered, and odd-length FA and both monounsaturated FA and SFA (Church, 1982; Jenkins, 1993). In addition, rumen microbes can take up dietary LCFA. Therefore, the FA reaching the small intestine are from both dietary and microbial sources (Jenkins, 1993).

Protein Digestion

Amino acids in the diet are predominately found as components of proteins, which need to be hydrolyzed prior to absorption by the host (Church, 1982). Unlike monogastrics and hindgut fermentors, in the ruminant, protein is subject to microbial modification prior to host enzymatic hydrolysis. Rumen microbes hydrolyze proteins forming peptides and amino acids. These amino acids and peptides can be directly incorporated into microbial protein or proteolytic bacteria can deaminate the amino acids forming organic acids and ammonia (Russell et al., 1983). Rumen microorganisms can also utilize free ammonia to produce protein. Nolan (1975) estimated that 30 to 80% of all dietary nitrogen cycles through the ammonia pool. Microbes are the major source of

protein available to the ruminant, with microbial protein comprising up to 80% of protein nitrogen flowing to the small intestine (Hogan, 1975; Cotta and Russell, 1982). By deaminating, transaminating, and synthesizing protein, the microbial population alters the amino acid profile of the diet, however, the amino acid profile of microbial protein itself remains fairly constant, with little change due to diet dependent variation (Church, 1969). Microbial protein is high in quality with a biological value of 66 to 87 and supplies adequate amounts of essential amino acids for host maintenance even on a diet with low protein quality (Owens and Zinn, 1988).

The primary factor in determining microbial protein production is energy availability in the rumen. The presence of starch stimulates microbial protein production more effectively than sugars, possibly due to slower release of high-energy substrates (Church et al., 1971). Ammonia concentrations in the rumen are rarely limiting. Optimal free ammonia for microbial protein synthesis is 5.0 mg/100ml. When insufficient energy is present for production of microbial protein and dietary protein is fermented as an energy source, ammonia accumulates (Doelle, 1975).

Nutrient absorption in the rumen

During early studies of the ruminant digestive tract it was assumed that no nutrients were absorbed from the rumen, because the rumen wall is comprised of stratified squamus epithelium and not mucosal cells (Church, 1969). These cells produce no mucus, are non-glandular, and form horny papillae that serve to increase the surface area of the rumen wall. In the rumen the papillae are most dense in the ventral portions of the caudal and ventral rumen sacs. It is assumed that this is where the majority of nutrient absorption occurs (Church, 1969). There are a limited number of compounds

that can cross the rumen wall, including acetate, propionate, butyrate, ammonia, and some lactate; in addition, Mg^{+2}, K^{+}, Na^{2+}, Cl^{-}, and some small amino acids may be absorbed.

The removal of end products from the rumen is important not only for host utilization, but to increase the rate of microbial digestion. Rapid removal of VFA helps drive fermentation toward end products. The VFA move across the rumen epithelium with the concentration gradient. As the VFA cross the epithelium they are metabolized, maintaining a chemical gradient between the rumen and blood in the portal vein (Stevens, 1970). The rate of VFA absorption across the rumen epithelium is influenced by rumen pH. At neutral pH rumen VFA are in the disassociated form but as rumen pH decreases the protonated form is favored and VFA cross the rumen epithelium faster.

The rumen also has the capability to absorb ammonia, ammonium ions, as well as some small amino acids, namely glycine. Ammonia crosses the rumen wall, is metabolized in the liver to urea through the urea cycle, and is recycled back to the rumen via saliva and blood. The rate of ammonia passage into the blood stream is influenced by the pH of the rumen. When rumen pH is low, ammonium ion formation is favored and ammonia absorption decreases. When rumen pH is high the uncharged ammonia passes rapidly into the blood stream. When levels of ammonia in the peripheral blood are greater than 0.6 - 0.9 M ammonia toxicity results (Lewis et al., 1957).

The Omasum

The omasum is located between the reticulum and the abomasum. It is the smallest organ in the ruminant digestive tract, making up about 2% of digestive tract

volume. It functions to regulate digesta flow into the abomasum and prohibit passage of large feed particles (Hungate, 1966). Like the reticulorumen, it secretes no mammalian enzymes and is lined with stratified squamus epithelial cells, but it has far fewer papillae than the rumen. It absorbs water, VFA, buffer salts, and amino acids from the digesta as it passes. While it is an organ that is small in volume, it has a large surface area. It is estimated that the surface area of the omasum is one third that of the total forestomach (Stevens and Stettler, 1966). The omasum has upward of one hundred folds or lamina omasi, which markedly increase the surface area, and a canal, providing direct access into the abomasum. This anatomy functions to trap larger particles while allowing smaller particles to pass (Church, 1969). The large surface area allows efficient uptake of water and VFA. The removal of large amounts of water creates negative pressure, which aids the flow of digesta from the rumen into the abomasum. In addition, it is estimated that 33-64% of VFA are absorbed in the omasum (Gray et al., 1954).

The Abomasum

The abomasum is the true gastric stomach. The physiology of the ruminant abomasum is similar to the gastric stomach of other species. The pH of the abomasum is between 2 and 3. The pyloric and cardiac regions produce mucus that protects the gastric mucosa from damage due to low pH. The fundus contains parietal cells that secret HCl and chief cells that secrete pepsinogen (Ganong, 1997). Inactive at secretion, pepsinogen is converted to pepsin at low pH. The function of the abomasum is to halt microbial fermentation and secrete and activate pepsin. In addition, the abomasum accumulates and stores digesta from the omasum and then releases it slowly to the small intestine.

The Small Intestine

While the rumen is general thought to be the site of structural carbohydrate digestion, up to 28% of NDF digestion occurs post ruminally (McCarthy et al., 1989). The majority of non-structural carbohydrates are completely fermented to VFA in the rumen. However, post-ruminal starch digestion can be important under certain dietary conditions. Karr et al. (1966) stated that on a high starch diet 16-38% of the starch may pass to the abomasum. In addition, protozoa are known to store starch, making it available post-ruminally. In the small intestine, pancreatic α amylase acts upon polysaccharides, hydrolyzing endo α 1, 4 linkages forming maltose and maltotriose and limit dextrans (Ganong, 1997). These products are digested to glucose at the brush border membranes by α dextrinase and maltase. The majority of carbohydrate absorption occurs in the duodenum and jejunum of the small intestine with lesser amounts in the ileum (Church, 1982). Galactose and glucose are transported via an active transport system and pass unchanged into the portal vein (Church, 1982). While ruminant digestion of carbohydrates in the small intestine is similar to that of monogastric species, there is one curious difference. The ruminant small intestinal tissue does not contain sucrase in the cells of the brush border membrane. The host could utilize sucrose if hydrolysis occurred in the abomasum, but sucrose that passes through the small intestine undigested would be used by the microflora in the hindgut.

In the very young ruminant, proteins can be absorbed by pinocytosis, however, after the first 48 hours of life there is little absorption of large peptides (Church, 1982). There are at least seven known amino acid transport systems in the enterocytes. Of these,

three use Na^+ co-transporters, two use Na^+/Cl^- transport, and two are Na^+ independent (Ganong, 1997).

The FA from microbial sources are located primarily in the cell membrane as phospholipids and FFA (Viviani, 1970). Therefore lipids that enter the small intestine of ruminants are comprised of about 70% saturated FFA, 10-20% phospholipids (**PL**) of microbial origin, and a small amount of digesta associated triglycerides; in addition there may be ruminally protected fat (Bauchart, 1993). The FFA present in digesta in the small intestine are fully protonated and therefore adhere to particulate matter. As the digesta moves down the tract, adhered FFA and PL gradually dissolve into the micellar layer. The secretion of bile into the duodenum favors the interaction between FFA and PL and liquid digesta, forming a crystalline phase. As the pH of the digesta rises, the crystalline phase disperses and associates with bile salts forming the micellar solution (Bauchart, 1993). The micellar solution is absorbed by the mucosal cells in the upper jejunum (15-25%) and the middle and lower jejunum (55-65%) (Bauchart, 1993). Protected lipid is digested in much the same manner as lipids in non-ruminants. Lipids released from the abomasum are mixed with bile and pancreatic secretions containing pancreatic lipase and colilipase (Church, 1982, Bauchart, 1993). The lipids are emulsified and form small particles, allowing greater exposure to lipases. The lipases digest the esterified lipids at the α 1 and 3 positions, forming 1, 2 diglycerides, β-monoglycerides and FFA (Palmquist and Jenkins, 1980; Church, 1982). These combine with salt, PL, and cholesterol micelles form mixed micelles. Short and medium chain FA are absorbed directly into the hepatic vein. Upon entering the intestinal epithelia LCFA are converted to coenzyme A derivatives and reassembled into triglycerides. The triglycerides, phospholipids, and

cholesterol esters are packaged into chylomicrons and enter the lymph via exocytosis (Church, 1982)

Lipid intake, dietary form (protected vs. unprotected), and saturation determine the extent of post-ruminal lipid digestion (Grummer, 1991). Generally, dietary fat is well digested by ruminants, and saturated fats tend to be better digested by ruminants than non-ruminants. However, when lipid intake exceeds 900 –1400 g/d, digestion decreases (Bines et al., 1978; Palmquist, and Conrad, 1978; Wrenn, et al., 1978; Grummer, 1991). Lipid digestibility tends to decrease as chain length increases (Steele and Moore, 1968; Andrew and Lewis, 1970; Gummer, 1991). Degree of saturation also effects digestibility. Evidence suggests that the presence of polyunsaturated fats in the diet leads to smaller micelle formation, and increased absorption efficiency (Steele, 1983; Grummer, 1991). Unsaturated LCFA have been observed to be more soluble in bile salts than SFA of equal chain length (Lough and Smith 1976; Smith and Lough. 1976). The intestinal epithelium contains desaturase activity, which can convert SFA to monounsaturated FA, this occurs primarily with C18:0 FA (Grummer, 1991). This assures a supply of unsaturated FA to the ruminant.

THE MAMMARY GLAND

Milk Component Production

Lactose Production

Lactose is the main constituent responsible for the osmolarity of milk. It is a disaccharide formed by the β 1, 4 linkage of galactose and glucose. Lactose synthesis begins with the phosphorylization of glucose to form G-6-P, which is then isomerized to form G-1-P. A UTP is combined with G-1-P at the expense of a high-energy bond, to

form UDP-glucose. Then UDP-galactose is formed by epimerization. Finally lactose is formed by the addition of a glucose catalyzed by lactose synthetase (Schmidt, 1971). In the mammary gland, lactose synthesis occurs in the Golgi and lactose is secreted into vesicles that are released into the alveoli from the apical membrane (Sutton, 1989). In general lactose concentrations in milk are relatively constant and extremely difficult to manipulate. However, severe underfeeding has been shown to modify milk lactose content, reduction in the forage:concentrate ratio has been shown to increase lactose concentration, and high fat supplementation has been shown to decrease lactose concentration by as much as 0.2% (Sutton, 1989).

Fat synthesis

De novo synthesis is responsible for all short chain FA in milk fat with carbon length C4-14 and half of all C16 milk FA. Milk fat is synthesized primarily from β-OH butyrate and acetate produced during rumen microbial fermentation (Grummer, 1991). Short chain FA are synthesized by stepwise addition of acetate molecules to propionate, β OH-butyrate, or acetate. Very little fat is synthesized from glucose (Schmidt, 1971). The remaining milk FA are derived from blood lipids. The LCFA for milk fat synthesis are taken up from blood plasma triglycerides (Steele, 1983). Butterfat typically contains some odd numbered and branched chained FA; these FA are of rumen microbial origin and not at all typical of plant FA (Church, 1982).

