ABBY ANN GAUTREAUX
The Effects of Supplemental Ruminally Undegradable Protein on Early Lactation Cows Fed Fat in the Form of Whole Cottonseed
(Under the Direction of HENRY E. AMOS)

Four multiparous Holstein cows were used in a 4 x 4 Latin square experiment with a 2x2 factorial arrangement of treatments. The objective of the experiment was to evaluate the effects of supplemental ruminally undegradable protein (RUP) on milk production, milk protein percentage and yield of cows receiving added fat in the form of whole cottonseeds (WCS). An evaluation of amino acid (AA) uptake by the mammary gland was also made. Treatments were 1) control, 2) WCS, 3) RUP, 4) WCS + RUP. The addition of WCS decreased milk protein production Supplemental RUP did not reverse the milk protein depression but the total effects may have been masked due to differences in CP intake.

INDEX WORDS: Fat, Whole Cottonseed, Milk Protein, Rumen Undegradable Protein

THE EFFECTS OF SUPPLEMENTAL RUMINALLY UNDEGRADABLE PROTEIN ON EARLY LACTATION DAIRY COWS FED FAT IN THE FORM OF WHOLE COTTONSEED

by

ABBY ANN GAUTREAUX

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ABBY ANN GAUTREAUX

Approved:

Major Professor: Henry E. Amos

Committee: Mark A. Froetschel
John K. Bernard

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
December 2001

To Mom, Dad, Greta, Jake, Sue and Jody.

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CHAPTER 1

INTRODUCTION

Ruminants are unique animals that have four compartments to their stomach. The first three compartments, the rumen, reticulum and omasum, provide an optimum environment for a variety of anaerobic microorganisms including bacteria, protozoa, fungi and yeast. This population of microorganisms allows the ruminant to carry out certain selected biochemical processes such as fermentation of structural and nonstructural carbohydrates (SC and NSC, respectively) and synthesis of microbial protein, B-vitamins and volatile fatty acids (VFA), among other functions. These fermentation and synthetic processes supply energy metabolites, proteins and vitamins for use by the ruminant animal.

One of the primary functions of the rumen microorganisms is the anaerobic fermentation of SC and NSC. These carbohydrates are fermented to volatile fatty acids (VFA) including acetic, propionic and butyric acids, which enter specific points of the glycolytic pathway or the Kreb's Cycle and are used for ATP production, long-chain fatty acid and cholesterol synthesis or gluconeogenesis by the ruminant. Synthesis of microbial crude protein (MCP) is a function of the microorganisms of major importance to the host animal. Synthesis of MCP from amino acids derived from the fermentation end products of dietary protein and non-protein nitrogen (NPN), and VFA from carbohydrate fermentation. The microorganisms use the products of carbohydrate fermentation and alpha-ketoacids, or certain VFA from the breakdown of dietary protein and energy (high-energy phosphate) to synthesize MCP from amino acids and NPN.

Although microbes are able to synthesize MCP and VFA, these products are insufficient to meet the needs of lactating animals when maximum productivity is desired. Also, the composition of the animal's diet affects the amount of protein synthesized and ratio of VFA produced, it is essential that diet composition, through selective formulation, be taken into account to provide the animal with a balanced ration that will complement rumen synthesis and meet their needs. Providing dairy cattle with balanced rations is extremely important and challenging. The goal in feeding dairy cattle is to produce large volumes of high quality milk with maximum percentages of protein, fat and lactose as economically as possible. Obviously, as more milk is produced, more total revenue is received from the sale of the product, but in today's changing marketing systems producers are being forced to concentrate on factors other than quantity of milk produced. Component pricing is now being used widely in the United States where either milk prices are based on percentages of either fat and (or) protein or there may be premiums involved with higher than average percentages of components in milk. In fact, of the 11 Federal Milk Marketing Orders, 7 of the orders use milk component pricing and the remaining 4 are strictly a fluid milk market. This pricing system is giving producers in certain areas an incentive to increase the amount of solids in milk, mainly fat and protein.

Milk yield and composition can be influenced by a number of factors including genetics, stage of lactation, environmental conditions and diet. Of these, diet is the easiest to control and manipulate. What cows eat has a direct effect on the yield and composition of their milk. With intensive genetic selection, proper ration formulation, and the economic use of resources, production and efficiency can be improved. In my

research, I will be looking at dietary factors affecting milk protein composition, mainly dietary protein and fat.

CHAPTER 2

REVIEW OF LITERATURE

Fat in the Diet

Fat from whole oilseeds, tallow or oil is sometimes added to rations to increase the energy density (Mcal NEL/kg diet). Fat is a more concentrated source of energy than cereal grains, and when fed to dairy cows may decrease the feeding of starches from grain. There are also critical times, such as in early lactation, in which a dairy cow's energy requirement is not met due to limited dry matter intake (DMI) where supplemental fat can assist in overcoming energy intake problems. Although the feeding of fat has its benefits, there are a number of negative effects that may occur with the addition of fat to diets, including depressed DMI, decreased rumen fermentation of fiber, and decreased milk protein and milk fat percentages.

Fat: Effects on Rumen Fermentation and Digestibility

One common problem that is often observed with the addition of fat to diets is decreased rumen fermentation of SC resulting in decreased digestibility and production of VFA. Devendra and Lewis (1974) presented some explanations for this decreased fermentation including the possibility that fats coat fiber and prevents microbial digestion. Since fats are hydrophobic in nature, they will usually bond to some amphiphilic substance, possibly feed particles or microbes, and prevent direct attachment of microbes to feed particles thus reducing the digestibility of SC (Jenkins, 1993). A second possible explanation for the decrease in digestibility is the possibility of fat

toxicity to the microbial population in the rumen. It has been shown that certain fatty acids inhibit growth of particular rumen bacteria in pure cultures (Henderson 1973, Maczulak 1981). Hydrophobic regions of lipid bilayers in cell walls of the microorganisms allow the long chained fatty acids to bind, disrupting the membrane and its function. Maczulak et al. (1981) studied the effects of palmitic, stearic, oleic and vaccinic acids on several species of rumen bacteria, all of which are major cellulose or starch digesters. Palmitic acid reduced growth of *Selenomonas ruminantium* and *Bacteroides succinogenes* (now *Fiberbacter succinogenes*) and inhibited the growth of *Bacteroides ruminicola*. Stearic acid had a lesser effect even only when applied at higher levels. Oleic acid at 0.0005% inhibited growth of gram-positive cellulolytic ruminococci. *Butyriovibrio Fibrisolvens* was also inhibited by stearic acid and higher levels of palmitic acid. Vaccinic acid at .01% inhibited *Ruminococcus albus* and *Ruminococcus Flavefaciens*. When cellulose was added to the cultures, inhibition of the microbes was reduced. It has also been reported that when fiber or feed particles were added to cultures, binding of the FA to the microbes was reduced (Jenkins, 1993). This indicates the necessity of sufficient fiber in rations for optimum growth and survival of microbes. The current recommendation for fiber, according to the NRC (2001), is at least 25% neutral detergent fiber should be included in rations.

Effect of Fat on Dry Matter Intake

Another effect of fat may be a decrease in DMI as noted in many studies including Grant and Weidner (1992), who fed whole soybeans at 11.6% of the dietary DM and reported a 7.1% decrease in DMI. Pantoja et. al (1994) reported a 14% decrease in DMI as unsaturated FA content of the diet increased from a saturated fat source with an iodine

value of 17.8 to a unsaturated source with an iodine value of 84.1 . The current recommendation for feeding added fat varies between 3 and 5% of the diet (Palmquist and Jenkins, 1980); at these lower levels there should be no adverse effect on rumen digestibility or DMI.

Saturation versus. Unsaturation

There are certain characteristics of fat that may cause these disruptions including the degree of saturation. Unsaturated FA are more toxic to RMO than saturated FA (Henderson 1973, Maczulak 1981). Unsaturated FA seem to have a greater effect than saturated FA on rumen fermentation and digestibility (Elliot et al.1997, Pantoja et al. 1994) by changing total and specific VFA production. Pantoja et al. (1994) fed three types of fat varying in degree of saturation including saturated tallow, tallow and an animal-vegetable blend with determined iodine values of 17.8,55.8, and 84.1, respectively. Changes in ruminal fermentation from addition of fats resulted in an increase in acetate and decrease in propionate as unsaturation decreased. There was also a decrease in DMI and in ruminal digestion of NDF with increased unsaturation. In a similar study, Elliot et al. (1997) fed tallow, partially hydrogenated tallow, and hydrogenated tallow with respective iodine values of 51.5,30.7 and 6.9. As unsaturation decreased there was an increase in total VFA production and acetate with a decrease in propionate. When compared to the control diet, the diet containing the hydrogenated tallow was the most similar in ruminal fermentation suggesting that fat source was the most inert. The results from these two studies agree with others where unsaturated fats have commonly had an effect on microbes to decrease production of acetic acid and methane but to increase the production of propionic acid (Jenkins 1993, Chalupa 1984).

The recommendation of the degree of saturation of fat added to rations is still unclear. Pantoja et al. (1994) recommends a source with saturation between tallow and saturated tallow, 17.8-55.8 iodine value, while Elliott et al. (1997) recommends a highly saturated tallow, approximately with a 6.9 iodine value.

Digestion of Fats

When fats are consumed with or in the diet, they are extensively hydrolyzed, up to 95% to FA and glycerol, by microbial lipases in a process known as lipolysis. Specifically, linseed oil and olive oil are hydrolyzed 95% and 70%, respectively (Garton et al., 1961). The proportion of unsaturated and saturated FA released from triglycerides is dependent on the fat source. Unsaturated FA are quickly hydrogenated by other microbes to saturated FA through biohydrogenation (Jenkins, 1993). The source of H for biohydrogenation may be either water or H produced from fermentation of carbohydrates. Although the majority of H produced in the rumen is used for VFA production and the reduction of CO_2 to CH_4, 1-2% of the H is used for biohydrogenation of FA (Czerkawski and Clapperton, 1984). *Butyrivibrio fibrosolvens,* one anaerobe that produces enzymes for biohydrogenation, uses water for an H source (Rosenfeld and Tove, 1971). The concentration of unsaturated FA in the rumen depends on the fat source supplied, the rates of lipolysis and biohydrogenation, and the formation of fatty acid salts (Jenkins, 1993). Gersen et al. (1983,1986,1988) determined that the rates of lipolysis and biohydrogenation are dependent upon maturity of the forage in the diet, the N content and particle size. Gersen et al. (1983) studied the effects of dietary N on lipolysis and hydrogenation by feeding sheep five high starch diets varying in N content from 0.72 to 3.72%. A linear relationship showed that as N increased so did the rate of lipolysis.

Hydrogenation was highest at moderate N levels between 1.72 and 2.47% N on a DM basis. To study the effects of forage maturity, Gersen et al. (1986) grazed sheep on three different ryegrass pastures varying in stage of maturity with CP levels of 13.8% for the immature, 8.1 for mature and 5.5% for senescent. Rates of lipolysis and hydrogenation decreased with maturity. In an in vitro study, hay was cut at lengths ranging in particle size from 0.1 to 2 mm and incubated in rumen digesta from sheep. The rates of lipolysis and hydrogenation were highest at particle lengths of 1 to 2mm (Gersen et al, 1988). It has also been reported that grain feeding may reduce lipolysis and hydrogenation due to a decrease in rumen pH (Lantham et al., 1972).

Influence of Added Dietary Fat on Milk Yield

Adding fat to the diets of lactating cows affects both milk yield and composition. Due to an increased energy density of the ration, added fat often supports a higher level of milk production as reported in studies by Casper and Schingoethe (1989), Christensen et al. (1994), Kim et al.(1991), and Weigel et al.(1997). Casper and Schingoethe (1989) reviewed studies where on an average the ether extract was increased from 2.4 to 5.3 with the addition of whole oilseeds, including sunflower seeds and extruded soybeans, and reported a 7.3% increase in milk production. The NE_L of the diets increased from 1.74 to 1.79 Mcal/kg of DM. Christensen et al. (1994) fed a 30% corn oil and 70% tallow mix to provide 5.4% of the total FA in the diet along with different levels of CP and reported an increase in milk yield for all diets containing added fat. The addition of the fat blend increased gross energy (GE) of the diet from 4.46 to 4.6 Mcal/kg of DM. Kim et al. (1991) fed extruded soybeans at 17% of DM on a 16% CP diet and reported increased milk yields from 33.0 to 35.8 kg/d. Weigel et al.(1997) fed 3.5% tallow, which increased

the GE content of the diet from 4.4 to 4.6 Mcal/kg, and increased milk production from 28.7 to 31.2 kg/d.

Influence of Added Dietary Fat on Milk Fat

The addition of fat has resulted in variable effects on milk fat composition. Bertrand et al. (1998) reported decreased milkfat percentages of 4.88 to 4.60 for cows fed diets containing whole cottonseed (WCS) at a rate of 12.9% of the TMR, which increased the ether extract from 2.0 to 4.4% of DM. On the other hand, Smith et al. (1981) reported an increase in milk fat percentage as the percentage of WCS in the diet increased from 5 to 15 and 25% of DM of the TMR with respective ether extract compositions of 3.01, 5.25, and 6.94% of DM. Milk fat concentration increased from 3.90% at 5% WCS to 4.52% at 25% WCS. Feeding other fat sources has resulted in increased milk fat percentages including Cant et al. (1993) who fed yellow grease and Christensen et al. (1994) who fed a blended fat. Cant et al. (1993) fed yellow grease at 4% of DM and increased milk fat production from 37.6 to 41.9 g/h. Christensen et al. (1994) used a blended fat source of 3% tallow and 30.1% high oil shelled corn to increase the ether extract from 3.5 to 6.8% which resulted in an increase of milk fat from 3.32 to 3.52%. The source of fat, saturated versus unsaturated, may be the reason for varied results seen on milk fat percentage with the addition of fat to rations. Milk fat depression occurs when the acetate: propionate ratio is lowered (Palmquist and Jenkins 1980), such as has been reported when unsaturated fats are fed (Chalupa 1984, Elliot 1997, Pantoja 1994). Changes in the VFA ratio (decreased acetate and increased propionate), cause adipose tissue to compete with mammary tissue for acetate and FA and to decrease the mobilization of adipose therefore reducing the FA available for milk fat synthesis (Davis and Brown, 1970). Once again, if

unsaturated fats could be provided without altering rumen fermentation, decreases in milk fat composition may be avoided. Calcium salts of FA and protected lipids are two possibilities to offset these effects of unsaturated fat. Palmquist and Jenkins (1980) note that the calcium level should be increased to 1% of DM to avoid milk fat depression and Smith et al. (1978) reported that feeding protected lipids increases milk fat percentage. Smith et al. (1978) fed diets containing 0, 15, and 30% protected tallow with respective ether extract values of 2.9, 8.4, and 14.3%. Milk fat percentage increased from 3.38 to 4.28 and 4.48%, respectively. The roughage content of the diet often effects milk fat composition. When cows are not getting sufficient fiber, alterations of VFA production and ratios and ruminal microflora population results in a decreased milk fat percentage (Latham et al., 1974). Adding fat to the diet may change the roughage: concentrate ratio and decrease roughage intake or if fat decrease DMI there could also be a decrease in the amount of roughage taken in.

Influence of Added Dietary Fat on Milk Protein

In an extensive review of literature, Emery (1978) reported that milk protein percentages increase .015 units/megacalorie of increased intake energy when supplied by grain or roughage. This does not seem to be the case if the energy is supplied by a fat source. Studies have repeatedly reported a decrease in milk protein percentages with the addition of fat to a diet. Emery (1978) reported that the addition of 4 to 12% fat usually decreased milk protein 0.1 to 0.3 percentage units and more specifically, Smith et al. (1981) fed WCS at 5, 15 and 25% of the DM of the TMR and noted that WCS at 15 and 25% decreased milk protein 0.1 percentage unit.

In one study (Bertrand et al., 1998), whole cottonseed was fed at 12.9% DM of the TMR to Jersey cows to increase the ether extract from 2.0 to 4.4%. This addition of WCS decreased the milk protein from 4.02 to 3.72%. It is often claimed that the reduction of milk protein percentage noted when fat is added is due to the dilution of the milk protein by an increased milk yield (Bertrand et al.,1998, Depeters and Cant, 1993). The diet is higher in NE_L and therefore supports a higher milk production, but milk protein synthesis does not keep up and the protein produced becomes diluted by the increased milk yield and milk protein percentage is decreased. In the study of Bertrand et al. (1998), the change in milk yield was not significant, indicating that some other mechanism(s) was responsible for the milk protein depression. DePeters and Cant (1992) proposed a formula to explain milk protein depression as a result of increasing fat intake of lactating cows. The formula they used is as follows: proportion of milk yield that contributes to the dilution of protein = (1- percentage increase in protein yield \ percentage increase in milk yield)*100. Using the formula with their own data where cows were fed 3.5% yellow grease, they concluded that only 34.5% of the change in milk yield was responsible for the milk protein depression. With this information researchers began looking for new explanations for the depression.

When cows are fed fat there is often a reduction in mammary gland uptake of amino acids (AA) as reported by Casper and Schingoethe (1989) and Cant et al. (1993a). This must be due to either a decrease in the supply of AA or the mechanism affecting the actual uptake of the AA. Both of the previous researchers reported a decreased arterial supply of AA. Uptake of AA has commonly been measured by the Fick equation: uptake of amino acids = arterial-venous difference of amino acids X mammary blood flow (Cant,

1993a). Mammary blood flow (MBF) is estimated using an indicator, possibly phenylalanine and tyrosine. These AA make good markers because their output in milk can be equated to mammary gland uptake. The Fick equation is based on the assumption of steady state conditions where there is no variation in blood flow, concentrations of arterial supply or uptake by tissues.

Blood flow is not only under central control, but also some local control where it is regulated by peripheral blood vessel vasodilators/vasoconstrictors release. Cant et al. (1993a) suggested that variations in MBF were results of local control and that changes are a response to vasodilator release, possibly adenosine, from the tissue itself into the interstitial fluid. Cows fed fat would have a higher energy concentration in blood and would decrease adenosine production, close precapillary sphincters, and decrease MBF. If this is the case, then it is understandable why milk protein composition decreases with added fat. First, energy content of the blood is increased, not AA content so decreased MBF may cause a reduction in the supply of AA reaching the udder. Also, if fat causes sphincters to close and ceases vasodilatation then there is a reduced surface area for AA transport. Another important point is that with the changes in MBF and AA concentration of the blood, the Fick equation may not be the best measurement for AA uptake.

Besides causing a reduction of blood flow to the mammary gland, fat may also have an indirect effect on other factors to complicate the problem of reduced substrates reaching the udder. Both Bovine Somatotrophin (BST) and to a less extent insulin have roles in the uptake of amino acids by the mammary gland (Brockman, 1986). Fat has been shown to decrease BST levels (Schneider, 1988) and more specifically, Cummins and Sartin (1987) showed that WCS decreases BST. Casper and Schingoethe (1989)

reported that the decrease in amino acid uptake may be due to endocrine regulation and potentially alleviated with the administration of BST. DePeters and Cant (1992) reported that BST also increased MBF going to the udder. If fat is decreasing MBF and also BST, resulting in a further reduction of MBF, along with a decreased uptake of AA, it would be difficult for milk protein production to continue at the same level.

It would be beneficial to be able to feed unsaturated fats to animals and not experience their negative effects on rumen digestion and on milk protein. One reason may include the availability or cost of particular fat source. WCS is an available source of fat often fed in the south and is usually cheaper than saturated tallow. Also, there has been an increase in consumer demand of products with a lowered proportion of saturated FA. It would be beneficial for both producers targeting a health conscious market and those consumers who are concerned about their well-being, to be able to feed unsaturated fats because it results in increased unsaturation of meat and milk (Bines et al., 1978, Mills et al., 1979).