The lipid component of milk is 97% triglycerides comprised of approximately 10% C14:0, 25% C16:0, and 10% 18:0. Glycerol is derived from glucose via the Emden-Myerhof Pathway as glycerol-3-P. Milk triglycerides are esterified from FFA by the smooth endoplasmic reticulum of the mammary secretory epithelial cells (Grummer,

1991). These cells also contain a desaturase, stearoyl-coenzyme A, which catalyzes the conversion of C18:0 FA to C18:1 (Grummer, 1991). The inhibition of this enzyme by *trans*-C18:1 has been implicated in milk fat depression with unsaturated fat and high carbohydrate feeding (Gayner et al., 1994). Because of the conversion of C18:0 to C18:1 in mammary tissue the ratio of C18:1 to C18:0 is 2 to 3:1 in milk versus 1:2 in plasma.

Like lactose and protein, milk fat is synthesized in the endoplasmic reticulum, packaged into vesicles at the Golgi, and secreted at the apical membrane. It is, unlike lactose and protein, packaged and secreted separately as fat globules. Because it is secreted separately, milk fat is the most widely variable constituent in milk and most subject to dietary manipulation (Sutton, 1989)

Protein synthesis

Milk protein is made up of 90-95% casein, β lactoglobulin, and α lactoglobulin, which are synthesized in the mammary gland (Schmidt, 1971). Essential amino acids for protein formation in the mammary come primarily from the blood and non-essential amino acids can come from the blood or can be synthesized in the mammary. Protein production takes place in the endoplasmic reticulum (Schmidt, 1971). After translation or uptake, proteins are transported to the Golgi and secreted at the apical membrane with lactose into the lumen of the alveoli (Sutton, 1989).

Effect of Feeding on Milk Composition

Effect of Fat Feeding on Milk Composition

High producing dairy cows need diets with high concentrations of net energy of lactation (NE_L) to support milk production. Increasing energy density with the addition of soluble carbohydrates at the expense of forage in the diet can lower rumen pH and

decrease acetate: propionate. Addition of fat to the diet can result in an increase in milk fat and FCM yield; however, results have been varied (Tackett et al., 1996; Kalscheur et al., 1997a). Increasing dietary energy by the addition of fats to the diet up to 4% of DMI can increase milk yield (Ashes et al., 1997). However, some studies have reported no increase in milk yield due to fat supplementation (Grummer, 1988; Schauff and Clark, 1989; Skaar et al., 1989; Knapp and Grummer, 1991; Tackett et al., 1996). Jenkins et al., (1996) observed that addition of soybean oil to the diet of lactating cows numerically increased milk yield but significantly decreased FCM yield and milk fat percentage. Knapp and Grummer (1991) reported increases in milk fat percentage and 3.5% FCM when prilled fat plus tallow was added to the diet at 5% of DMI. Addition of fat to the diet tends to be more effective for improving performance in high producing and multiparous dairy cattle (Tackett et al, 1996).

Changes in milk FA composition due to dietary fat feeding are related to the degree of protection of the fat, fat type, and FA composition (Ashes et al., 1997). Unprotected unsaturated fats are subject to biohydrogenation in the rumen and tend to suppress fiber digestion. Reduced fiber digestion leads to lower acetate production, milk yield and milk fat (Ashes et al., 1997). Increasing dietary fat in the ruminant diet tends to decrease *de novo* production of FA in mammary tissue, leading to lower ratios of milk short chain FA (C6:0 to C14:0) (Ashes et al., 1997). This decrease in short chain FA is offset by an increase in milk LCFA, which originate from the diet or adipose tissue mobilization. Attempts to quantify the relationship between dietary FA and milk FA led Hermansen (1995) to state that the proportion of C12:0, C14:0, and C16:0 in milk fat was relative to those present in the diet; levels of these FA in the milk were also negatively

correlated to dietary FA supply, the negative effect on C12:0, C14:0, and C16:0 milk FA content increased with increasing dietary FA chain length. However, with the short chain FA (< C12) and C16:1, factors other than FA supply comprised 60% of variation in milk FA composition. Grummer (1991) reported the milk C18:C16 was strongly correlated to the C16:C18 in the diet. Changes in C18:0 and C18:1 milk FA composition were also highly correlated to total FA supply (Grummer, 1991; Hermansen, 1995). Clapperton and Banks (1985) reported that the diet-dependent changes in C18:1 were 1.7 times greater than those of C18:0 (Hermansen, 1995). In addition, rumen-protected unsaturated fat increases the proportion of unsaturated:SFA in milk (Ashes et al., 1997). Increasing the levels of C18 mono and polyunsaturated fat in dairy products has proposed human health benefits and increases the spreadability of butter. Because of the high activity of mammary gland desaturase, the possibility of increasing C18:0 FA in the diet to increase the levels of monounsaturated FA in milk has been proposed (Grummer, 1991).

Attempts to manipulate FA profile using lipid supplementation have had varied degrees of success. Lipid feeding influences the FA profile of milk but attempts to produce a more "ideal" milk fat for human health have not been successful. The "ideal" milk fat contains higher levels of mono and polyunsaturated FA and much lower levels of SFA than typical milk fat (Grummer, 1991). In addition, higher levels of short chain FA (< C12), lower levels of C12:0 and C14:0 FA, and higher levels of C18:n and omega FA are desirable (Grummer, 1991). Addition of canola, sunflower, or high oleic sunflower oil to the diet increased C18:0 and C18:1 FA 55-80%. However, there was also a 20-49% decrease in C4:0 to C:16 FA. Dietary manipulation of milk LCFA has been hampered by ruminal hydrogenation and mammary desaturase activity (Middough et al,

1988, Murphy, and McNeill, 1988; Grummer, 1991). In addition, the most plentiful FA in the blood is C18:2, however, very little is found in the triglyceride fraction, which is where most LCFA for milk fat synthesis are derived (Steele, 1983). While additional of C18:n, especially C18:2, would improve the human health benefits of dairy products, high C18:2 milk has properties that make it undesirable. Dairy products with higher levels of unsaturated fats are more subject to spontaneous off-flavor; high C18:2 milk fat oxidizes quickly if antioxidants are not added immediately. Butter produced from high C18:2 milk is more spreadable but has negative taste and texture characteristics above 4^{o}C, while cheese produced with high C18:2 milk has poor flavor, texture, and visual appeal (Palmquist et al., 1993)

Addition of concentrates to the diet at the expense of forages also tends to increase the lipid content of the diet. The FA contained in concentrates are highly unsaturated. Incomplete microbial biohydrogenation of these lipids, resulting in formation of *trans*-C18:1 FA, has been implicated in milk fat depression (Kalscheur et al., 1997b). The addition of buffers has been shown to alleviate milk fat depression caused by higher concentrate diets, decrease the abomasal flow of *trans*-C18:1 FA to the duodenum, and reduce the levels of *trans*-C18:1 fat in the milk (Kalscheur et al., 1997a). Addition of 500 g of 49% *trans*-C18:1 directly to the diet has been shown to reduce milk fat percentage from 3.9 to 3.1%, while the addition of 500 g of *cis*-18:1 had no effect on milk fat percentage (Selner and Schultz, 1980). Further evidence of the effects of *trans*-C18:1 FA is demonstrated in the lack of milk fat depression in cows on high concentrate diets that did not exhibit elevated *trans*-C18:1 milk fat levels (Gaynor et al., 1995). With high-grain low-forage diets the number of lipolytic and biohydrogenating bacteria

are reduced, this may increase the level of milk unsaturated FA (Latham et al., 1972; Palmquist and Jenkins, 1980).

Milk protein percentage tends to be depressed by supplemental dietary fat (Tackett et al., 1996; Kalscheur et al., 1997a). However, milk protein yield often remains stable (Tackett et al., 1996). Where milk yield is increased, these changes may be due to a dilution effect. However, some data shows decreased protein yield by cows supplemented with fat (Harrison et al., 1995). The most significant reduction in protein appears to be in the casein fraction (Dunkley et al., 1977). There have been several theories proposed for explaining decreased protein percentages and or yield sometimes seen with supplemental fat feeding. Dunkley et al. (1977) suggested that the addition of fat to the diet might decrease microbial protein production, leading to reduced protein availability. Smith et al. (1978) proposed that the addition of fat to the diet reduced the availability of glucose to the mammary. Another theory is that the addition of fat to the diet increases insulin resistance, impairing amino acid transport by the mammary. Still another theory is that fat feeding reduces pituitary release of BST and this in turn reduces mammary amino acid uptake (Coppock and Wilks, 1991).

Effect of Forage Feeding on Milk Composition

Because acetate is the main precursor of short chain FA in the mammary gland, an ample supply of forage NDF in the diet is necessary to maintain milk fat levels. At least 19-21% effective NDF is considered the minimum level necessary to prevent milk fat depression (Ashes et al., 1997).

Replacement of large amounts of forage in the diet with concentrate causes an increase in milk *trans*-C18:1 FA and milk fat depression (Storry and Rook, 1965).

Forage length is also important in maintaining milk fat percentage. Finely chopped forages are not adequate, a mean length of 0.6 to 0.8 cm has been proposed as the minimum needed to maintain normal rumen fermentation (Woodford et al., 1986; Sutton, 1989). Small particle size increases the rate of ruminal passage, decreasing rumen retention time, pH, and fiber digestion, leading to lower acetate production and decreased acetate:propionate.

Milk protein yield tends to decrease with increasing forage levels in the diet. Protein and non-structural carbohydrate intake tend to be lower in high fiber diets and may be responsible for these decreases (Tackett et al., 1996). Lower NE_L content of higher forage diets may decrease microbial protein yield and decrease protein availability to the mammary gland, if additional rumen by-pass protein is not provided. The non-essential amino acids plus arginine, methionine, cysteine, histidine, valine, threonine, and tryptophan are gluconeogenic (Church, 1982). Therefore, reduced production of propionate in the rumen may increase the utilization of gluconeogenic amino acids for glucose production and reduce their availability to the mammary gland.

EFFECTS OF FEEDING OF WHOLE COTTONSEED ON DAIRY CATTLE PERFORMANCE

Whole cottonseed (**WCS**) is a byproduct of cotton production. The nutrient profile, as well as, its relatively low cost makes it a beneficial ration ingredient for lactating cow diets. It is high in protein, 22.5%, EE, 17.8%, and NDF, 26% and is approximately 35% forage and 65% concentrate (Coppock and Wilks, 1991; NRC, 2001). By substituting WCS for other concentrate ingredients the energy density of the diet can be maintained or increased while increasing the effective fiber of the diet.

Coppock et al. (1987) stated after reviewing eighteen trials involving cottonseed feeding, that addition of WCS up to 25% of the DM did not depress DMI. McNamara et al. (1995) observed that addition of WCS to a TMR with grass silage and alfalfa hay increased DMI and net energy intake. However, several studies have reported decreased DMI with WCS feeding (Coppcock et al., 1985; Mohamed et al., 1988).

The oil in WCS is not rumen-protected and is greater than 50% unsaturated (Grummer, 1991). However, because of the physical characteristics of the seed the lipid is released slowly into the rumen, allowing for more complete biohydrogenation. This allows WCS to be fed at much higher levels than other unsaturated, unprotected lipid sources. Despite this, some changes in rumen fermentation have been reported. The addition of WCS to a corn/alfalfa silage based diet was shown to increase ruminal propionate, reduce ruminal acetate:propionate and protozoa counts, and reduce rumen pH; these negative results were reversed by roasting the WCS prior to feeding (Mohamed et al, 1988). Smith et al, (1981) reported the addition of WCS increased nitrogen, energy, and ether extract digestibility but had no effect on fiber digestion.

The effects of WCS on milk production are variable. Anderson et al., (1979) reported WCS increased yield of FCM, decreased milk protein percentage, but had no effect on fat percentage. The addition of WCS to a corn/alfalfa silage-based diet by Mohamed et al. (1988) had no effect on milk protein percentage, milk yield, or FCM yield and reduced the rate of acetate incorporation into adipose tissue. Addition of up to 20% WCS to an alfalfa-based diet, increased milk fat content, total milk solids, FCM yield, and fat yield, and decreased milk protein and SNF (DePeters et al., 1985). While Smith et al. (1981) stated that 15 and 25% WCS in an alfalfa-based diet, increased milk

fat percentage, milk fat yield, and FCM yield, decreased milk protein percentage and SNF. Harrison et al. (1995) observed increased milk fat percentage, milk fat production per day, and milk energy in early to mid-lactation cows with the addition of WCS to a barley silage-based diet.