Feeding Ruminally Inert Fats or Calcium Salts

One possibility to increase the passage of unsaturated FA through the rumen is the addition of calcium salts that bind to the carboxyl group of the FA and remove it from solution, making these FA unable to attach to microbes or other particles. Protected lipids may be another method of delivering unsaturated FA through the rumen allowing them to escape hydrogenation. Unsaturated FA can be coated with protein and then treated with formaldehyde to pass through the rumen without becoming saturated (Scott et al., 1971). The HCl in the abomasum degrades the protein coat and allows the unsaturated FA to be absorbed through the intestinal wall.

Dietary Protein

Another possible solution to reversing milk protein depression may be altering the dietary protein content. Supplying an animal with adequate dietary protein is essential for optimum production. The amount of protein required may be dependent on a number of factors including the size of the animal, reproductive status, milk production and the level of protein degradation in the rumen (NRC, 1989). When protein enters the rumen it is either degraded by rumen microorganisms (RMO) to ammonia and alpha-keto acids or VFA, or it escapes rumen degradation and is digested and absorbed in the small intestines. Rumen microorganisms use the products of dietary protein degradation and energy from carbohydrate fermentation to produce MCP. The extent to which dietary protein is degraded is dependent upon a number of factors including the specific properties of the protein source, the retention time in the rumen, rate of proteolysis, level of feeding and processing effects (Clark et al., 1987, Satter, 1986, Tamminga, 1979). When discussing specific properties of protein, structure and solubility should be taken into effect. The structure of a protein will determine the access given to proteolytic enzymes for degradation (Satter, 1986). Extensive crosslinkages will decrease access and therefore decrease degradability. Also, the class of protein, whether it is an albumin, globulin, prolamine or glutelin, will affect its degradability. Prolamines and glutelins have a larger mass and disulfide bond crosslinkages that decrease their degradability (Clark et al., 1987). The retention time or time the protein is in the rumen, will increase degradation (NRC, 1985). Retention time can be influenced by variations among species, diet ingredients, particle size, and environmental temperature. The level of feeding may effect degradability by influencing retention time as shown by Tamminga (1979), who

increased DMI from 8.2 to 12.9 kg/cow/day to increase the amount of protein bypassing degradation. Other feeding factors may include roughage: concentrate ratio or environmental temperature. When feedstuffs are processed they are often exposed to heat. Heat from ensiling, drying, pelleting or extrusion may decrease the degradability of proteins by causing them to dehydrate, decrease the surface area and decrease area for microbial attack (Satter, 1986, Chalupa, 1974)). As heat increases, rumen undegradability increases but so does the intestinal digestion of proteins. If heating processing is used, it should be done to the point to where the increase in undegradable protein is not offset by a decrease in digestibility.

Feeding Rumen Undegradable Protein

There is a definite need for RUP in a diet of young ruminants and high producing cows. Ruminants have three sources of metabolizable protein including MCP, RUP from the diet and endogenous protein from tissue and enzymes. Microorganisms are only able to synthesize enough protein to support maintenance and lower levels of production (~20 kg milk/day) (NRC, 1989). For higher levels of production, protein must come from one of the other two sources, preferably RUP.

Feeding RUP can have many benefits including increased production, decreased CP requirement because the same amount of amino acids can be supplied by a protein source that has a higher RUP content, change the rate of body tissue mobilization during early lactation, and possibly maintain milk protein percentage and yield with decreased dietary CP. In a study by Wright et al. (1998) three levels of RUP were fed at 4.5, 14.9, and 29.1% of DMI. Both milk yield and protein yield increased linearly with the RUP supplementation. Milk yield increased from 22.4 to 26.3 Kg/d and milk protein from 0.69

to 0.83 kg/d when comparing the high and low supplements. By balancing for necessary amino acids and feeding high levels of RUP, the researchers were also able to reduce nitrogen waste. In another study by Kalscheur et al. (1999), CP and RUP levels were evaluated in early, mid, and late lactation cows. The researchers showed that feeding higher levels of RUP only had a beneficial effect in early lactation animals. The RUP levels were 35.5, 41.4, and 46.4% of total CP and milk production increased linearly with increasing RUP from 32.7 kg/d on the low RUP diet to 36.1 kg/d on the high RUP diet. .

Supplemental RUP could possibly maintain milk protein composition when fat is added to the ration. Researchers have seen variable results by changing the protein supplied, whether by addition of amino acids, amount of CP, or and increase in the supply of RUP. In a study by Kincaid and Cronrath (1993), WSC was used along with calcium salts of long chained fatty acids to increase the ether extract from 3.1 to 6.8%. They reported a drop in milk protein from 3.38 to 3.28%, a difference of 0.1%. The researchers tried to reverse the depression in milk protein percentage by supplying ruminally undegradable zinc methionine and lysine at 5.0 and 6.25 grams/day respectively, but recorded 3.10% milk protein and were unsuccessful.

Kim et al. (1991) added extruded soybeans to diets containing 15.7 and 17.5% CP to increase the ether extract from 2.6 to 5.5% and 5.1% respectively, and noted a slightly depressed milk protein percentage. In the diet containing 15.7% CP milk protein dropped from 2.92 to 2.88%, a difference of only 0.04%. The CP was increased to 17.5% with the addition of soybean meal to determine if supplying extra crude protein would reverse the depression and in this case it did not. The reported milk protein for this diet was 2.83%.

Bertrand et al.(1998) fed WCS and reported a depression in milk protein percentage. The milk protein depression was reversed by supplying 20 g of Smartamine M and 10 g of Smartamine ML/cow/d to the diet, which delivered 10 g each of Methionine and lysine postruminally. This addition of ruminally protected AA to the diet containing fat increased the milk protein from 3.72 to 4.18%, which was 0.16% higher than that of the control diet.

Conclusion

Both fat and protein are essential components of dairy rations that have the capability to affect milk production and composition. Feeding fat increases the energy density of rations, which helps to insure animals are meeting the energy needs in times of limited DMI. Supplemental fat has been shown to have negative effects on rumen digestibility and on milk protein composition. Protein is a necessity for both maintenance and production, and is often a limited nutrient in early lactation dairy cows. The majority of the dairy cow's protein supply comes from either MCP or RUP, in which the proportions of each are dependent upon a number of factors. Increasing the percentage of UIP in dairy rations has been an effective method used to increase milk production. It has also been used to overcome various problems such as a reducing body tissue mobilization in early lactation cows and more recently to reduce excretion of nitrogen waste into the environment. I am particularly interested in the use of RUP to reverse the depression of milk protein associated with the addition of fat to dairy rations. Previous studies, in which individual amino acids or RUP sources were supplied to dairy cows, have reported various results indicating the need for further research on the subject. If it were possible to feed an available and inexpensive fat source to increase energy intake and milk

production, along with an RUP source that supplied sufficient protein to maintain milk protein yield, it would be economically efficient.

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CHAPTER 3

THE EFFECTS OF SUPPLEMENTAL RUMINALLY UNDEGRADABLE PROTEIN ON EARLY LACTATION COWS FED FAT IN THE FORM OF WHOLE COTTONSEED[1]

[1]Gautreaux, A.A., H.E. Amos, M.A. Froetschel, and V. Pattarajinda. 2001. To be submitted to the Journal of Dairy Science.

ABSTRACT

Four multiparous Holstein cows were used in a 4 x 4 Latin square experiment with a 2 x 2 factorial arrangement of treatments. The objective of the experiment was to evaluate the effects of supplemental ruminally undegradable protein (RUP) on milk production and milk protein percentage and yield from cows receiving added fat in the form of whole cottonseeds (WCS). An evaluation of amino acid (AA) uptake by the mammary gland was also made. Concentrates were 1) control with supplemental crude protein (CP) from soybean meal (SBM) and cottonseed meal (CSM), 2) supplemental CP from SBM and CSM with 26.9% WCS, 3) supplemental CP from a RUP mix containing distillers dried grain with solubles, Menhaden fish meal, and heated SBM, 4) supplemental CP same as diet 3 with 24.9% WCS. Cows remained on each treatment for 21d and sample collection was in the last 7d of each period. Dietary concentration of CP was lower ($p=0.01$) and RUP was higher ($p=0.01$) due to supplemental RUP. Diets differed in NDF ($p=0.01$), ADF ($p=0.02$) and EE ($p=0.03$) concentration due to the addition of WCS. DMI was similar among treatments but CP intake was lower ($p=0.02$) due to RUP supplementation and RUP intake was lower ($p=0.02$) due to added fat. Milk production was different due to WCS ($p<0.01$) and supplemental RUP ($p=0.02$). Milk protein production (kg/d) was different ($p=0.01$) due to the addition of WCS.

INTRODUCTION

In striving for optimal productivity, dairy farmers place strenuous metabolic and nutrient intake demands on their cattle. Often times, dairy cows cannot meet these demands due to limited DMI, which prevents them from reaching their NE_L requirement. Increasing the energy density of the diet by the addition of fat has become a common

practice to aid dairy cattle in reaching their energy requirements. Unfortunately, a number of negative effects may be observed with the addition of fat to the diet and these effects may be dependent on the source of the added fat.

One commonly observed effect of added fat is a 0.1 to 0.3 percentage unit depression in milk protein composition (Emery, 1978). Specifically, WCS, a popular fat source in the south, caused a 0.1 percentage unit decrease in milk protein composition (Smith et al.,1981) In the past, a slight decrease in milk protein may not have been considered significant, but recent changes in the pricing of milk have brought attention to this adverse effect of added fat. Component pricing, where milk prices are based on fat and protein yield, is being employed in 7 of the 11 Federal Milk Marketing Orders in the United States.

Recent research has made many attempts to explain this decrease in milk protein and to reverse the depression of milk protein by varying the protein component of the diet. A possible explanation for the depression is there is a decreased amount of AA reaching the mammary gland due to decreased mammary blood flow caused by the addition of fat to diets (Cant et al., 1993a). Researchers have attempted to supply additional amounts of Met and Lys (Kincaid and Cronrath,1993) or increase the amount of CP in the diet (Kim et al.,1991) to reverse the effect of added fat, but were unsuccessful. Feeding increased amounts of RUP has been shown to increase both milk production and milk protein composition (Wright et al, 1998, Kalscheur et al, 1999). Bertrand et al. (1998) was successful in reversing milk protein depression when undegradable Met and Lys were added to diets of cows fed WCS at 12.9% of dietary DM. Our objective was to study the effects of adding fat in the form of WCS

accompanied with an increase supply of RUP on milk protein and to evaluate variations of amino acid availability and uptake by the mammary gland.

MATERIAL AND METHODS

Four multiparous Holstein cows were used in a 4x4 Latin square experiment with a 2 x 2 factorial arrangement of treatments to determine the effects of supplemental RUP on percentage and yield of milk protein and of other milk components, total milk yield, and plasma AA concentrations and other metabolites of cows fed diets containing added fat from whole cottonseed (WCS). Experimental concentrate (C) ingredient compositions are given in table 1. Percentage of ground shelled corn in the C was varied depending upon WCS addition. Concentrate protein supplementation and WCS addition were arranged as follows: 1) diet 1 (control)-all supplemental CP from soybean meal (SBM) and cottonseed meal (CSM) with no WCS, 2) diet 2-supplemental CP from SBM + CSM with 26.9% WCS, 3) diet 3-all supplemental CP from a RUP mix containing distillers dried grains with solubles (DDGS), Menhaden fish meal (MFM) and heated SBM (H-SBM) each providing equal CP and no WCS, and 4) diet 4-supplemental protein as in diet 3 with 24.9% WCS.

Cows were housed in a free-stall barn equipped with a Calan gate feeding system (American Calan, Northwood, NH) for measuring individual feed intake. Sorghum silage (SS) provided all roughage and was blended with respective C to make TMR's, which were fed twice daily at 0800 and1600h. Each experimental period was for 21d and divided into a 7d diet and metabolic adjustment period, 7d preliminary period and a 7d sample collection period.

The C were formulated to meet the NRC (1989) recommendations for NE_L, CP, Ca, P, Mg, S, and K for a 635 kg Holstein cow producing 31.7 kg of milk containing 3.5% milkfat that was not supplied by 9.97 kg DM from the SS (8.1 % CP; 1.2 Mcal NEL/kg). For cows producing over 31.7 kg of milk, C and SS were increased in a R:C ratio of 48:52, 50:50, 46:54, and 48:52 on a DM basis for diets 1,2,3,and 4, respectively. In addition, total DM was offered in amounts necessary to provide 5% orts daily and was provided to permit cows to regain any needed body condition.

Dry matter intake was measured daily throughout each 21 d period of the study. Samples of C, SS, and TMR were taken at each feeding and frozen by week of collection for later analyses. Orts were collected prior to the am feeding, sampled and frozen for analyses. Samples of C, SS, TMR, and orts were dried at 55° C until they reached a constant weight, and were then ground through a 2 mm Wiley Mill screen (Arthur W. Thomas Company, Philadelphia, PA) for analyses. Samples were analyzed for DM (AOAC, 1984), N using a Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI), NDF and ADF according to Robertson and Van Soest (1981) and ether extract with a soxhlet apparatus as described by Sukhija and Palmquist (1988). Samples of C, SS, TMR, and orts were hydolyzed in 6 N HCL under N gas for 24 h (Amos e. al., 1976) and analyzed for AA with a Beckman 6300 AA Analyzer (Beckman Instruments, Inc, Palo Alto, CA).

Fecal samples were taken on d 15, 16, 17, 18, 19, and 20 of each period at 0600, 0800, 1000, 1200, 1400, and 1600h, respectively. Samples were composited by period for each cow. Fecal samples were dried at 55° C until they reached a constant weight, were then ground through a 2 mm Wiley Mill screen (Arthur W. Thomas Company, Philadelphia,

PA), and stored for analyses. Samples were analyzed for DM (AOAC, 1984), NDF and ADF as described by Robertson and Van Soest (1981), and N with a Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Apparent digestibility of DM was determined by using indigestible acid detergent fiber (IADF) as an internal marker. Portions of the TMR and fecal samples were analyzed by a 120 h in vitro fermentation procedure using a Daisy II Incubator (Ankom Technology, Fairpart, NY) as described by Bernard and McNeill (1991). The apparent digestibility coefficient (ADC) for DM was calculated as follows, where MD indicates the marker in the diet and MF indicates the marker in the feces.

$$\% \text{ ADC-DM} = 100 * (1.00 - \text{MD/MF})$$

The ADC for EE, NDF and ADF were calculated as follows, where NF indicates the component in feces and ND indicates the component in the diet.

$$\%\text{ADC} = 100 \times 1.00 - [(\text{MD/MF}) \times (\text{NF/ND})]$$

Production of MCP was estimated based on NE_L intake (NRC, 1989) and the equation was adjusted to discount fat contribution to NE_L:

$$\text{kg BCP} = 6.25\{-30.93+11.45[\text{NEL intake}-(\text{kg fat intake}*2.65)]\}/1000$$

Metabolizable true protein (MTP) was estimated as follows, taking into account the proportion of true protein and the digestion coefficient of RUP and MCP (NRC, 1985):

$$\text{kg MTP} = (\text{kg RUP}*0.8*0.8)+(\text{kg MCP}*0.8*0.85)$$

Cows were milked twice daily and milk weights recorded at each milking. Milk samples were collected on d 0, 7, 14, and 21 at both the am and pm milkings. Samples were sent to Southeast DHI Laboratory, McDonough, GA for analysis of fat. A Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI) was used to determine milk

protein content. Milk samples were analyzed for total solids using a Labcono Freeze Dryer (Labcoco, Kansas City, MO), ash by heating lyophilized sample at 550° C for 8 h in a Isotemp Muffle Furnace (Isotemp Research, Inc, Charlottesville, VA), and AA for milk samples hydrolyzed as previously given using a Beckman 6300 AA Analyzer (Beckman Instruments, Inc, Palo Alto, CA). Lactose was estimated by subtracting the protein, fat, and ash from total solids.

On d 20 of each period, catheters were placed in the subcutaneous milk vein and jugular vein to sample the blood supply. On d 21 blood samples were taken at 20 minute intervals for 12 h. Blood was collected in Vaccutainer tubes containing NaF and Na Oxalate and composited by h. Blood samples were analyzed for glucose (Sigma Diagnostics Procedure # 315, Sigma, St. Louis, MO), triglycerides (Sigma Diagnostics Procedure # 339, Sigma, St. Louis, MO), and Non-esterfied Fatty Acids (Waco kit #990-75401, Waco Pure Chemical Industries, Osaka, Japan). Samples were also analyzed for free AA using a Beckman 6300 AA Analyzer (Beckman Instruments, Inc, Palo Alto, CA). Jugular vein samples were assumed to represent the systemic blood and arterial-venous differences of AA were calculated by subtracting the mammary AA concentration from the jugular AA concentration.

Data were treated by analysis of variance using the GLM procedures of SAS (1988) for a 4 x 4 Latin square design to determine differences due to treatment. The three degrees of freedom due to treatment were separated by using the following orthogonal contrast: 1) RUP versus No RUP, 2) WCS versus No WCS, and 3) RUP x WCS interaction.

RESULTS AND DISCUSSION

Table 1 gives the composition of treatment concentrates used in this study. Treatment 1 was considered a control and did not contain WCS or supplemental RUP. Treatments 2 and 4 contained WCS and a supplemental RUP mixture was added to treatments 3 and 4. Diets were formulated to be isonitrogenous. The supplemental RUP mixture used in diets 3 and 4 consisted of 19% Menhaden fishmeal, 55% distillers dried grain with solubles, and 26% heat-treated soybean meal, which as a percentage of total CP had RUP concentrations of 65, 47, and 65 percent, respectively. The RUP mixture was formulated to have 55.1% RUP as a percentage of total CP. A cottonseed meal and soybean meal mixture was used for supplemental protein in diets 1 and 2, the low RUP treatments. On average, these treatments (1 and 2) were calculated to have 37.3% RUP as a percentage of CP, 17.8 percentage units lower that treatments 3 and 4.

Data in Table 2 gives a partial composition of the TMR's used in this study. Diets were different ($p=0.01$) in CP concentration due to RUP supplementation. Diets containing supplemental RUP were 1.3 percentage units lower in CP concentration upon analysis than treatments 1 and 2. Reasons for this discrepancy between calculated CP and actual CP analysis are not apparent. Diets differed in RUP percentage due to both RUP ($p<0.01$) and WCS ($p=0.02$) addition. The addition of supplemental RUP to treatments 3 and 4 increased the RUP concentration, as a percentage of total CP, by 8.8 percentage units. The NDF ($p=0.05$), ADF ($p=0.03$), and EE ($p=0.03$) concentrations were highest for diets containing WCS. Treatments 2 and 4, with WCS, were 2.8 percentage units higher in NDF, 2.8 percentage units higher in ADF, and 1.9 percentage units higher in EE.

Table 3 shows the DM and dietary component intakes and digestibilities by treatment from TMR's fed in this study. Intakes were similar for DM, NDF, ADF, EE and NEL. The addition of WCS to diets 2 and 4 increased EE intake by 0.2 kg/d and decreased DMI by 1.5 kg/d and NEL intake by 1.6 Mcal/d. Treatments differed in CP intake ($p=0.02$) due to RUP supplementation and in RUP intake ($p=0.02$) due to WCS addition. The supplemental RUP added to treatments 3 and 4 decreased the total CP intake by 0.4 kg/d when compared to mean intakes for treatments 1 and 2. CP intake may also have been influenced by decreased DMI. There was no increase in RUP intake due to RUP supplementation because of decreased DMI and CP percentage, but WCS decreased RUP ($p=0.02$) intake by 0.2 kg/d.