The effects of adding WCS on milk FA profile have been well documented. Mohamed et al. (1988) noted decreased milk C14:0 and C16:0 and increased milk C18:0 andC18:1. DePeters et al., (1985) also demonstrated increased weight percentage of milk C18:0 and C18:1. While Smith et al., (1981) observed WCS decreased weight percentage of milk short chain FA, increased weight percent of C18:0, C18:1, C:18:2 in milk fat, but had no effect on C18:2 milk FA yield. Overall effects of feeding WCS on milk FA profile in this study showed a 50% reduction in mammary gland FA synthesis, but a two-fold increase in yield of milk C18:0 and C18:1, and a four-fold increase in milk trans-C:18:1 due to increased dietary FA uptake.

EFFECTS OF THE ADDITION OF FREE FATTY ACIDS TO THE DIET

Oilseeds used for oil production are relatively low in FFA content. However, oilseeds that are high in FFA content and therefore unsuitable for oil production are often sold as feed for livestock. In addition, some commercial waste greases are low in cost and could be utilized in ruminant diets, but are also high in FFA. Some mechanical processes for the treatment of oilseeds have also been reported to increase the rate of FFA release during fermentation. However, there are few data on the effects of dietary FFA on ruminant digestion.

Chulupa et al., (1984) reported that the addition of FFA to *in vitro* ruminal fermentations decreased VFA production. All individual FFA measured were reported to

decrease acetate:propionate, except for C18:0 and C20:2. Lauric acid (C12:0) was shown to be the most damaging to fermentation; causing a 69% decrease in total VFA production and inducing a 40:1 acetate:propionate ratio. Reddy et al., (1994) demonstrated that extrusion increased the rate of FFA release from soybeans. Inclusion of extruded soybeans in *in vitro* fermentations of alfalfa hay decreased NDF and ADF digestibility verses raw soybeans. In addition, there was reduced biohydrogenation of C18:1, C18:2, and total C18 FA. Plascencia et al. (1991) reported that increasing the FFA content of yellow grease increased the ADG, DMI, and feed efficiency of Holstein steers on a feedlot diet. These changes according to the author were due to increased palatability of the diet. There was no change in ruminal, post ruminal, or total tract digestibility of N, ADF, OM, or lipid with increasing FFA level. However, higher FFA content increased ruminal starch digestion and the increasing FFA acid content of the diet had a quadratic effect on rumen lipid biohydrogenation. Rumen lipid biohydrogenation percentages were 61, 71, 61, and 64 for 0, 15, 28, and 42% FFA in yellow grease respectively. The FFA also had a quadratic effect on the molar proportions of acetate, and propionate, and estimated methane production in the rumen. Palmquist and Conrad (1978) noted increased milk production with addition of 6% hydrolyzed fat (50% FFA); however, there was no increase in production with 20% hydrolyzed fat and increasing the level of hydrolyzed fat also decreased the fat digestibility from 81% to 56%.

Previous research has shown the addition of FFA sources to the diet alters rumen fermentation. Animal studies have been limited and have focused on the effects of FFA in grease on rumen fermentation and production. There has been no investigation of the

effects of FFA in oilseed on rumen digestion. This is especially important in dairy nutrition because WCS has become such a popular ration ingredient for lactating cows.

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INTRODUCTION

Whole fuzzy cottonseed is often added to the diet of lactating dairy cattle as a source of fat, protein, and fiber. Addition of fat to dairy rations increases the energy density of the diet in support of high levels of milk production; however, fat can also have a negative impact on fiber digestion. High levels of unsaturated fatty acids (FA) in the diet have been shown to be toxic to certain rumen microbes and can coat feed particles preventing fibrolytic microbes from attaching to fiber, subsequently depressing fiber digestion (MacLeod and Buchanan-Smith, 1972; Eastridge and Firkins, 1991).

Prime WCS used for oil production cannot contain more than 1.8% FFA in the oil (NCPA, 1997). Since non-prime WCS is not desirable for oil production, it is often sold as livestock feed. It is thought that the concentration of unsaturated FFA may be responsible for disruption of normal rumen fermentation (Jenkins, 1993).

The ability of lipid sources to alter milk FA profile is well documented (Hermansen, 1995). Various lipid sources, especially those containing polyunsaturated fat, have been implicated in spontaneous oxidation and off-flavors in milk (Timmons et al., 2001). Due to the high level of polyunsaturated lipid in WCS and the possibility of reduced biohydrogenation of FFA in the rumen, high FFA WCS could be detrimental to milk quality.

Previous research has shown the addition of FFA sources to the diet alters rumen fermentation. Animal studies have been limited and have focused on the effects of FFA in grease on rumen fermentation and production. There has been

no investigation of the effects of FFA in oilseed on rumen digestion. This is especially important in dairy nutrition because WCS has become such a popular ration ingredient for lactating cows. Therefore experiments were undertaken to evaluate the effects of high free fatty acid whole cottonseed on the performance of Holstein cattle.

CHAPTER 2

EFFECTS OF FEEDING HIGH FREE FATTY ACID WHOLE COTTONSEED ON THE PERFORMANCE OF LACTATING DAIRY CATTLE.[1]

[1] Sullivan, H. M., J. K. Bernard, H. E. Amos, and T. C. Jenkins. To be submitted to Journal of Dairy Science.

ABSTRACT

*Twenty-four multiparous cows were used in a 10 wk randomized block design trial to evaluate the effects of feeding whole cottonseed (**WCS**) containing increasing concentrations of free fatty acids (**FFA**) on DMI, milk yield and composition, milk fatty acid profile, nutrient digestibility, and plasma insulin, glucose, and AA concentrations. Two lots of WCS containing either 3 or 12% of the oil as FFA were blended to provide WCS with 3, 6, 9, and 12% FFA. Cottonseeds were fed at 12.5% of dry matter in a wheat silage based total mixed ration. There was no difference in DMI, milk yield, milk protein percentage, lactose, or SNF. Milk fat percentage was lower in the diet supplying 6% FFA. Intake of ADF increased linearly with increasing FFA. Intake of CP, NDF, and apparent DMD were not affected by treatment. Apparent NDF digestibility was highest for the 3 and 6% FFA treatment and CP digestibility was highest for the 3 and 9% FFA treatment. Digestibility of ADF increased linearly with increasing dietary FFA. Concentrations of milk C6:0 decreased with increasing dietary FFA, whereas milk C16:1 increased linearly as FFA increased. Concentrations of milk C8:0, C10:0, and C12:0 exhibited a cubic response to treatment because the 6% FFA was higher than 3 and 12% FFA and 9% FFA was lowest. Differences in milk fatty acid concentration and nutrient digestibility suggest changes in rumen fermentation. However, these changes did not impact production at the FFA levels fed in this experiment.*

INTRODUCTION

Whole fuzzy cottonseed is often added to the diet of lactating dairy cattle as a source of fat, protein, and fiber. Addition of fat to dairy rations increases the energy density of the diet in support of high levels of milk production; however, fat can also have a negative impact on fiber digestion. High levels of unsaturated fatty acids (FA) in the diet have been shown to be toxic to certain rumen microbes and can coat feed particles preventing fibrolytic microbes from attaching to fiber, subsequently depressing fiber digestion (MacLeod and Buchanan-Smith, 1972; Eastridge and Firkins, 1991). In contrast to other fat sources, WCS can be fed at rates up to 15% of the diet without drastically disturbing fiber digestion because

the FA contained in the seed are released slowly in the rumen and biohydrogenated so they are not toxic.

Prime WCS used for oil production cannot contain more than 1.8% FFA in the oil (NCPA, 1997). Since non-prime WCS is not desirable for oil production, it is often sold as livestock feed. It is thought that the concentration of unsaturated FFA may be responsible for disruption of normal rumen fermentation (Jenkins, 1993). Because the oil in WCS is 70% unsaturated, high levels of FFA in WCS may have a negative effect on fiber digestion (Martinez et al., 1991; DePeters and Cant, 1992).

The ability of lipid sources to alter milk FA profile is well documented (Hermansen, 1995). Various lipid sources, especially those containing polyunsaturated fat, have been implicated in spontaneous oxidation and off-flavors in milk (Timmons et al., 2001). Due to the high level of polyunsaturated lipid in WCS and the possibility of reduced biohydrogenation of FFA in the rumen, high FFA WCS could be detrimental to milk quality. The objectives of this study were to determine the effects of feeding WCS supplying increasing concentrations of FFA on DMI, milk yield and production, nutrient digestibility and intake, and plasma glucose, insulin, and amino acids in lactating dairy cows.

MATERIALS AND METHODS

Two lots of WCS differing in FFA concentrations were obtained from gins in South Georgia and transported to The University of Georgia Dairy Center in Athens, GA. One lot of WCS contained 3% of oil as FFA and the second lot contained 12% of oil as FFA. Experimental diets (Table 2.1) were formulated for

a 670 kg lactating cow producing 32 kg/d of milk with 3.5% milk fat and 3.3% protein with 0.45 kg of BW gain / d. Dietary treatments consisted of four concentrations of FFA in WCS and were achieved by stepwise substitution of the low FFA WCS, with off-quality, high FFA (FFA = 12% of oil in seed) WCS. Concentrations of FFA in lots of WCS were determined using NCPC (1997) guidelines.

Twenty-four multiparous lactating Holstein cows were used in a 10 wk randomized design trial. Cows were managed according to procedures approved by The University of Georgia Institutional Animal Care and Use Committee. Cows were fed the control diet for 14 d prior to the start of the 56 d trial period. Cows were housed in a free stall barn and fed twice daily behind Calan gates (American Calan, Inc., Northwood, NH) at 105% of the previous day's intake. Cows were blocked according to current milk yield (range = 32.7 to 49.9 kg/d; mean = 37.8 kg/d) and assigned to one of four blocks of six cows each. Cows within blocks were assigned randomly to one of four dietary treatments at the end of the 14 d preliminary period. The amount of feed offered and orts were recorded daily. Feed and orts were sampled daily and composited weekly by treatment. Pooled samples were dried at 55^{o}C for 72 h, ground to pass through a 2mm Wiley mill screen (Arthur W. Thomas Co., Philadelphia, PA), and stored in glass jars for analyses. Fecal grab samples were collected on three consecutive d during wk 5 at 12 h intervals. Sampling schedule was advanced 2 h each day during collection period. Samples were dried, composited by cow, and stored for analyses. Ether extract of feed and orts was determined by soxhlet extraction

with petroleum ether, while fecal samples were extracted using petroleum ether acidified with 10% glacial acetic acid (Palmquist and Conrad, 1978). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) of the TMR, feces, and orts were determined by the method of Robertson and Van Soest (1981) using an Ankom 220 Fiber Analyzer (Ankom Technology Co., Fairport, N.Y.). Nitrogen was determined using a Leco FP528 (Leco Corp., St. Joseph, MI) and CP was estimated (%N X 6.25). Dry matter, CP, EE, NDF, and ADF digestibility was calculated during wk 5 using indigestible ADF (IADF) as a marker (Henderson et al., 1985). The IADF of TMR and feces were determined using a Daisy II 200 Rumen Fermentor (Ankom Technology) (Anonymous, 1998).

Milk weights were recorded twice daily at each milking and BW were recorded on three consecutive d during the last wk of the pretrial period and the last wk of experimental period. Milk was analyzed during the pretrial and wk 2, 3, 4, 5, and 6, using pooled samples from morning and evening milking, for milk fat and protein using infrared spectrometry (DHIA, Belleview, FL). Total solids of lyophilized samples were determined using a Virtis Freezemobile 12 (Virtis Co Inc., Gardiner, N.Y.). The solids were ashed in a muffle furnace (Lindberg, Watertown, WI) for 3 h at 510^{o}C to determine the mineral content. Lactose percentage was determined by difference. Milk fat from samples collected during wk 5 and 7 was isolated by centrifugation at 21,000 x *g*, 15 min. The milk FA were converted to methyl esters and FA profile was determined by the method of Kramer et al. (1997) using Hewlett Packard 5890A equipped with a 30 x 0.25 mm (i.d.) polyamino glycol capillary column (Spelco Inc., Bellefonte, PA).