High values for NDF and ADF concentration reported in Table 2 may have limited DM and nutrient intake (Table 3) and may have negatively affected digestibility. Actual values for NDF varied from 48.7 to 52.6 % and dietary concentrations of ADF ranged from 27.3 to 30.2 %. Although fiber is required in the diets of ruminants, the amount is often disputed and is dependent upon a number of factors including type of forage, particle size, total DM consumption, and production level of the animal (NRC, 1989). The NRC (1989) suggest that cows require 28 % NDF and 21 % ADF for the first three weeks of lactation and 25 and 19 %, respectively, in later lactation, with 75 % of NDF coming from supplied forage. On the other hand, Kawas et al. (1991) and Mertens (1983) recommends that cows between 10 to 26 weeks of lactation receive 28 to 31 % NDF and cows later in lactation receive 34 to 38 % NDF. The values of NDF and ADF percentage for the diets being used in the study well exceeded these recommendations. Waldo (1986) and Mertens (1994) state that NDF is the single best predictor of DMI. NDF is negatively

correlated with DMI (Mertens 1994, Waldo 1986) and Allen (2000) reported that when diets exceeded 25 % NDF, there is a general decline in DMI.

Dietary NDF concentrations were high in this study, their effect on DMI may have been over estimated. Mertens (1994) suggests that non-forage fiber sources, including soybean hulls and WCS which were used in the diets, that contain over 40 % NDF only have 30 % the filling effect of other forages and should be discounted 70%. Other fiber sources, with less than 40% NDF, should be assigned a NDF value of 12%. The sorghum silage used in the study was 66.3% NDF and comprised 48, 50, 46, and 48 % of TMR's for diets 1 through 4. Thus, 65.1, 64.1, 60.4, and 60.3% of NDF intakes, for diets 1 through 4, was supplied by sorghum silage. The majority of the remaining portion of NDF intake was probably supplied by soybean hulls and corn gluten feed for diets 1 and 3 and soybean hulls, WCS, and corn gluten feed for diets 2 and 4. The NRC (1989) reports that soybean hulls have 67 % NDF and WCS has 44 % NDF. Since they are both nonforage fiber sources with NDF values over 40 %, their contribution to NDF intake should be discounted 70%, as should that for corn gluten feed. The calculated NDF values adjusted, according to Mertens (1994), are 35.5, 29.4, 37.6, and 31.9 % for diets 1 through 4. Thus NDF should not have been a major factor limiting DMI.

Diets did not differ in DM, CP, NDF, and ADF digestibilities (Table 3) although DM digestibility decreased 2.7 percentage units by the addition of WCS, and CP digestibility was 4.0 percentage units lower for those diets containing the RUP supplement. Ether extract digestibility was decreased by RUP ($p=0.01$). Smith et al. (1981) fed WCS at 0, 5, 15, and 25 % of DM and also reported no difference in DM digestibility; however, the addition of WCS increased CP and EE digestibility.

Amino acid composition (g AA/100g CP) of the TMR's used in this study is given in Table 4. Diets were lower in Arg (p=0.02) due to RUP and higher for WCS (p=0.04). Diets without the RUP treatment had 1.3 more g Arg/g CP than diets with the higher RUP concentrations. This is expected because CSM and SBM used in diets 1 and 2 are higher in Arg concentration (4.33% and 3.8%) than DDGS and MFM (1.12% and 3.7%). Concentrations of Met were higher (p=.03) with supplemental RUP compared with control because of the higher Met concentrations provided by MFM. Menhaden fish meal has a Met concentration of 1.7%, over three fold higher than CSM (0.51%) and over two fold higher than SBM (0.75%) used in diets 1 and 2. Schwab (1997) emphasized the importance of the Lys to Met ratio in diets fed to dairy cattle and suggested that Lys and Met be fed in a 3:1 ratio. The Lys:Met ratios for diets 1 through 4 were 4.1, 3.6, 2.4, and 2.9. The Lys:Met ratios for the diets with RUP supplementation were 1.2 units lower. There was an interaction between RUP and WCS supplementation for the concentrations of Thr (p=0.02) and Tyr (p=0.01). The diet containing both WCS and RUP had the greatest concentrations of Thr (3.6 g/100g CP) and Tyr (2.4 g/100g CP), although the addition of either WCS or RUP to the diets, on average, decreased Thr by 0.6 g/100g CP and Tyr concentration by 0.2g/100g CP.

Table 5 shows the actual AA intakes among treatments, as related to CP and DM intake. Total AA, essential AA (EAA), and non-essential AA (NEAA) intakes were different (p=0.02, p=0.01, p=0.03, respectively) due to RUP supplementation. Total AA intake was greatest for cows receiving the control diet and lowest for those animals receiving the RUP supplement, due to a lower intake of DM and CP. This resulted in lower intakes of His (p=0.02), Leu (p=0.02), Lys (p=0.01), Phe (p<0.01), Thr (p=0.03),

and Val (p=0.03), due to RUP treatment. Intakes for specific NEAA were different for Asp (p<0.01), Glu (p=0.01), and Tyr (p=0.01). Intake for those animals fed the treatments with RUP supplement were 171.6 g AA/d lower in EAA, 201.0 g AA/d lower in NEAA, and 374.8 g AA/d lower in total AA. Interactions between WCS and RUP were observed for the intakes of: Lys (p=0.05), Phe (p=0.01), Val (p=0.04), Asp (p=0.01), and Glu (p=0.05). All of the interactions occurred where the main effect of RUP was highly significant and were probably influenced by this significance.

Table 6 gives milk production, milk composition, and milk component yield among treatments. Milk production was lower for cows fed diets containing supplemental RUP (p=0.02) and WCS (p<0.01). Animals receiving WCS produced the least milk; 1.35 kg/d less than cows not receiving additional fat. Other studies reported increased milk yield when fat was added to the diet (Casper and Schingoethe, 1989, Christensen et al., 1994, Kim et al., 1991, Weigel et al., 1997). Failure to observe increased milk yields in this study is probably due to the failure to significantly increase the EE and NE_L intakes (Table 3) and the decreased DM and CP intakes noted earlier. Supplemental RUP decreased milk production by 1.0 kg/d. Wright et al. (1998) and Kalscheur et al. (1999) reported linear increases in milk production as RUP concentration was increased.

Percentage solids, protein, fat, lactose and ash were similar among treatments (Table 6). Milk from cows receiving WCS had 0.17 percentage units less milk protein than milk from those not receiving added fat. Total production of fat, lactose, and ash were also similar among treatments. Total milk solids and protein production were lower (p=0.03 and p<0.01) when WCS was added to the diet. Those animals receiving WCS produced 0.11 kg/d less milk protein than those fed the control or RUP diet. The numerical

decrease in milk protein percentage and the significant decrease in milk protein production observed in cows receiving added fat in the diet, agrees with other research where added fat depressed milk protein percentage (Bertrand et al., 1998, Casper and Shingoethe, 1989, Kim et al., 1991, Kincaid and Cronrath, 1993). The 0.17 percentage unit decrease in milk protein composition is similar to other reported values. Emery (1978) reported a 0.1-0.3 percentage unit drop in milk protein when various sources of added fat were used. Smith et al. (1981) reported specifically, that WCS at 15 and 25% DM decreased milk protein percentage by 0.1 percentage unit.

The addition of supplemental RUP to the diet containing WCS had no effect on milk protein percentage or production. The failure to observe an increase in milk protein production may be due to a number of things, including an insufficient increase in RUP intake or a decrease in CP intake. Results from other research where increasing RUP was used to reverse milk protein depression varies. Kincaid and Cronrath (1993) were unsuccessful when they tried to increase ruminally undegradable Met and Lys at 5.0 and 6.25 g/d respectively. On the other hand, Bertrand et al. (1998) were successful in reversing milk protein depression by supplying 10 g/d of both Met and Lys postruminally. The researchers increased milk protein from 3.72% to 4.18%. DePeters and Cant (1992) suggest that failure to increase milk protein yield when feeding RUP is due to an insufficient increase in limiting AA flow postruminally or intestinal AA absorption.

In an attempt to explain observed decreases in milk and milk protein production, adequacy of NE_L and protein intake were evaluated. The cows had an average BW of 689 kg. For cows fed RUP as compared to those receiving no RUP supplement, milk yield

was 25.9 kg/d containing 3.7% milkfat and 26.9 kg/d at 3.8% milkfat, respectively. The NRC (1989) recommends that a cow weighing 700 kg receive 10.30 Mcal NE_L for maintenance and 0.71 Mcal NE_L/ kg milk containing 3.7% milkfat and 0.72 Mcal NE_L/kg milk containing 3.8% milkfat. Based on the recommendations, the cows fed RUP supplemented diets would need 28.7 Mcal NE_L/d and those cows fed diets without RUP supplementation needed 29.7 Mcal NE_L/d. Actual NE_L intake was 31.9 and 32.9 Mcal/d, respectively. These data indicate that NE_L intake was 3.2 Mcal/d in excess of these recommendations for milk production and maintenance. A similar comparison of NE_L intake of cows with and without WCS feeding showed that cows weighing 700 kg and producing 25.6 kg/d of milk containing 3.7% milkfat (WCS) would need 28.5 Mcal NE_L/d; where as cows receiving the diets without WCS produced 27.3 kg/d of milk containing 3.8% milkfat and needed 30.0 Mcal NE_L/d, according to NRC (1989) recommendations. Actual NE_L intakes were 31.6 (WCS) and 33.4 (no WCS) Mcal/d, an excess of 3.1 and 3.4 Mcal/d of the amount recommended for milk and maintenance.

Adequacy of protein intake was evaluated and indicated that cows fed diets with RUP supplementation produced 25.9 kg/d of milk containing 3.7% milkfat; whereas those fed diets without RUP supplementation produced 26.9 kg/d of milk containing 3.8% milkfat. These cows would require 0.449 kg of dietary CP for maintenance and 0.0864 and 0.0876 kg CP/kg of milk at these respective milkfat percentages. Thus, cows fed RUP would need 2.7 kg of dietary CP/d and those fed diets without supplemental RUP would need 2.8 kg CP/d. Actual CP intakes were 2.8 and 3.2 kg/d. These CP intakes, though lower than expected, especially with respect to RUP supplementation, should not have limited milk yield. A final comparison with respect to WCS feeding versus no WCS feeding

indicates that cows fed WCS produced 25.6 kg/d of milk containing 3.7% milkfat and cows fed diets without WCS produced 27.3 kg/d of milk at 3.8% milkfat. According to the NRC, these cows would need 0.449 kg/d CP for maintenance and 0.0864 and 0.0876 kg/d CP/kg milk at each respective milkfat percentage. Thus cows fed WCS would have needed 2.7 kg/d CP and those fed no WCS would have needed 2.8 kg/d CP. Actual intakes were 2.8 and 3.1 kg/d CP, respectively. Overall NE_L and CP intakes appear adequate for the cows used in the study on a BW and milk production basis but the high fiber content of the diets noted earlier may have limited DMI.

Protein adequacy was also evaluated by estimating microbial crude protein (MCP, NRC, 1989) production and metabolizable true protein (MTP, NRC 1985), as previously described. MCP production was estimated to be 2.2, 1.9, 1.9, and 1.9 kg/d for treatments 1, 2, 3, and 4 respectively. There were no differences in MCP production due to WCS or RUP treatment (SE=0.1). MTP for treatments 1 through 4 were calculated to be 2.3, 2.0, 2.1, and 2.1 kg/d. There were no treatment differences in MTP (SE=0.1). It was assumed that MTP has a biological value of 65% (NRC, 1985) and that half of the cows' metabolizable protein requirement for maintenance, 0.225 kg/d, would be met by MTP (NRC, 1989, Satter and Roffler, 1975)). With maintenance taken into account, AA left for milk production were 1.3, 1.1, 1.2, and 1.1 kg/d for treatments 1 through 4. Cows on treatments 1, 2, 3, and 4 produced milk with the following amounts of milk protein in kg/kg of milk: 0.032, 0.029, 0.031, and 0.030, respectively. This implies that with the given values of AA available for milk production, cows should have been able to produce 40.6, 37.9, 38.7, and 36.7 kg of milk/d for treatments 1 through 4. Actual milk production

was 28.0, 25.9, 26.6, and 25.3 kg/d, indicating sizable excesses in AA and protein available for milk and milk protein production.

Pattarajinda (2001) evaluated the importance of a MTP/Mcal of NE_L ratio and in a review and determined that milk production was significantly correlated ($r^2 = 0.34$, $p<0.05$) with this ratio. His review indicated that the MTP/Mcal NE_L was ~70g/Mcal for cows producing ~34 kg milk/d; where as, the ideal ratio for cows producing ~40 kg milk/d was ~ 80 g MTP/Mcal NE_L. The MTP/Mcal NEL ratios for this study were 66.8, 61.8, 67.5, and 65.2 for treatments 1 through 4. There was a difference (p=0.01) due to WCS addition, where the diets containing WCS had MTP/Mcal MEL ratios that were 3.7 unit higher. The higher ratios for diets 1 and 3 may have contributed to higher milk production, 1.35 kg/d more than animals receiving diets 2 and 4.

The AA output in milk is shown in Table 7 and follows a pattern similar to milk protein production. Total AA ($p<0.01$), EAA (p=0.01), and NEAA (p=0.01) yield was lowest for diets containing WCS. Animals not receiving WCS had 119.5 gAA/d more total AA, 49.4 gAA/d more EAA, and 70.1 gAA/d more NEAA, which was expected from the decreased CP intake and decreased milk protein production. There was an interaction (p=0.03) between WCS and RUP on AA output of EAA, when both WCS and RUP were added to diets EAA output was 60.3 gAA/d more than the average of when either WCS or RUP was added alone.. Output of AA differed for the following EAA due to WCS addition: His (p=0.05), Ile ($p<0.01$), Leu ($p<0.01$), Lys ($p<0.01$), Met ($p<0.01$), and Phe ($p<0.01$). Output of AA differed for the following NEAA: Ala (p=0.01), Glu ($p<0.01$), Gly (p=0.01), Pro (p=0.02), Ser (p=0.04), and Tyr ($p<0.01$). Output of AA was not related to AA intake or plasma AA concentration and not affected by supplemental

RUP. Although the control diet did have the highest AA intake and output, the diet containing only the supplemental RUP (treatment 3) had the lowest intake but higher output than those diets containing WCS (treatments 2 and 4).

Table 8 gives the jugular AA concentration among treatments. These data are given to present an overall estimation of the free AA supply to the post liver body and mammary tissue. There was no difference in total AA, EAA, or NEAA. There was also no difference in specific EAA or NEAA except for HYP ($p=0.02$), which was higher for cows fed WCS. There was also an interaction between RUP and WCS on jugular concentration of HYP; the addition of WCS and RUP to diets resulted in a HYP concentration that exceeded all other treatments.

Table 9 gives the mammary AA concentrations among treatments and follows the same pattern as jugular concentration. The mammary vein AA data is presented for comparison to jugular AA concentrations so that an estimation of mammary gland AA uptake could be made. Essential AA ($p=0.02$) were higher due to WCS. There were only differences in Arg ($p=0.01$) and Met ($p=0.03$) when looking at specific AA. Supplemental RUP decreased Arg concentration 1.1 μmol/dl. Cows fed the control diet had the highest jugular and mammary concentration of total AA and EAA, followed by the diets containing WCS, and finally the diet containing only the supplemental RUP. There were interactions between WCS and RUP for mammary concentrations of NEAA ($p=0.02$), total AA ($p=0.01$), Ile ($p=0.02$), Thr ($p=0.04$) and Tyr ($p=0.03$). In situations where interactions occurred, the addition of both WCS and RUP to treatment 4 increased concentrations of mammary AA above that found when either one alone, WCS or RUP, was added and above the average of both of those treatments. Jugular and mammary AA

concentrations reflect AA intake. The control diet had the highest intake and plasma concentrations of AA and the RUP diet had the lowest of both intake and plasma concentrations of AA.

Table 10 gives the arterial-venous differences in AA among treatments. There were no differences in total AA, EAA, NEAA, or any specific AA and uptake seemed unrelated to diet AA, AA uptake, or AA output. Uptake was highest for treatment 3, which actually had the lowest AA intake and plasma concentration. Treatment 1, which had the highest AA intake and plasma concentration, ranked third in AA uptake. AA uptake did not follow the same pattern of milk protein production. WCS had no effect on AA uptake. Cant et al. (1993a) and Casper and Schingoethe (1989) reported decreased AA uptake by the mammary gland when cows are fed added fat. Cant et al. (1993a) also suggest that fat decreases mammary blood flow (MBF) due to increased blood energy content which decreases adenosine production and closes precapillary sphincters. Failure to see a decrease in AA uptake by the mammary gland may be a result of failing to increase the EE and NE_L intakes. Therefore, we did not increase the energy content of the blood.

Table 11 gives hematocrit values, jugular and mammary metabolite concentrations, and a comparison of differences in jugular and mammary vein plasma concentrations of glucose and triglycerides. Hematocrit was 1.8 percentage units lower ($p=0.03$) for those animals fed the treatments with supplemental RUP. There was a negative interaction effect on hematocrit, where the addition of WCS and RUP decreased hematocrit 2.8 percentage units lower than when either was added alone. There were no treatment differences in jugular or mammary concentration of plasma metabolites or in estimated jugular-mammary vein differences of plasma triglycerides and glucose. However in

comparing the jugular and mammary vein differences in glucose, jugular vein concentrations are consistently higher, indicating glucose uptake by the mammary gland for lactose production. In a similar comparison of differences between jugular and mammary vein concentrations of plasma glucose, all treatments except 4, were high in jugular plasma glucose indicating triglyceride conversion to milkfat. Values reported for concentrations of NEFA were slightly higher than what was reported by other researchers. Christensen et al. (1994) reported values ranging from 166 to 191μeq/l for cows receiving added fat that were sampled from a tail vessel.

CONCLUSION

Milk production and milk protein production was decreased when WCS was added to diet. These observations may be due to failure to significantly increase EE or NE_L intake in diets containing WCS, which was accompanied by decreased DMI. The latter may have been due to which high NDF concentrations in the diets. Although we did increase the RUP concentrations of the diets we were unable to increase RUP intake and furthermore, decreased CP intake, so were unsuccessful in determining if supplementing RUP can reverse milk protein depression. The decrease in CP intake was due to decreased DMI and lower CP in diets than what was formulated. Fat had no effect on AA uptake or MBF but may be due to minimal increases in EE percent and EE intake.