Blood samples were collected from a coccygeal vein in preheparinized and NaF tubes at 0, 2, and 4 h after A.M. feeding during wk 2 of the pretrial period and wk 3 and 6 of the experimental period. Samples were prepared for glucose and insulin analyses by refrigeration (8 h at 4^{o}C) and centrifugation (3000 x *g*, 20 min). Plasma glucose was analyzed by a Tinder colormetric method (procedure #315, Sigma, St. Louis, MO). Insulin was determined by radioimunoassay using a ^{125}I competitive binding method (ICN Pharmaceuticals Inc., Costa Mesa, CA). Plasma samples from wk 6 were prepared for AA analysis by deproteinizing with 5% sulfasalicylic acid, freezing for 12 h at -4^{o}C, and centrifuged at 5000 x *g,* 20 min. Plasma AA were determined by HPLC using a Beckman 1600 amino acid analyzer using norleucine as an internal standard (Beckman Instruments Inc., Fullerton, CA).

The experimental period began on April 11th and ended on June 6th 2001. Climatic data was obtained from hourly readings taken at Athens Ben Epps Airport Weather Station. Maximum and minimum temperature and relative humidity (**RH**) data were obtained and maximum and minimum temperature humidity index (**THI**) was calculated using the formula: THI = F^{o}-(0.55-(0.55*(RH/100)))*(F^{o}-58) (NOAA, 1976).

The DMI and milk production data were analyzed using the Proc Mixed procedure for repeated measures of SAS (SAS, 1996). The statistical model included the effect of treatment, days in milk, and wk and data collected in the pretrial period was used as a covariate. Milk FA profile was analyzed using the Proc Mixed procedure for repeated measures of SAS with the model including

treatment and wk. Digestibility and wk 5 intake data were analyzed using the general linear model (GLM) procedure of SAS. Heat stress data was analyzed using the Proc Mixed procedure for repeated measures, with the model containing treatment, days in milk, maximum and minimum temperature, maximum and minimum relative humidity, and maximum and minimum THI and interactions between treatment and temperature, relative humidity, and THI. Treatment effects for all data were tested using one degree of freedom by the contrast statement of SAS. Contrasts were 1) linear effect of FFA level; 2) quadratic effect of FFA level; and 3) cubic effect of FFA level.

RESULTS AND DISCUSSION

The chemical composition of experimental diets was similar among treatments (Table 2.1). Chemical composition and FA profile of WCS is presented in Table 2.2. The composition of the two lots of WCS was also similar, with the only noticeable difference between the two lots being a slight higher C16:0 percentage and a slightly lower EE and C18:2 percentage in the 12% FFA WCS.

There were no differences among treatments in average weekly DMI (Table 2.3); however, there was a ($P < 0.05$) treatment by week interaction. Initially, during wk 1 intake decreased linearly with increasing FFA levels in the diet. After wk 1, DM intake tended to be higher for cows fed the 6 and 12% FFA treatments as compared to those fed the 3 and 9% FFA treatments. These results suggest that after an adjustment period there were no negative effects from the addition of high FFA WCS on the palatability of the diet. The change in BW was

similar for all treatments. Milk yield was similar for all treatments and averaged 33.5 kg/d. Previous research has shown a high negative correlation between milk protein percentage and addition of fat to the diet (Sporndly, 1989; DePeters and Cant, 1992). Negative effects on milk protein percentage have been observed regardless of fat source (whole oilseeds, protected fat, or free oil) (DePeters and Cant, 1992). In the current study, there were no significant differences in milk protein percentage or yield among treatments (Table 2.3).

There was no difference in percentage and yield of milk lactose, ash, total solids, solids-not-fat, or yield of energy corrected milk. Addition of unprotected fat to dairy rations can cause depression in milk fat percentage. However, WCS has been shown to have variable effects on milk fat percentage. Wu et al. (1994) reported changes in milk FA profile, but no significant change in milk fat percentage with the addition of WCS. Van Horn et al. (1984) observed that milk fat depression with the addition of WCS was more severe for a low protein diet (14%) than a high protein diet (18%). In the current study, milk fat percentage was higher ($P < 0.05$) for cows fed the 9% FFA WCS than those fed the other treatments; however, the milk yield for this treatment group was also numerically lower. Yield of milk fat was similar for all treatments, so the higher milk fat percentage observed with 9% FFA WCS was most likely due to differences in dilution.

Data in Table 2.4 and 2.5 show the effects of dietary FFA levels on milk FA. Typically, increases in the dietary fat content have been shown to reduce concentrations of C6 through C14 milk FA due to the inhibition of mammary *de*

novo FA synthesis (Palmquist et al., 1993; Jenkins et al., 1996). Concentrations of C6:0 decreased linearly ($P < 0.05$) with increasing dietary FFA level. The C8:0 ($P < 0.05$), C10:0 ($P < 0.02$), and C12:0 ($P < 0.01$) milk FA exhibited a cubic response to treatment, with the 6% FFA WCS diet having higher percentages than the other three treatments. Total short chain FA exhibited a cubic response to treatment ($P < 0.001$) because the 6% FFA WCS treatment had the highest percentage, the 9% FFA WCS treatment had the lowest percentage, and the 3 and 12% FFA WCS treatment had intermediate concentrations of short chain FA. It should be noted that while the 6% FFA WCS treatment had the highest percentages of short chain milk FA, this treatment also had numerically higher DMI; therefore, there may have been more VFA precursors available for *de novo* mammary gland fat production and there may have been fewer negative effects on fiber digestion.

Long and medium chain FA (> C13) content of milk fat from cows fed diets containing 3, 6, 9, and 12 % FFA WCS is presented in Table 2.5. The C16:1 milk FA exhibited a linear ($P < 0.05$) response to treatment, with the 9 and 12% FFA WCS diets having higher concentrations than the 3 and 6% FFA WCS diets. Concentration of C16:0 increased linearly ($P < 0.05$) with increasing FFA from WCS. Total medium chain FA increased linearly ($P < 0.01$) with increasing FFA in the diet. Generally off-flavors due to spontaneous auto-oxidation are associated with increases in milk C18:2 and C18:3 FA, and these FA are often increased with addition of oilseed to the diet (Hermansen, 1995). There was no difference in C18:2 and C18:3, but C18:2 was numerically higher when 6% FFA

WCS was fed. Total long chain FA, total unsaturated FA and total saturated FA were also not affected by treatment. Depression of milk short chain FA with WCS feeding is well documented (Smith, et al., 1981; DePeters et al., 1985; Harrison, et al., 1995). These results suggest that elevated FFA levels may magnify the depression of milk short chain FA synthesis seen with WCS feeding, especially at the 9% FFA level. However, milk long chain FA remained relatively unchanged with increasing dietary FFA levels. The changes in milk FA profile observed in this study would not be expected to impact milk quality or flavor.

Nutrient intake and digestibility measured during wk 5 were affected by WCS FFA level (Table 2.6). Intake of ADF increased linearly ($P < 0.02$) with increasing dietary FFA; whereas, DM, CP, EE, and NDF intakes were similar for all treatments. Apparent CP digestibility was higher ($P < 0.01$) for diets containing 3 and 9% FFA WCS than diets with 6 or 12% FFA WCS. The NDF digestibility was lowest ($P < 0.05$) for diet containing 9% FFA WCS, but digestibility of ADF increased linearly ($P < 0.01$) with increasing dietary FFA levels. There was no difference in EE digestibility. Intakes of EE were similar, so differences in NDF, and ADF digestibility would be expected to be related to the form of the FA. However, it should be noted that there was a small but significant increase in ADF intake with increasing FFA levels. Others have shown that increasing the fiber content of a diet can mitigate the fermentation problems normally associated with increased unprotected dietary fats (Doreau et al., 1991; Tackett et al., 1996; Mir, 1998).

There were no differences among treatments in average plasma glucose level (Fig. 2.1). However, plasma glucose exhibited a highly significant treatment x wk x h interaction ($P < 0.001$). During wk 3 the cows fed 3, 9, and 12% FFA WCS had an elevated glucose concentration at 2 h post feeding, while all 4 treatments exhibited lower plasma glucose levels post-feeding during wk 6.

The effect of treatment on average plasma insulin levels was quadratic ($P < 0.005$), with the 6 and 9% FFA diets having higher concentrations than the 3% or 12% FFA diets. In wk 3, the 6% FFA treatment had the highest plasma insulin concentration (Table 2.7). The plasma insulin concentrations of the cows fed the 9% FFA WCS were intermediate to those fed 6% and 3% FFA WCS, and the cows fed 12% FFA WCS were lowest. In wk 6, the 3% treatment had a higher concentration of plasma insulin at 2 h postfeeding than the other three treatments. With the exception of the 3% treatment, in wk 6, all treatments had lower plasma insulin concentrations at 2 and 4 h postfeeding than at 0 h postfeeding in wk 6 or 2 and 4 h postfeeding in wk 3. The differences in plasma glucose and insulin between wk 3 and 6 could be attributed to changes in intake due to the cows being later in lactation, or because the trial began in early Spring and as the trial progressed the cows were consuming more of their feed at night due to increased heat stress.

When environmental factors were added to the model the effect of treatment on DMI was cubic ($P < 0.05$) because 3 and 9% FFA WCS treatments had lower DMI than 6 and 12% FFA WCS treatments. There was an interaction between treatment and maximum temperature ($P < 0.0001$), maximum THI ($P <$

0.05), maximum RH ($P < 0.0001$). Intake decreased ($P < 0.05$) as minimum daily THI increased. Intake decreased ($P < 0.0001$) as maximum THI increased.

Plasma AA concentrations as measured during wk 6 are reported in Table 2.8. Plasma EAA were different among treatments. Arginine concentrations tended to be higher for the 3% FFA treatment. Plasma lysine exhibited a cubic ($P < 0.02$) effect, with the 3% treatment having the highest concentrations and the 12% FFA treatment having the lowest concentrations. Phenylalanine concentrations decreased linearly ($P < 0.02$) as the FFA concentrations increased. Plasma isoleucine ($P < 0.05$), and leucine ($P < 0.02$) concentrations were highest for 3% FFA treatment. Plasma valine concentrations were lower ($P < 0.005$) for the 6, 9, and 12% FFA treatments than the 3% FFA treatment. Plasma threonine, methionine, and histidine were similar among treatments. Total EAA tended to have linear ($P < 0.07$), cubic ($P < 0.09$), and quadratic ($P < 0.07$) responses to treatment. Total EAA were higher ($P < 0.05$) for the 3% FFA WCS treatment.

Plasma NEAA were also affected by treatment. Plasma glycine tended to be higher with the 6% FFA treatment and lower with the 9% FFA WCS treatment than the 3 and 12% FFA WCS treatment. Concentrations of cystithionine were highest ($P < 0.0002$) for the 12% FFA treatment. A cubic ($P < 0.02$) effect was observed for plasma ornithine concentrations because of higher concentrations for 3 and 9% FFA WCS than 6 or 12% FFA WCS. Plasma serine, glutamine glutamate, proline, alanine, cysteine, and tyrosine were similar among treatments.

Total NEAA exhibited a linear ($P < 0.02$) response to treatment because concentrations of NEAA decreased with increasing FFA from WCS. Total AA

exhibited a linear ($P < 0.01$) and quadratic ($P < 0.07$) response to treatment because total AA concentration decreased with increasing FFA from WCS. Plasma branched chain AA exhibited a linear ($P < 0.05$) and quadratic ($P < 0.005$) effect, with the 3% FFA WCS treatment having higher ($P < 0.005$) plasma branched chain AA concentrations than the other three treatments. Mohamed et al., (1989) reported that addition of free soybean oil and whole soybeans significantly lowered plasma isoleucine, ornithine, and leucine concentrations; however, the addition of free oil and whole cottonseed to the control diet had no effect on plasma amino acids.

Feed AA profiles were similar among the treatment diets (Table 2.9), however, plasma branched chain AA leucine, isoleucine and valine were higher, and plasma arginine had a tendency to be higher for the 3% FFA WCS treatment. Decreases in plasma branched chain AA concentrations can indicate reduced MCP synthesis (Mohamed et al., 1989). Lower plasma AA concentrations for the 6, 9, and 12% FFA WCS treatments indicate a change in N metabolism. As noted previously this change in N metabolism did not alter milk protein production in this study; however, this change could be detrimental in cows producing more milk and therefore requiring more AA for mammary gland protein synthesis.

Results of this experiment suggest that feeding whole cottonseed containing up to 12% FFA does not depress intake or milk production. Increasing the concentrations of FFA from WCS magnified the depression of *de novo* mammary gland fatty acid synthesis often seen with WCS feeding. However,

there were no differences among treatments in milk long chain fatty acids. Therefore, the addition of high FFA WCS would not impact milk quality. In addition, there were no negative effects of feeding high FFA WCS on total tract fiber digestibility. Differences in milk fatty acid composition, plasma AA concentrations, and nutrient digestibility suggest potential changes in rumen fermentation; however, these changes did not negatively impact the production measures tested during this study. Therefore, whole cottonseed containing up to 12% FFA oil in seed, can be fed to lactating dairy cattle at up to 12.5% of diet DM without detrimental effects.

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Table 2.1. Ingredient and partial chemical composition of total mixed ration containing whole cottonseed (WCS) with increasing concentrations of free fatty acids (FFA).

	% FFA in WCS			
	3	6	9	12
Ingredient	----------% of DM----------			
Wheat silage	44.9	44.9	44.9	44.9
Ground corn	24.5	24.5	24.5	24.5
3% FFA WCS	12.5	8.3	4.2	----
12% FFA WCS	----	4.2	8.3	12.5
Soybean meal 48% CP	9.2	9.2	9.2	9.2
Protein supplement[1]	3.8	3.8	3.8	3.8
Mineral premix[2]	2.1	2.1	2.1	2.1
Diet Chemical Composition	---------- % ----------			
DM	42.5	42.6	42.2	42.2
CP	19.8	18.8	19.6	19.0
NDF	46.5	47.2	46.8	47.8
ADF	29.8	29.1	28.4	29.6
EE[3]	6.35	6.34	6.37	6.39
NE_l, Mcal/kg[4]	0.79	0.79	0.79	0.79

[1]Protein supplement was composed of 60% menhaden fish meal and 40% distillers grains with solubles.

[2]Premix contained 34.4% CP from urea 86.7% ash; 24.50% Ca; 3.68% P; 1.27% Mg; 0.08% K; 3.03 % Na; 4.60% Cl; 0.31% S; 11.67 ppm Co; 665 ppm Cu; 4,622 ppm Fe; 58 ppm I; 2,039 ppm Mn; 14.69 ppm Se; 1,943 Se, 133,070 IU/lb Vit A; 53,230 IU/lb Vit D; and 665 IU/lb Vit E.

[3] Ether extract.

[4] Determined using NRC (1989) values.

Table 2.2. Partial chemical composition of whole cottonseed (WCS) containing 3 and 12% free fatty acids (FFA).

	% FFA in WCS	
	3	12
Ingredient	----------% of DM----------	
DM	88.83	88.04
CP	26.67	24.80
NDF	43.24	43.26
ADF	30.34	30.80
EE[1]	16.99	16.15
FA Profile	---------- % of FA ----------	
C10:0	0.1	0.05
C14:0	0.7	0.7
C16:0	22.0	24.3
C16:1	0.5	0.6
C18:0	1.0	2.5
C18:1	16.0	15.6
C18:2	54.3	51.8
C20:0	0.2	0.3
C18:3	0.2	0.2
C9t11	0.06	0.06

[1] Ether extract.

Table 2.3. Dry matter intake, milk yield and composition of lactating Holstein cows fed diets containing increasing amounts of free fatty acids (FFA) from whole cottonseed (WCS).

	% FFA in WCS				
	3	6	9	12	SE
DMI[1], kg/d	22.2	22.7	21.9	22.3	0.7
Milk, kg/d	32.9	35.7	32.0	33.8	4.2
Fat, %	3.48	3.32	3.67	3.41	0.08
Protein, %	2.63	2.74	2.85	2.81	0.09
Lactose, %	5.39	5.30	5.37	5.22	0.15
Total solids, %	12.22	12.01	12.61	12.18	0.25
Ash, %	0.69	0.71	0.71	0.69	0.01
Solids-not-fat, %	8.72	8.74	8.92	8.74	0.18
ECM[2], kg/d	32.7	33.2	33.5	33.1	1.6

[1] Average DMI during 8 wk experimental period.
[2] Energy-corrected milk (3.5% fat, 3.2% protein).

Table 2.4. Milk short chain fatty acid composition of lactating Holstein cows fed diets containing increasing levels of free fatty acid (FFA) from whole cottonseed (WCS).

	% FFA in WCS				
	3	6	9	12	SE
	---------- % of total fatty acids ----------				
C4:0	3.62	3.61	3.28	3.4	0.15
C6:0[a]	2.38	2.55	2.25	2.27	0.07
C8:0[b]	1.30	1.49	1.25	1.26	0.05
C10:0[c]	2.56	3.06	2.50	2.60	0.10
C11:0	0.04	> 0.001	0.04	0.04	0.02
C12:0[c]	2.70	3.23	2.69	2.79	0.10
Short Chain FA[1b]	12.62	13.91	12.03	12.43	0.22

[a] Linear ($P < 0.05$).

[b] Cubic ($P < 0.05$).

c Cubic ($P < 0.02$).

[1] Fatty acids less than 14 carbons.

Table 2.5. Milk medium and long chain fatty acid composition of lactating Holstein cows fed diets containing increasing levels of free fatty acid (FFA) from whole cottonseed (WCS).

	% FFA in WCS				
	3	6	9	12	SE
	---------- % of total fatty acids ----------				
C14:0	10.13	10.81	10.31	10.36	0.21
C14:1	1.22	1.30	1.29	1.22	0.08
C15:0	0.90	0.90	0.89	0.90	0.02
C16:0[a]	28.74	27.94	30.01	30.33	0.74
C16:1[a]	0.94	0.92	1.20	1.18	0.09
C17:0	0.52	0.52	0.51	0.51	0.02
Medium Chain FA[1b]	42.50	42.38	44.15	44.40	0.60
C18:0	14.97	14.37	14.27	14.43	0.59
t18:1	2.01	2.13	2.03	2.17	0.08
C18:1	20.7	19.6	20.3	19.4	0.65
C18:2	2.09	2.26	2.11	2.07	0.08
C18:3	0.37	0.38	0.36	0.36	0.05
C20:0	0.24	0.21	0.24	0.23	0.01
C9t11	0.36	0.37	0.38	0.39	0.06
CLA	0.12	0.13	0.12	0.14	0.03
C22:0	0.08	0.07	0.05	0.06	0.02
C22:1	0.08	0.05	0.05	0.04	0.02
Total Long Chain FA[2]	41.02	39.57	40.00	39.32	0.60
Unsaturated FA	27.88	27.15	27.85	26.93	0.51
Saturated FA	68.26	68.71	68.32	69.23	0.58

[a]Linear ($P < 0.05$).

[b]Linear ($P < 0.01$).

[1] Fatty acids with 14 to 18 carbons.

[2] Fatty acids greater than 18 carbons.

Table 2.6. Effect of diets containing increasing amounts of free fatty acids (FFA) from whole cottonseed (WCS) on nutrient intake and digestibility.

	% FFA in WCS				
	3	6	9	12	SE
[1]Intake, kg/d	---------- kg/d ----------				
DM	21.3	23.0	21.9	22.0	0.9
CP	3.5	3.4	3.6	3.3	0.1
NDF	10.4	11.7	10.2	10.7	1.0
ADF[a]	6.0	6.8	6.8	7.1	0.3
EE	3.2	3.3	3.3	3.1	0.1
Digestibility	---------- % DM ----------				
DM	50.3	51.2	49.7	49.6	1.0
CP	54.1	48.0	53.2	46.7	1.1
NDF	39.7	42.8	35.4	38.6	0.7
ADF[b]	35.1	36.7	39.9	45.6	1.6
EE	51.0	55.7	55.8	49.8	4.1

[a] Linear ($P < 0.02$).
[b] Linear ($P < 0.01$).
[1]Average wk 5 intake.

Fig. 2.1. Effect of free fatty acids (FFA) from whole cottonseed (WCS) on blood glucose concentrations in lactating Holstein dairy cattle.

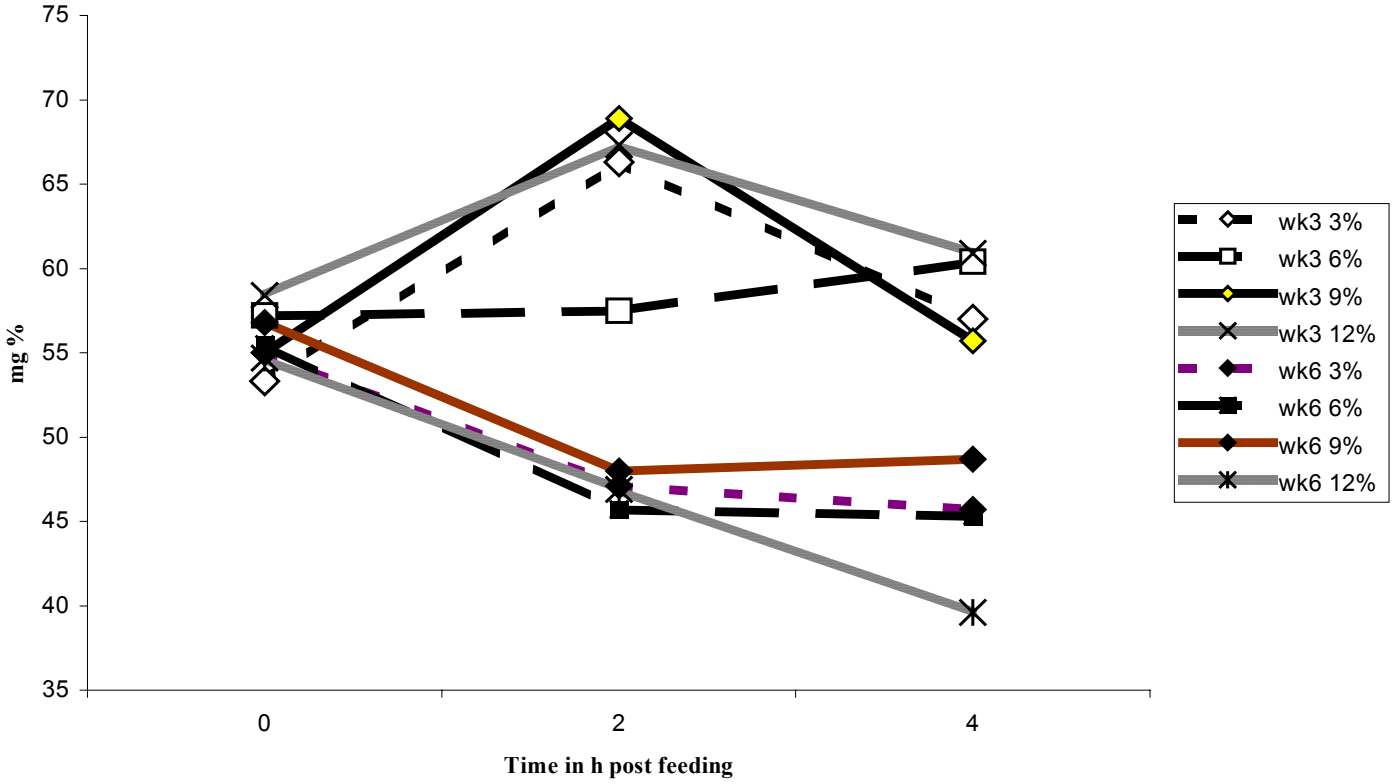

Table 2.7. Effects of feeding whole cottonseed (WCS) with increasing concentrations of free fatty acids (FFA) on plasma insulin levels post feeding in lactating Holstein dairy cattle.