TABLE 1. Ingredient Composition of Concentrates Varying in Ruminally Undegradable Protein and Lipid Content Fed to Early Lactation Cows

Ingredient	Treatments			
	Control	WCS	RUP	WCS+RUP
	(%)			
Corn gluten feed	16.9	17.9	15.9	16.6
Soybean hulls	16.9	17.9	15.9	16.6
Whole cottonseed	-	26.9	-	24.9
Ground shelled corn	33.7	10.8	34.3	13
Cottonseed meal	10.6	11.2	-	-
Soybean meal	19.2	13.4	-	-
Distillers dried grain	-	-	17.7	14.4
Menhaden fish meal	-	-	6.2	5.1
Heated soybean meal	-	-	8.5	6.9
Limestone	1.1	1.3	0.7	0.5
Dicalcium phosphate	0.4	0.5	-	0.8
Salt	1.2	1.3	1.2	1.2
Vitamin premix [1]	+	+	+	+
Mineral premix [2]	+	+	+	+
Zinc methionine [3]	+	+	+	+

[1] provided 8820, 1102, and 17 IU/kg of DM of vitamins A, D, and E, respectively

[2] provided the following in mg/kg of DM: 57 Ca, 25 Cu, 25 Fe, 60 Mn, 60 Zn, 0.3 Co, 1.25 I, and 0.3 Se

[3] provided .4 mg/kg of DM of Zinc methionine (ZinPro Corp, Eden Prairie, MN)

TABLE 2. Partial Composition of Diets Varying in Ruminally Undegradable Protein and Lipid Content Fed to Early Lactation Cows

	Treatments					Contrast[a]		
Item	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(%)							
CP	15.3	15.0	13.7	14.0	0.4	0.01	NS [b]	NS
RUP	37.3	34.6	47.4	42.3	0.2	0.01	0.02	NS
NDF	48.7	51.7	50.1	52.6	1.1	NS	0.05	NS
ANDF [2]	35.5	29.4	37.6	31.9				
ADF	27.3	30.2	27.3	29.9	0.9	NS	0.03	NS
Ether Extract	2.9	4.8	3.6	5.4	0.6	NS	0.03	NS
	(Mcal/kg)							
Conc NEL [1]	1.8	1.9	1.9	2				
TMR NEL [1]	1.5	1.6	1.6	1.6				

[a] Contrast RUP=RUP vs. no RUP, WCS= WCS vs. no WCS, Int= RUP x WCS

[b] NS=not significant ($p>.05$)

[1] estimated from NRC (1989) values and diet composition given in Table 1

[2] adjusted according to Mertens (1994)

TABLE 3. Dry Matter and Nutrient Intakes of Cows Fed Diets Varying in Ruminally Undegradable Protein and Lipid Content

	Treatments					Contrast[a]		
Item	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
Intake	(kg/d)							
DMI	22.3	20.1	20.4	19.7	0.8	NS[b]	NS	NS
CP	3.4	3.0	2.8	2.8	0.2	0.02	NS	NS
RUP	1.3	1.1	1.3	1.2	0.1	NS	0.02	NS
NDF	10.9	10.4	10.3	10.4	0.5	NS	NS	NS
ADF	6.1	6.1	5.7	6.0	0.3	NS	NS	NS
Ether Extract	0.6	0.9	0.8	0.8	0.0	NS	NS	NS
NEL Mcal/d	34.1	31.7	32.3	31.5	1.2	NS	NS	NS
Digestion	(%)							
DM	61.1	58.9	61.4	58.2	2.0	NS	NS	NS
CP	69.3	69.0	64.9	65.4	2.8	NS	NS	NS
NDF	48.0	48.1	49.8	48.8	2.1	NS	NS	NS
ADF	49.1	47.7	40.8	47.1	5.5	NS	NS	NS
Ether Extract	78.4	69.8	68.8	77.0	3.2	NS	NS	NS

[a] Contrast RUP=RUP vs. no RUP, WCS= WCS vs. no WCS, Int= RUP x WCS

[b] NS=not significant (p>.05)

TABLE 4. Amino Acid Composition of Diets Varying in Ruminally Undegradable Protein and Lipid Content Fed to Early Lactation Cows

	Treatments					Contrast [a]		
AA	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(gAA/100g CP)							
Arg	4.1	4.7	2.9	3.9	0.3	0.02	0.04	NS [b]
His	2.1	2.1	2.0	2.2	0.1	NS	NS	NS
Ile	6.8	6.4	5.9	7.1	0.6	NS	NS	NS
Leu	3.5	3.3	2.9	3.4	0.1	NS	NS	NS
Lys	4.1	4.0	3.3	4.0	0.2	NS	NS	NS
Met	1.0	1.1	1.4	1.4	0.1	0.03	NS	NS
Phe	3.6	3.8	2.9	3.5	0.2	NS	NS	NS
Thr	3.4	3.2	2.9	3.6	0.1	NS	NS	0.02
Val	4.5	4.2	3.7	4.6	0.2	NS	NS	NS
Total EAA	33.2	32.7	23.9	32.1	3.2	NS	NS	NS
Ala	5.5	5.3	5.0	6.1	0.4	NS	NS	NS
Asp	8.1	7.5	5.6	7.3	0.5	NS	NS	NS
Cys	0.3	0.3	0.7	0.3	0.2	NS	NS	NS
Glu	15.4	14.8	10.8	14.5	1.3	NS	NS	NS
Gly	4.2	4.0	3.9	4.7	0.2	NS	NS	NS
Pro	4.6	4.4	4.5	5.2	0.4	NS	NS	NS
Ser	4.0	3.8	3.3	4.0	0.2	NS	NS	NS
Tyr	2.3	2.3	2.1	2.4	0.1	NS	NS	0.01
Total NEAA	44.4	42.3	32.5	43.2	4.5	NS	NS	NS
Total AA	77.6	75.1	55.9	75.1	8.0	NS	NS	NS

[a] Contrast RUP=RUP vs. no RUP, WCS= WCS vs. no WCS, Int= RUP x WCS

[b] NS=not significant (p>.05)

TABLE 5. Amino Acid Intake of Cows Fed Diets Varying in Ruminally Undegradable Protein and Lipid Content

	Treatments					Contrast[a]		
AA	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(gAA/d)							
Arg	143.1	140.2	90.6	124.7	13.2	NS[b]	NS	NS
His	72.7	63.2	53.3	58.0	3.5	0.02	NS	NS
Ile	234.1	194.4	196.5	196.0	10.5	NS	NS	NS
Leu	118.8	99.5	87.7	92.1	5.1	0.02	NS	NS
Lys	141.9	120.5	101.6	110.4	5.4	0.01	NS	0.05
Met	36.0	32.4	33.8	32.9	2.2	NS	NS	NS
Phe	124.9	114.0	87.2	96.7	2.6	<.01	NS	0.01
Thr	115.1	95.1	87.0	92.2	4.6	0.03	NS	NS
Val	153.3	127.4	116.5	126.1	6.1	0.03	NS	0.04
Total EAA	1139.7	986.7	854.2	929.0	41.1	0.01	NS	NS
Ala	187.5	158.8	166.4	171.2	8.4	NS	NS	NS
Asp	276.6	227.2	182.6	201.8	10.2	<.01	NS	0.01
Cys	10.0	7.6	6.3	3.5	1.8	NS	NS	NS
Glu	527.9	447.2	370.4	404.5	20.3	0.01	NS	0.05
Gly	142.5	121.4	123.8	128.6	6.7	NS	NS	NS
Pro	159.4	133.6	149.6	151.0	8.8	NS	NS	NS
Ser	135.5	109.0	100.3	107.3	7.4	NS	NS	NS
Tyr	80.3	69.3	61.7	62.6	3.1	0.01	NS	NS
Total NEAA	1519.7	1274.2	1161.5	1230.5	61.0	0.03	NS	NS
Total AA	2659.4	2265.2	2015.7	2159.5	100.6	0.02	NS	NS

[a] Contrast RUP=RUP vs. no RUP, WCS= WCS vs. no WCS, Int= RUP x WCS
[b] NS=not significant (p>.05)

TABLE 6. Milk Production and Composition of Cows Fed Diets Varying in Ruminally Undegradable Protein and Lipid Content

Item	Treatments					Contrast[a]		
	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(kg/d)							
Milk	27.97	25.85	26.56	25.27	0.32	0.02	<.01	NS[b]
Protein	0.89	0.76	0.82	0.74	0.02	NS	0.01	NS
Fat	1.05	0.98	0.98	0.92	0.07	NS	NS	NS
Solids	3.30	2.86	3.19	3.07	0.10	NS	0.03	NS
Lactose	1.12	0.91	1.17	1.16	0.09	NS	NS	NS
Ash	0.24	0.22	0.23	0.21	0.01	NS	NS	NS
	(%)							
Protein	3.21	2.95	3.08	3.01	0.11	NS	NS	NS
Fat	3.83	3.80	3.70	3.68	0.27	NS	NS	NS
Solids	11.86	11.11	12.05	12.27	0.34	NS	NS	NS
Lactose	4.01	3.51	4.42	4.61	0.31	NS	NS	NS
Ash	0.85	0.85	0.85	0.82	0.03	NS	NS	NS

[a] Contrast RUP=RUP vs. no RUP, WCS= WCS vs. no WCS, Int= RUP x WCS

[b] NS=not significant (p>.05)

TABLE 7. Amino Acid Output in Milk from Cows Fed Diets Varying in Ruminally Undegradable Protein and Lipid Content

	Treatments					Contrast [a]		
AA	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(gAA/d)							
Arg	20.0	17.1	20.8	20.5	2.2	NS [b]	NS	NS
His	19.9	15.2	18.3	16.6	0.7	NS	0.05	NS
Ile	72.6	54.6	66.8	60.3	2.6	NS	<.01	NS
Leu	37.7	28.5	33.2	28.5	1.8	NS	0.01	NS
Lys	60.6	45.3	55.3	50.1	2.3	NS	<.01	NS
Met	16.8	12.9	16.2	14.3	0.5	NS	<.01	NS
Phe	36.5	27.7	33.5	30.3	1.4	NS	<.01	NS
Thr	32.2	24.4	29.6	30.8	2.5	NS	NS	NS
Val	47.4	40.3	43.3	39.0	2.9	NS	NS	NS
Total EAA	348.7	265.8	318.2	302.3	12.3	NS	0.01	0.03
Ala	24.6	18.4	22.4	20.4	1.0	NS	0.01	NS
Asp	47.7	42.6	52.2	41.7	4.0	NS	NS	NS
Cys	2.1	1.5	2.0	1.6	0.2	NS	NS	NS
Glu	171.4	129.1	156.9	133.1	7.1	NS	<.01	NS
Gly	14.9	11.2	13.5	12.4	0.6	NS	0.01	NS
Pro	70.0	54.3	65.4	59.0	3.3	NS	0.02	NS
Ser	41.0	31.0	37.6	36.5	2.1	NS	0.04	NS
Tyr	37.5	28.1	34.3	31.0	1.3	NS	<.01	NS
Total NEAA	409.1	316.1	382.5	335.3	17.6	NS	0.01	NS
Total AA	757.8	582.0	700.1	637.6	27.5	NS	<.01	NS