	% FFA from WCS			
Item	3	6	9	12
Wk 3	----------μunits/ml ----------			
0 h	14.68	19.95	17.43	12.41
2 h	18.51	25.67	21.19	20.82
4 h	20.94	24.29	26.03	19.86
Wk 6				
0 h	17.55	19.47	19.56	17.94
2 h	20.72	15.75	16.57	17.50
4 h	15.56	17.67	17.21	15.59

[1] Pooled standard error = 1.74

Table 2.8. Effect of feeding increasing concentrations of free fatty acids (FFA) from whole cottonseed (WCS) on plasma amino acid levels.

	% FFA in WCS				
	3	6	9	12	SE
EAA	----------μM/dL----------				
Arginine	8.64	7.26	7.34	7.15	0.50
Histidine	3.93	3.14	3.50	3.54	0.35
Isoleucine	23.79	17.58	17.36	19.26	1.23
Leucine	15.18	11.22	11.28	11.86	0.94
Lysine[c]	8.78	6.79	7.40	5.82	0.63
Methionine	4.12	3.84	3.84	3.97	0.19
Phenylalanine[a]	3.91	3.35	3.37	3.01	0.18
Threonine	7.87	6.70	8.17	7.72	0.53
Tryptophan	2.97	7.12	7.38	5.48	2.60
Valine	25.84	18.44	17.44	20.45	1.49
Total EAA	105.03	85.43	87.09	87.50	5.15
NEAA					
Alanine	20.09	19.30	18.34	17.88	1.85
Glutamine	149.38	146.58	144.38	122.25	8.56
Glutamate	5.62	5.87	5.90	5.72	0.48
Glycine	30.01	33.27	27.66	29.64	1.70
Ornithine[c]	4.77	3.81	4.41	3.89	0.26
Proline	6.80	6.00	5.91	6.33	0.48
Serine	6.54	6.92	6.77	6.26	0.39
Tyrosine	3.50	5.27	2.82	2.94	0.91
cystithionine	0.19	0.21	0.20	0.29	0.01
Cysteine	4.86	5.05	4.85	5.06	0.17
Total NEAA[a]	230.01	230.48	220.94	198.73	7.92
Total AA[b]	335.04	315.92	308.03	286.23	10.47
BCAA[1]	64.81	47.24	46.09	51.57	3.47

[a] Linear ($P < 0.02$)
[b] Linear ($P < 0.01$)
[c] Cubic ($P < 0.02$)
lacking a common superscript differ ($P < 0.05$).
[1]Branched chain AA.

Table 2.9. Amino acid profile of treatment diets containing increasing levels of free fatty acids (FFA) from whole cottonseed (WCS).

	% FFA			
Item	8	11.3	14.7	18
	---------- mg AA/ g of DM----------			
EAA				
Thr	5.6	5.7	5.7	5.3
Val	0.4	0.4	0.5	0.4
Met	2.3	2.3	2.2	2.0
Ile	6.6	6.7	6.4	6.3
Leu	11.4	11.4	11.4	10.7
Phe	6.6	6.7	6.5	6.2
His	3.5	3.5	3.5	3.3
Lys	6.0	6.0	5.8	5.6
Arg	9.7	9.9	9.5	8.6
Total	52.1	52.6	51.5	48.4
NEAA				
Tyr	4.7	4.6	4.7	4.3
Cys	3.8	3.8	3.7	3.6
Asp	12.8	13.0	12.8	12.4
Glu	26.2	26.3	26.0	24.9
Gly	7.4	7.6	7.5	7.4
Ser	7.5	7.5	7.7	7.1
Ala	9.5	9.6	9.5	8.9
Pro	10.8	10.8	10.8	10.2
Total	82.7	83.2	82.7	78.8
Total	134.8	135.8	134.2	127.2

CHAPTER 3

EFFECTS OF FEEDING HIGH FREE FATTY ACID WHOLE COTTONSEED ON RUMEN FERMENTATION AND AMINO ACID FLOW TO THE ABOMASUM OF HOLSTEIN STEERS.[2]

[2] Sullivan, H. M. J. K. Bernard, and H. E. Amos. To be submitted to J. Dairy Sci.

ABSTRACT

Total mixed ration diets containing 12.5% whole cottonseed (WCS) with 8, 11.3, 14.7, and 18% free fatty acids were fed to four ruminally and abomasally cannulated Holstein steers in a 4 x 4 Latin square design. Diets were fed for ad libitum intake. Each experimental period consisted of 10 d for ration adjustment and 4 d for sampling. There was no difference in DM, OM, or ADF intake. Flow of OM and DM to the abomasum was not affected by treatment. Ruminal DM digestibility was lower for the 11.3% FFA treatment. Intake of NDF increased linearly with increasing FFA level of the diet. Digestibility of NDF and kg of NDF digested in the rumen was not affected by treatment. There was no significant difference in ADF intake or digestibility. Intake of N responded cubically to treatment. Total flow of N to the abomasum was numerically higher for the 11.3 and 18% FFA WCS treatments. Flow of microbial N/d exhibited cubic and quadratic responses to treatment because of higher flow for the 14.7% FFA WCS treatment and lower flow for the 18% FFA WCS treatment. Flow of non-microbial N was lower and microbial N flow higher for the 14.7% FFA WCS treatment compared with the other treatments. Apparent and true efficiency of microbial N production exhibited a cubic response to treatment because of higher efficiency for the 14.7% FFA WCS treatment and lower efficiency for the 18% FFA WCS treatment. Average pH decreased linearly with increasing levels of FFA from WCS. Molar percentages of acetate, propionate, butyrate, isovalerate, and valerate were not significantly affected by treatment. Isobutyrate concentrations decreased linearly with increasing dietary FFA. Ruminal A:P increased linearly with increasing dietary FFA. Flow of valine to the abomasum was higher for the 11.3% FFA WCS treatment, and lower for the 18% FFA treatment. Total AA flow, and total NEAA were not different among treatments. Total EAA flow was higher for the 11.3 % FFA WCS treatment than the 18% FFA WCS treatment.

INTRODUCTION

Cottonseed is an important feed ingredient for lactating dairy cattle; previous research has suggested it may be worth twice its cost in lactating dairy cow diets, because it provides a unique blend of fat, protein, and fiber (White, 1983). Whole cottonseed (**WCS**) with free fatty acid (**FFA**) levels higher than 1.8% of oil in seed is undesirable for oil production and is often sold as livestock feed (NCPA, 1997). However, limited data exists on the possible implications of the inclusion of high FFA WCS in the diet of dairy cattle.

High levels of dietary unsaturated fatty acids (**FA**) in the diet can be toxic to certain rumen microbes and can coat fiber particles, preventing fibrolytic bacteria from attaching and subsequently depressing fiber digestion (MacLeod and Buchanan-Smith, 1972; Eastridge and Firkins, 1991). Jenkins (1993) stated that the level of unsaturated FFA in the rumen might be the determining factor in disruption of normal rumen fermentation. Oilseeds are high in unsaturated fatty acids; therefore, high levels of FFA in WCS could have a negative effect on fiber digestion (Martinez et al., 1991; DePeters and Cant, 1992). Reddy et al., (1994) reported that increasing the rate of FFA release in soybeans by extrusion reduced NDF and ADF digestibility in mixed culture *in vitro* ruminal fermentations.

It is well documented that the addition of WCS to the diet can lower milk protein percentage (Anderson et al; 1979; Smith, et al., 1981; DePeters et al., 1985). Decreased milk protein percentage observed for diets containing supplemental fat has been attributed to reduced microbial crude protein production (Dunkley, 1977). Reduced microbial protein production could result from replacement of ruminally fermentable carbohydrates with unavailable fat, which is not fermented and does not provide energy in support of microbial protein synthesis, reduced microbial efficiency due to growth uncoupling, or a reduction in protozoa and fibrolytic bacteria populations. High FFA WCS could increase the negative effects of WCS on membrane function or protozoal and fibrolytic bacterial populations by increasing the rate of release of FFA into the rumen liquor. Therefore, an experiment was undertaken to determine the effects

of high FFA WCS on reticuloruminal fermentation and flow of nutrients to the abomasum in cannnulated Holstein steers.

MATERIAL AND METHODS

Four 350 kg Holstein steers fitted with ruminal and abomasal cannulae were used in a 4 x 4 Latin square to evaluate the effects of increasing FFA levels in WCS. Two lots of WCS differing in FFA concentration were obtained and transported to The University of Georgia Dairy Cattle Research Center in Athens, Georgia. Diets were formulated (Table 3.1) to meet the requirements of a 670 kg lactating cow producing 32 kg/d of milk containing 3.5% fat and 3.3% protein plus 0.45 kg of BW gain/d. The FFA content of the WCS was elevated by increasing the moisture content of the 12% FFA WCS to 20% for 72 h before drying to approximately 90% DM. Samples were evaluated for FFA content according to NCPA (1997). Whole cottonseed containing either 8 or 18% of the oil as FFA were blended to provide 8, 11.3, 14.7, and 18% FFA. Steers were cannulated and managed according to procedures approved by The University of Georgia Institutional Animal Care and Use Committee. Experimental periods were 14 d in length with 10 d for ration adjustment and 4 d for sample collection. Steers were housed in a tie stall barn and allowed 2 h per d exercise in a dry lot. A TMR was fed twice daily at 110% of previous day's intake. Feed and orts were recorded daily and nutrient intake calculated using d 7 to 14 of each treatment period. Samples of feed and orts were collected on d 10 to 14 of each experimental period and composited by steer within period.

Ruminal and abomasal samples were collected on d 10 to 14 at 12 h intervals. The sampling schedule was advanced three hours each day. Rumen fluid was collected and strained through four layers of cheesecloth, analyzed for pH, and frozen for later analysis. Rumen fluid samples were analyzed for VFA concentrations using a Varian 3400 Gas Chromatograph (Varian, Walnut Creek, CA). A composite rumen fluid sample was formed by combining 25 mL from each collection time. Bacteria were isolated from the composited rumen fluid by centrifugation 10,000 x *g*, 15 min. The bacterial isolate was lyophilized and stored for analysis. Abomasal samples were frozen for storage and later composited by steer within treatment and lyophilized to dryness.

Feed, orts, and abomasal samples were ground to pass through a 1 mm screen using a Wiley mill (Authur H. Thomas, Philadelphia, PA) and analyzed for NDF and ADF (Robertson and Van Soest, 1985), ash (AOAC, 1984), DM, and total N (FP-528, Protein/Nitrogen Analyzer, Leco Corp. St. Joseph, MI). Ether extract of feed and orts was determined using soxhlet extraction with petroleum ether. Reticuloruminal nutrient digestibility and abomasal flow was calculated using indigestible ADF (IADF) as a marker (Henderson et al., 1985). The IADF of feed, orts, and abomasal samples were determined using a Daisy II 200 Rumen Fermentor (Ankom Technology Co., Fairport, N.Y.) (Anonymous, 1998).

Feed and abomasal samples were refluxed in 6 N HCL under 99.9% pure N_2 atmosphere for 24 h and then dried under reduced pressure (Amos et al., 1976). Hydolysates were resuspended in a diluting buffer and analyzed for AA acids using a Beckman 1600 automated AA analyzer (Beckman Instruments, Inc.,

Fullerton, CA) with norleucine as an internal standard. Purine concentrations in the abomasal and bacterial isolate samples were determined using the procedure of Zinn and Owens (1986).

Data were analyzed using the general linear models (GLM) procedure of SAS (SAS, 1996). Using the model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + E_{ijkl}$$

where Y_{ikkl} = observation, μ = population mean, T_i = treatment effect (i = 1 to 4), P_j = period effect (j = 1 to 4), S_k = steer effect (k =1 to 4), and E = residual error. Rumen VFA and pH data were analyzed using the repeated measures feature of Proc Mixed. The model included steer, treatment, period, and hour, and the interaction between treatment and hour. Linear, quadratic, and cubic contrasts of FFA content were included in all analyses.

RESULTS AND DISCUSSION

Chemical composition of experimental diets was similar (Table 3.1). Chemical composition and FA profile of WCS contained in the treatment diets is presented in Table 3.2. The only marked difference in the two lots of WCS was a lower slightly higher EE, C16:0, and C18:0 and a slightly lower C18:1 and C18:2 concentration in the 18% FFA WCS.

There was no difference in DM and OM intake among treatments, which averaged 9.4 and 8.7 kg/d, respectively (Table 3.3). Previous research by Plascencia et al., (1999) reported increased palatability of feedlot diets containing grease high in FFA. Apparent ruminal DM digestibility (kg/d) tended to be

lowest for the 11.3% FFA WCS treatment and highest for the 8% FFA WCS treatment, resulting in cubic ($P < 0.08$) effect. There was a quadratic trend ($P< 0.08$) for the percentage of DM digested in the rumen due to lower digestibility for the 11.3% FFA WCS treatment. There were no differences among treatments in flow of DM or OM to the abomasum. Intake of NDF decreased linearly ($P < 0.005$) with increasing dietary FFA level. Apparent ruminal digestion of NDF was similar for all treatments. Equal amounts of NDF were digested in the reticulorumen for all treatments, resulting in a trend for a higher ($P < 0.07$) proportion of NDF consumed being digested as FFA of WCS increased because of a corresponding linear ($P < 0.01$) decline in NDF intake. This is in direct contrast to the findings of Reddy et al., (1994) who reported decreased NDF digestion when extruded soybeans were added to *in vitro* ruminal fermentations. However, it should be noted that rupturing the soybean micelle by extrusion allows rapid exposure of microbes to fatty acids, whereas the physical form of WCS causes more gradual dispersion of fatty acids. Intake of ADF was also similar among treatments and averaged 2.63 kg/d. Ruminal digestion of ADF averaged 1.60 kg/d and was similar among treatments. Ruminal digestibility of ADF was similar among treatments. Keele et al., (1989) did not observe a difference in rumen ADF digestibility when WCS or extruded soybeans were included in diets fed to non-lactating Holstein cows.

Total N intake exhibited a cubic ($P < 0.05$) response to treatment due to higher intakes for the 8 and 14.7 % FFA WCS treatments than for the 11.3 and 18% FFA WCS (Table 3.4). Because the diets were isonitrogenous, this increase

in N intake is primarily due to non-significant increases in DMI and small difference in dietary N content. Total flow of N to the abomasum was not different among treatments, despite small differences in intake. Previous research has shown decreased rumen protein digestibility, increased N flow, and decreased rumen ammonia concentration in response to supplemental lipid (Ikwuegbu et al., 1982). Flow of microbial N (g/d) exhibited a cubic ($P < 0.03$) response to treatment, with microbial N flow highest for the 14.7 % FFA WCS and lowest for the 18% FFA WCS. Keele et al., (1989) reported a decrease in the flow of microbial N to the duodenum when oilseeds were fed. Additionally, Avila et al., (2000) reported increased microbial nitrogen flow when yellow grease (2.90% FFA) was substituted for tallow (5.50% FFA). Flow of non-microbial N was not different among treatments but was numerically lower for the diet containing 14.7% FFA WCS. A cubic response ($P < 0.005$) was observed for microbial efficiency because of apparent efficiencies with 18% FFA WCS.

Several studies have reported improved microbial efficiency with the addition of fat to the diet in conjunction with decreases in feed deamination (Czerkawski et al., 1975; Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984; Boggs et al., 1987). These authors have postulated that these results are due to an increase in the solids dilution rate and/or reduced N recycling due to lowered protozoal population numbers. Apparent microbial efficiency was lower in this study than most of the data available for comparison; however it should be noted that those experiments were performed using lactating cows with much higher DMI than the steers used to collect the data presented here. Murphy et al.,

(1987) reported apparent microbial efficiencies of 17.3 in lactating Swedish Red and White cows consuming approximately 14.5 kg/d of a 40% concentrate 60% hay diet. This efficiency increased to 26.6 with the addition of 2 kg/d of crushed rapeseed to the diet. Avila et al., (2000) reported a non-significant increase in apparent microbial efficiency from 20.1 to 27.6 when tallow was replaced with yellow grease in lactating dairy cows consuming greater than 23 kg DM/d. In the same study, true microbial efficiency increased from 16.45 to 22.2 with the substitution of tallow with yellow grease.

Average pH decreased linearly ($P < 0.05$) with increasing levels of FFA from WCS (Table 3.5). Ruminal pH over time is presented in Figure 3.1. Rumen pH at 3 h postfeeding decreased linearly ($P < 0.01$) with increasing FFA concentrations in WCS. Ruminal pH was lower at 12 h postfeeding for the 11.3 ($P < 0.05$) and 14.7% ($P < 0.002$) FFA WCS treatments and tended to be lower for the 18% FFA WCS treatment in relation to the 8% FFA WCS treatment. There was no difference among treatments in pH at 6, 9, 12, 15, and 21 h post A.M. feeding. Maximum and minimum rumen pH was not affected by treatment.

Total ruminal VFA responded cubically ($P < 0.002$) to treatment with the 11.3% FFA treatment lower than the other three treatments (Table 3.5). Molar percentages of acetate were not different among treatments and averaged 66.5 %. Propionate responded cubically ($P < 0.05$) to treatment, with higher concentrations for the 8 and 14.7% FFA WCS treatments. Butyrate concentrations were numerically lower with increasing dietary FFA level. Keele et al., (1989) reported that increasing levels of extruded soybeans decreased molar proportions

of butyrate and increased molar proportions of propionate in *in vitro* fermentations. Avila et al., (2000) reported that supplemental fat in the diet from yellow grease or tallow tended to increase acetate concentration, tended to decrease butyrate concentration, and increase acetate:propionate.

Increasing FFA from WCS resulted in a linear decrease ($P < 0.0002$) in isobutyrate concentrations. Ruminal isovalerate and valerate concentrations were similar among treatments. Ruminal A:P increased linearly ($P < 0.02$) with increasing dietary FFA. Previous researchers have reported increased ruminal A:P with the addition of WCS to the diet (Moody and Cook, 1961; Moody and Barnes, 1966). Ruminal fluid branched chain VFA concentrations were numerically higher with the 14.7% FFA WCS and the 18% FFA WCS had numerically lower branch chain VFA. The changes in rumen VFA concentrations reported from this study are not indicative of reduced fiber digestion, but may reflect changes in rumen N metabolism.

The AA concentration of the experimental diets is presented in Table 3.7 and flow of AA to the abomasum are reported in Table 3.6. No differences were observed among treatments in flow of isoleucine, leucine, phenylalanine, histidine, and lysine flow to the abomasum. Arginine flow tended ($P < 0.10$) to be lower for the 18% FFA WCS treatment. Threonine flow tended ($P < 0.14$) to be higher for the 11.3 and 14.7% FFA WCS treatment, and lower for the 18% FFA WCS. Abomasal valine flow was higher ($P < 0.05$) for the 11.3% FFA WCS, and lower for the 18% FFA treatment. Total EAA tended ($P < 0.06$) to be higher for 11.3% FFA WCS and tended to be lower for 18% FFA WCS than 8 % FFA WCS,

and the overall treatment effect tended to be quadratic ($P < 0.11$). Flow of glycine, alanine, and cysteine were similar among treatments. Abomasal flow of aspartate tended ($P < 0.14$) to increase in steers fed the 11.3 and 14.7% FFA WCS treatment, and tended to decrease for steers fed the 18% FFA WCS treatment. The effect of increasing dietary FFA levels on serine flow was quadratic ($P < 0.05$). Glutamate flow tended to be quadratic ($P < 0.09$). Proline flow was higher ($P < 0.05$) for the 14.7% FFA WCS treatment than for the 18% FFA WCS treatment. Tyrosine flow tended ($P < 0.15$) to be highest for the 11.3 and 14.7% FFA WCS treatments. Total NEAA tended to exhibited a quadratic ($P < 0.10$) response because of higher NEAA flow for 14.7% FFA WCS and lower NEAA flow for the 18% FFA WCS, than the other two treatments. Total AA flow tended to have a quadratic ($P < 0.10$) response to treatment.

Concentrations of EE were similar among diets so any changes in rumen metabolism would be expected to be from form of the FA. Changes in nutrient intake and digestibility were observed. Changes were also observed AA flow and microbial efficiency. Changes in ruminal fluid branched chain VFA concentrations were observed for the 14.7% FFA WCS which correspond to a decrease in flow of non-microbial N and a trend for increased NH_3 flow (Table 3.7), which are all indicative of increased dietary AA deamination. However, a similar effect was not seen for the 18% FFA WCS. Instead the 18% FFA WCS treatment group had numerically lower branched chain VFA concentration and numerically higher non-microbial N flow, which is more indicative of reduced feed AA deamination. Reduced AA deamination has been reported with

antibiotic growth uncouplers (Newbold et al., 1990). Other effects reported for antibiotic growth uncouplers, such as lower acetate production, higher propionate production and lower A:P ratios were also not observed in the data from this study.

This data did not indicate reduced fiber digestion from addition of high FFA WCS to the diet. The changes in microbial CP production and microbial efficiency indicate that high FFA WCS may have an effect on rumen fermentation when FFA concentrations reach 18%. However, many of the trends were cubic or quadratic and it is difficult to predict what effect these changes might have on lactating dairy cow production. In addition, the changes in microbial N production observed were offset by an apparent reduction in feed protein deamination. There was no effect of high FFA WCS on flow of lysine and methionine to the abomasum; however, the 18% FFA WCS reduced ($P < 0.06$) total flow of EAA to the abomasum which could have a negative impact on protein synthesis in the mammary gland in high producing dairy cows.

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Table 3.1. Ingredient and partial chemical composition of total mixed ration containing whole cottonseed (WCS) with increasing concentrations of free fatty acids (FFA).

	% FFA in diet			
	8	11.3	14.7	18
Ingredient	----------% of DM----------			
Wheat silage	44.9	44.9	44.9	44.9
Ground corn	24.5	24.5	24.5	24.5
8% FFA WCS	12.5	8.3	4.2	-
18% FFA WCS	-	4.2	8.3	12.5
Soybean meal 48% CP	9.2	9.2	9.2	9.2
Protein supplement[1]	3.8	3.8	3.8	3.8
Mineral premix[2]	2.1	2.1	2.1	2.1
Chemical Composition Diet				
DM	41.52	39.09	41.54	42.09
CP	19.93	19.43	19.55	19.36
NDF	48.80	48.21	48.30	48.94
ADF	28.66	28.89	29.79	29.29
EE	6.07	6.01	5.91	5.95
NE_l, Mcal/kg DM[3]	1.7	1.7	1.7	1.7

[1]Protein supplement was composed of 60% menhaden fish meal and 40% distillers grains with solubles.
[2]Premix contained 34.4% CP from urea 86.7% ash; 24.50% Ca; 3.68% P; 1.27% Mg; 0.08% K; 3.03% Na; 4.60% Cl; 0.31% S; 11.67 ppm Co; 665 ppm Cu; 4,622 ppm Fe; 58 ppm I; 2,039 ppm Mn; 14.69 ppm Se; 1,943 Se, 133,070 IU/lb Vit A; 53,230 IU/lb Vit D; and 665 IU/lb Vit E.
[3]Calculated using NRC (2000) values.

Table 3.2. Partial chemical composition of whole cottonseed (WCS) with increasing concentrations of free fatty acids (FFA).

	% FFA in WCS	
	3	18
Ingredient	---------- % of DM ----------	
DM	88.8	88.0
CP	26.7	25.3
NDF	43.2	43.3
ADF	30.3	30.8
EE[1]	17.0	16.2
FA Profile	---------- % of FA ----------	
C10:0	0.1	< 0.01
C14:0	0.7	0.6
C16:0	22.0	23.1
C16:1	0.5	0.5
C18:0	1.0	2.4
C18:1	16.0	14.6
C18:2	54.3	52.6
C20:0	0.2	0.3
C18:3	0.2	0.2
C9t11	0.06	0.05

[1] Ether extract.