[a] Contrast RUP= RUP vs. No RUP; WCS= WCS vs. No WCS; Int= RUP x WCS interaction

[b] NS=not significant (p>.05)

TABLE 8. Jugular Vein Concentration of Plasma Amino Acids

	Treatments					Contrast [a]		
AA	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(μmol/dl)							
Arg	6.3	6.0	4.8	5.9	0.5	NS[b]	NS	NS
His	3.3	3.1	3.1	3.1	0.1	NS	NS	NS
Ile	7.4	7.1	6.4	8.2	0.6	NS	NS	NS
Leu	8.4	7.6	9.0	9.5	0.7	NS	NS	NS
Lys	6.0	5.2	5.9	5.8	0.6	NS	NS	NS
Met	4.2	4.0	4.1	4.1	0.4	NS	NS	NS
Phe	3.5	3.3	3.3	3.4	0.2	NS	NS	NS
Thr	6.7	5.7	5.7	6.3	0.4	NS	NS	NS
Val	15.5	15.1	13.9	15.5	1.2	NS	NS	NS
Total EAA	61.3	57.0	56.2	61.1	4.3	NS	NS	NS
Ala	18.2	18.4	17.6	18.3	1.3	NS	NS	NS
Cys	6.6	7.2	6.6	6.8	0.4	NS	NS	NS
Gln	64.3	63.1	66.0	63.9	3.5	NS	NS	NS
Glu	6.2	6.6	6.1	6.9	0.6	NS	NS	NS
Gly	23.9	24.8	25.5	27.5	2.3	NS	NS	NS
Hyp	44.5	44.7	41.1	53.3	1.7	NS	0.02	0.02
Pro	5.1	4.9	4.1	7.0	1.0	NS	NS	NS
Ser	6.0	5.4	6.1	6.1	0.6	NS	NS	NS
Tyr	3.6	2.8	2.9	3.4	0.3	NS	NS	NS
Total NEAA	180.9	182.2	178.8	183.2	15.2	NS	NS	NS
Total AA	242.1	239.2	235.0	244.3	18.7	NS	NS	NS

[a] Contrast RUP= RUP vs. No RUP; WCS= WCS vs. No WCS; Int= RUP x WCS interaction

[b] NS=not significant ($p<.05$)

TABLE 9. Mammary Vein Concentration of Plasma Amino Acids

	Treatments					Contrast [a]		
AA	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(μmol/dl)							
Arg	3.8	3.8	2.2	3.3	0.2	0.01	NS	NS [b]
His	2.5	2.3	2.0	2.3	0.1	NS	NS	NS
Ile	4.8	4.3	3.5	4.6	0.2	NS	NS	0.02
Leu	4.7	4.0	4.5	5.4	0.4	NS	NS	NS
Lys	2.6	2.5	2.3	2.6	0.3	NS	NS	NS
Met	2.3	1.5	2.0	1.7	0.2	NS	0.03	NS
Phe	2.2	2.0	1.9	2.2	0.1	NS	NS	NS
Thr	5.2	3.9	3.6	4.2	0.3	NS	NS	0.04
Val	12.2	11.4	9.0	11.5	0.7	NS	NS	NS
Total EAA	40.3	35.6	31.0	37.6	1.6	NS	0.02	NS
Ala	15.9	15.9	13.3	16.3	0.7	NS	NS	NS
Cys	6.5	5.7	6.7	5.2	0.4	NS	NS	NS
Gln	52.3	49.2	43.9	50.4	2.6	NS	NS	NS
Glu	3.7	3.4	3.0	3.5	0.2	NS	NS	NS
Gly	23.7	22.6	22.0	25.4	1.6	NS	NS	NS
Hyp	37.0	34.5	31.5	35.9	2.3	NS	NS	NS
Pro	4.2	4.1	3.1	3.1	1.1	NS	NS	NS
Ser	4.4	3.9	3.8	3.6	0.2	NS	NS	NS
Tyr	2.1	1.6	1.4	2.0	0.2	NS	NS	0.03
Total NEAA	151.9	141.8	130.1	152.0	4.6	NS	NS	0.02
Total AA	192.1	177.4	161.1	189.6	4.5	NS	NS	0.01

[a] Contrast RUP= RUP vs. No RUP; WSC= WCS vs. No WCS; Int= RUP x WCS interaction

[b] NS=not significant (p>.05)

TABLE 10.Differences in Jugular and Mammary Vein Plasma Amino Acid Concentrations

	Treatments					Contrast [a]		
AA	Control	WCS	RUP	WCS+RUP	SE	RUP	WCS	Int
	(μmol/dl)							
Arg	2.5	2.1	2.6	2.6	0.5	NS[b]	NS	NS
His	0.8	0.8	1.2	0.8	0.2	NS	NS	NS
Ile	2.6	2.8	2.9	3.6	0.4	NS	NS	NS
Leu	3.7	3.6	4.5	4.2	0.5	NS	NS	NS
Lys	3.4	2.7	3.5	3.3	0.5	NS	NS	NS
Met	1.9	2.5	2.1	2.4	0.4	NS	NS	NS
Phe	1.3	1.3	1.5	1.3	0.2	NS	NS	NS
Thr	1.5	1.8	2.1	2.1	0.3	NS	NS	NS
Val	3.4	3.7	4.9	4.1	0.9	NS	NS	NS
Total EAA	21.0	21.4	25.2	23.6	3.5	NS	NS	NS
Ala	2.3	2.5	4.2	2.0	1.2	NS	NS	NS
Cys	0.1	1.5	-0.1	1.6	1.0	NS	NS	NS
Gln	12.0	14.0	22.1	13.6	3.8	NS	NS	NS
Glu	2.5	3.2	3.1	3.5	0.6	NS	NS	NS
Gly	0.3	2.2	3.5	2.1	1.5	NS	NS	NS
Hyp	7.5	10.2	9.6	17.4	3.0	NS	NS	NS
Pro	0.9	0.8	1.0	3.9	1.5	NS	NS	NS
Ser	1.5	1.5	2.2	2.5	0.3	NS	NS	NS
Tyr	1.5	1.2	1.5	1.5	0.2	NS	NS	NS
Total NEAA	29.0	40.4	48.7	31.2	13.9	NS	NS	NS
Total AA	50.0	61.8	73.9	54.8	17.2	NS	NS	NS

[a] Contrast RUP=RUP vs. No RUP; WCS= WCS vs. No WCS; Int= RUP x WCS interaction

[b] NS=not significant ($p<.05$)

TABLE 11. Jugular and Mammary Plasma Metabolites

Metabolite	Treatments				SE	Contrast [a]		
	Control	WCS	RUP	WCS+RUP		RUP	WCS	Int
	(%)							
Hematocrit	32.7	35.3	33.1	31.4	0.6	0.029	NS [b]	0.011
	(mg/dl)							
Jugular glucose	62.3	58.4	54.2	56.8	3.7	NS	NS	NS
Mammary glucose	50.3	50.6	47.8	44.5	4.4	NS	NS	NS
A-V glucose	12.0	7.8	6.4	12.6	3.8	NS	NS	NS
	(mg/dl)							
Jugular TG	9.3	11.4	8.3	9.2	0.9	NS	NS	NS
Mammary TG	7.3	8.6	7.5	9.4	0.9	NS	NS	NS
A-V TG	2.0	2.8	0.8	-0.2	1.7	NS	NS	NS
	(mEq/l)							
Jugular NEFA	0.2	0.3	0.2	0.2	0.1	NS	NS	NS
Mammary NEFA	0.2	0.3	0.2	0.2	0	NS	NS	NS

[a] Contrast RUP= RUP vs. No RUP; WCS= WCS vs. No WCS; Int= RUP x WCS interaction

[b] NS=not significant (p>.05)

CHAPTER 4

CONCLUSION

Diet formulation for dairy cows are extremely important and have profound effects on milk production. The feeding of added fat and supplemental RUP are common practices in dairy nutrition. When fat is added to rations, milk production is often improved but milk protein depression occurs, decreasing the value of the product in certain markets. Milk protein depression may be due to a decreased amino acid uptake by the mammary gland observed when fat is fed. This observation is caused by fat increasing the energy content of blood and decreasing MBF through local control by vasoconstriction. The purpose of this study was to determine the effects of WCS on milk production, milk component production, AA uptake by the mammary gland, and if increasing the RUP concentratration of diets would reverse milk protein depression by increasing the amino acid concentration of blood going to the mammary gland.

The addition of WCS to diets fed to multiparous Holstein cows in early lactation decreased milk production and milk protein production. The failure to increase milk production, as noted in other studies when fat was fed, was probably due to a failure to increase the EE and NEL intake of the cows receiving the WCS treatments. Also, the high NDF concentrations of diets may have had a negative effect on DMI for those cows receiving WCS. The milk protein depression observed when WCS was added to rations was expected, but no differences were noted for total AA concentrations of mammary blood or estimated A-V differences. Although, the RUP concentration of the diets was

increased, we were unable to increase actual RUP intake and furthermore decreased intake of total CP. Decreased CP intake can be attributed to decreased DMI and decreased CP concentration of diets containing supplemental RUP. Diets were formulated to be isonitrogenous and the difference in the formulated CP content and the actual CP content of the diets containing the RUP supplement is unknown. With the decreased DMI and CP intake, we were unable to increase the AA concentration of blood going to the mammary gland and therefore did not reverse milk protein depression by supplying supplemental RUP in this study.

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