Table 3.3 Effects of increasing levels of free fatty acids (FFA) from whole cottonseed (WCS) on nutrient intake, abomasal flow, and reticuloruminal digestibility in Holstein steers.

	% FFA					Contrasts		
Item	8	11.3	14.7	18	SE	Linear	Quad ratic	Cubic
DM								
Intake, kg/d	9.67	9.21	9.32	9.23	0.27	0.39	0.48	0.51
Apparent ruminal digestion, kg/d	4.89	4.13	4.74	4.71	0.17	0.65	0.07	0.08
DMD, %	50.47	44.88	50.90	51.06	1.37	0.15	0.08	0.12
Abomasal Flow	4.79	5.09	4.58	4.53	0.20	0.21	0.36	0.61
OM								
Intake, kg/d	8.96	8.57	8.75	8.61	0.25	0.55	0.63	0.37
Abomasal flow	4.79	5.09	4.58	4.53	0.20	0.21	0.36	0.61
NDF								
Intake, kg/d	4.84	4.42	4.48	4.45	0.14	<0.005	0.36	0.09
Apparent ruminal digestion, kg/d	3.10	2.82	2.98	3.09	0.13	0.76	0.19	0.57
% of NDF intake	64.45	63.88	66.78	69.44	1.52	0.07	0.25	0.31
ADF								
Intake, kg/d	2.65	2.50	2.60	2.57	0.14	0.90	0.65	0.56
Apparent ruminal digestion, kg/d	1.64	1.52	1.61	1.64	0.9	0.77	0.40	0.59
% of ADF intake	61.98	60.37	61.89	63.89	1.40	0.27	0.24	0.90

Table 3.4 Effects of increasing levels of free fatty acids (FFA) from whole cottonseed (WCS) on intake, passage, and digestibility of total N and nitrogenous compounds in the reticulorumen of Holstein steers.

Item	% FFA				SE	Contrasts		
	8	11.3	14.7	18		Linear	Quadratic	Cubic
N intake, g/d	316.87	303.49	318.67	301.66	8.01	0.31	0.49	0.01
Flow to abomasum:								
Total N, g/d	192.18	198.38	190.20	200.56	10.65	0.81	0.59	0.55
% of N intake	62.93	67.03	62.15	66.98	2.33	0.68	0.19	0.88
Microbial N, g/d	66.14	63.23	75.98	56.81	2.79	0.53	0.04	0.03
Non Microbial N, g/d	126.04	135.14	114.22	143.74	12.53	0.72	0.26	0.44
Microbial N synthesis efficiency, g N / kg OM digested								
Apparent	15.03	16.60	17.70	12.88	0.56	0.14	0.07	<0.01
True	17.70	20.03	21.45	14.77	0.84	0.16	0.09	<0.01

Table 3.5. Effect of increasing levels of free fatty acids (FFA) from whole cottonseed (WCS) on rumen VFA concentrations.

	% FFA				
Item	8	11.3	14.7	18.0	SE
pH[a]	6.34	6.27	6.20	6.23	0.02
Total VFA[b]	97.24	107.33	93.52	99.47	2.38
	---------- Molar Proportion----------				
Acetate	66.30	64.32	68.12	67.28	1.34
Propionate	18.57	17.98	18.63	17.44	0.39
Butyrate	11.14	11.04	10.89	10.71	0.26
Isobutyrate[a]	2.16	2.20	1.98	1.47	0.12
Isovalerate	2.09	1.97	2.34	1.92	0.22
Valerate	1.75	1.20	1.96	1.55	0.30
Total BCVFA[1]	6.00	5.38	6.28	4.95	0.59
A:P[c]	3.60	3.59	3.69	3.90	0.09

[a] Linear response ($P < 0.05$).
[b] Cubic response ($P < 0.05$).
[c] Linear response ($P < 0.02$).
[1] Branched chain VFA.

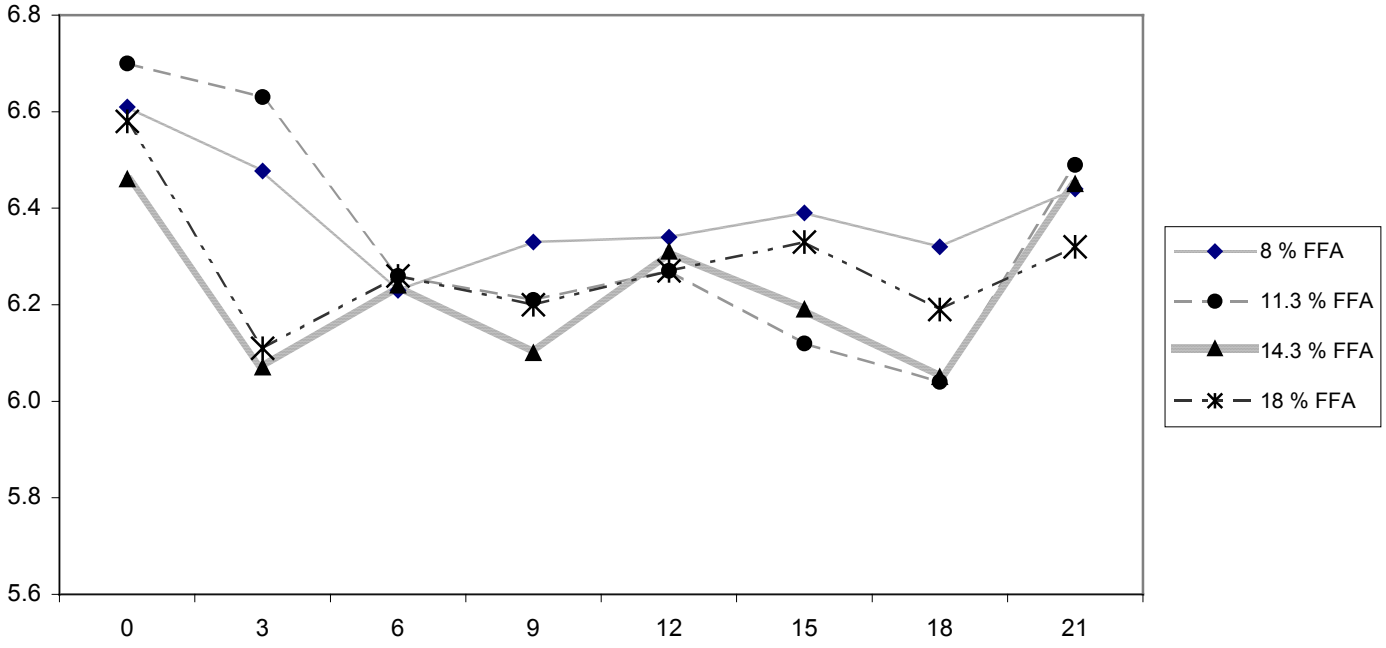
Table 3.6. Grams of amino acids reaching the abomasum of Holstein steers fed diets containing increasing levels of free fatty acids (FFA) from whole cottonseed (WCS).

	% FFA				
	8	11.3	14.7	18	SE
	g AA/ d				
EAA					
Thr	44.08	46.62	48.98	41.73	2.50
Val	41.15	45.89	40.23	35.29	2.06
Met	21.01	28.98	24.11	23.22	3.38
Ile	46.66	47.85	49.72	44.72	3.20
Leu	78.94	82.28	84.66	74.99	4.41
Phe	53.34	54.23	51.42	49.73	2.26
His	20.84	21.71	21.55	20.02	1.01
Lys	63.21	65.81	63.74	60.12	2.95
Arg	49.27	51.75	50.45	44.38	2.40
Total EAA	374.42	398.51	385.87	352.48	14.45
NEAA					
Tyr	37.38	39.21	41.33	35.05	2.29
Cys	10.55	107.49	110.75	100.43	7.41
Asp	89.45	95.26	102.03	84.93	5.47
Glu	129.38	136.68	145.47	122.07	7.26
Gly	48.07	49.96	52.60	45.52	2.85
Ser	46.45	49.17	52.40	43.49	2.01
Pro	36.35	37.99	40.83	32.36	2.09
Ala	58.42	61.30	64.79	55.78	3.53
Total NEAA	456.05	480.31	510.52	429.22	25.68
Total AA	874.54	925.44	945.38	823.44	41.95
NH_3	14.69	16.48	22.41	16.09	1.90

Table 3.7. Amino acid intake of steers fed diets containing increasing levels of free fatty acids (FFA) from whole cottonseed (WCS).

	% FFA			
Item	8	11.3	14.7	18
	---------- g AA/ d ----------			
EAA				
Thr	52.2	50.7	47.5	47.1
Val	6.8	3.7	3.7	3.7
Met	23.2	22.1	19.6	20.3
Ile	58.0	57.1	52.2	49.8
Leu	112.1	105.9	98.8	93.2
Phe	76.4	59.9	52.2	63.7
His	32.9	43.3	49.4	28.61
Lys	65.8	52.5	60.6	64.6
Arg	53.2	69.1	68.0	73.8
Total EAA	510.6	464.2	452.0	444.9
NEAA				
Tyr	23.2	22.1	19.6	20.3
Cys	43.5	39.6	33.6	34.2
Asp	118.9	111.4	103.5	103.4
Glu	216.6	189.7	182.7	185.5
Gly	67.7	60.8	63.4	61.8
Ser	64.8	57.1	55.0	58.1
Ala	91.9	103.2	92.3	81.2
Pro	78.3	80.1	69.0	69.2
NEAA	704.9	664.04	618.8	613.8
EAA	510.6	464.1	452.0	444.9
Total AA	1215.5	1128.23	1070.9	1058.7

Figure 3.1. Effects of 8, 11.3, 14.7, and 18 % FFA whole cottonseed on rumen pH over time.



CHAPTER 4

SUMMARY

Results of this experiment suggest that feeding whole cottonseed containing up to 12% FFA does not depress intake or milk production. Increasing the concentrations of FFA from WCS magnified the depression of *de novo* mammary gland fatty acid synthesis often seen with WCS feeding. However, there were no differences among treatments in milk long chain fatty acids. Therefore, the addition of high FFA WCS would not impact milk quality. In addition, there were no negative effects of feeding high FFA WCS on total tract fiber digestibility. Differences in milk fatty acid composition, plasma AA concentrations, and nutrient digestibility suggest potential changes in rumen fermentation; however, these changes did not negatively impact the production measures tested during this study. Therefore, whole cottonseed containing up to 12% FFA oil in seed, can be fed to lactating dairy cattle at up to 12.5% of diet DM without detrimental effects.

Feeding WCS up to 18% FFA, oil in seed caused small but significant changes in nutrient intake and digestibility. Changes were also observed AA flow and microbial efficiency. Changes in ruminal fluid branched chain VFA concentrations were observed for the 14.7% FFA WCS which correspond to a decrease in flow of non-microbial N and a trend for increased NH_3 flow (Table 3.7), which are all indicative of increased dietary AA deamination. However, a similar effect was not seen for the 18% FFA WCS. Instead the 18% FFA WCS treatment group had numerically lower branched chain VFA concentration and

numerically higher non-microbial N flow, which is more indicative of reduced feed AA deamination. Reduced AA deamination has been reported with antibiotic growth uncouplers (Newbold et al., 1990). Other effects reported for antibiotic growth uncouplers, such as lower acetate production, higher propionate production and lower A:P ratios were also not observed in the data from this study.

This data did not indicate reduced fiber digestion from addition of high FFA WCS to the diet. The changes in microbial CP production and microbial efficiency indicate that high FFA WCS may have an effect on rumen fermentation when FFA concentrations reach 18%. However, many of the trends were cubic or quadratic and it is difficult to predict what effect these changes might have on lactating dairy cow production. In addition, the changes in microbial N production observed were offset by an apparent reduction in feed protein deamination. There was no effect of high FFA WCS on flow of lysine and methionine to the abomasum; however, the 18% FFA WCS reduced ($P < 0.06$) total flow of EAA to the abomasum which could have a negative impact on protein synthesis in the mammary gland in high producing dairy cows.