Effect of Protein Source on Milk Compositition of Cows

Fed Low Fiber, High Grain Diets

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Dairy Science

in

Dairy Science

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June 5, 1987

Blacksburg, Virginia

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(ABSTRACT)

Thirty primiparous Holstein cows in midlactation (106± 24 d) were randomly assigned to one of six diets in an incomplete block design. Each cow recieved two of six diets.Cows received the first diet for 32 d, then were offered a different diet for 32 d. received two of six diets. There were 5 observations per diet per period for a total of ten observations per treatment. Diets were 12% CP soybean meal (LSB), 20% CP soybean meal (HSB), 20% CP fishmeal (FM), 20% CP corn gluten meal (CG), 20% CP CG:SB, and 20% CP FM:SB. All diets were > 75% TDN and ~ 16% acid detergent fiber. Milk yield was not different between treatments. Milk fat percent, milk lactose percent, and fat corrected milk yield were higher in cows on CG (3.13%, 5.30%, 23 kg) in contrast to cows receiving FM (2.11%, 5.00%, 20.5 kg). Milk protein percent was not different, but milk protein yield was different across treatments. Rumen ammonia concentrations differed significantly with HSB (13.5 mg/dl) higher than LSB (7.33 mg/dl), indicating the increase in ruminally degraded protein. Plasma urea concentrations reflected rumen ammonia concentrations with HSB (16.2 mg/dl) higher than LSB (6.4 mg/dl). Results suggest that protein source can affect milk composition in cows fed low fiber diets.

Acknowledgements

The author wishes to express his sincere appreciation to the following people:

Dr. C.E. Polan for his support, assistance, and counsel during the Master's program. His help has made the past two years a tremendous experience.

Dr. J.H. Herbein, Dr. B.G. Cassell, and Dr. W.E. Vinson for their suggestions and support throughout the study and during the preparation of this manuscript.

Dr. K.E. Webb and Dr. M.L. McGilliard for their thoughts and insights in planning and conducting this project.

My fellow graduate students for their help and moral support over the past two years.

Mr. C.N. Miller and staff of the Dairy Research Center for the superb technical support and help in conducting the animal trial portion of this project.

My wife Vickie for her love, support, and sacrifices which have been given to the author from the heart for his success.

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Introduction

Low milk fat syndrome is of great importance due to its metabolic and economic significance. Low fiber diets depress milk fat percent and are associated with shifts in rumen volatile fatty acid concentrations. Lower percentage of ruminal acetate and higher percentage of propionate are characteristic in cows suffering milk fat depression. Abnormal volatile fatty acid concentrations can result from shifts in substrate availability for microbial fermentation. These products of fermentation can have a pronounced effect on animal performance. Exact mechanisms of control on milk composition by rumen volatile fatty acid concentration shifts have been studied but are not fully understood. Present theory suggests increased propionate stimulates gluconeogenesis, increased milk production, and insulin release. Gluconeogenesis would increase glucose available for milk production. Insulin coupled with increased glucose availability stimulates lipogenesis and depresses lipolytic activity of adipose tissue. This shift in lipid metabolism would decrease fatty acid availability for milk triglyceride synthesis. Increased milk volumes due to increased energy availability for milk synthesis can result in depressed milk fat percent. Decreased milk fat synthesis can result in decreased milk fat yields and also result in depressed milk fat percent.

In addition to dietary fiber influence, protein level and post - ruminal protein supply can also influence animal performance and milk composition by affecting microbial activity and/or animal performance. Protein digestion can be broadly divided into three general areas of digestion. First,

dietary, recycled microbial, and endogenous protein are subjected to microbial proteolysis in the rumen with subsequent synthesis of microbial protein. Second, microbial, dietary, and endogenous protein are subjected to proteolysis of the abomasum and small intestine with absorption occurring in the small intestine. Finally, previously undigested and unabsorbed protein can be partially hydrolyzed by hindgut microorganisms and utilized for microbial synthesis.

Until recent years, protein nutrition focused on meeting nitrogen requirements of rumen microbes. New work has indicated a benefit of supplying protein post -ruminally in order to improve protein quality with respect to amino acid composition and level available to the animal . Methods have been developed to protect protein from ruminal proteolytic enzymes providing intact feed protein for digestion and absorption by the small intestine. Numerous protein sources have been found to largely by - pass microbial degradation reaching the lower gut unaltered. By - passing microbial digestion in the rumen could enrich amino acid supply at the small intestine. These protein sources and levels of protein need to be studied further to determine effects of feeding on milk yield and composition. Further work on protein sources could give information that might allow feeding of high energy diets with low fiber to maximize milk production while minimizing decreased milk fat percent and yield.

Objectives

Therefore, this study was designed to determine if dietary protein sources would affect milk composition in cows fed low fiber, high grain diets. In particular, soybean meal, a protein source with a high degree of ruminal degradation, was compared to corn gluten meal and fishmeal, protein sources with lower ruminal degradation. Primiparous animals fed low fiber diets during mid-lactation were used as they were predisposed to depressed milk fat synthesis. Response in milk fat percent and production to protein source could be determined.

Literature Review

Effects of Low Fiber Diets Low - fiber, high - grain diets have been associated with low milk fat syndrome and rumen acidosis. (Davis,1967; Jenny et al.,1974; Slyter,1976; Van Soest,1964). High grain diets contain high levels of easily solubilized starches which are readily fermented. High levels of readily fermentable starches can cause decreased rumen pH (Slyter,1976; Zanartu,1983; Jorgensen and Schultz,1965). Acidic rumen pH is a result of substrate availability changes with concurrent shifts in microbial fermentation. Slyter (1976) suggests glucose is released from soluble starch faster than it can be utilized by rumen microbes. Glucose concentrations increase in rumen fluid causing an increase in lactic acid formation. Consequently, accumulation of lactic acid drives rumen pH down inhibiting lactic acid metabolism in certain bacterial strains.

Excessive numbers of lactate producing bacteria, such as *Streptococcus bovis*, and inadequate lactate metabolism are involved in rumen acidosis in sheep. Grubb and Dehority (1975) changed sheep diets from 100% orchardgrass hay to 60% cracked corn, 40% hay. Diet shift was accompanied with a decrease in numbers of cellulose digesting microbes with a concurrent decrease in rumen dry matter turnover. Furthermore, Mackie and Gechrist (1979) increased corn grain and molasses from 10 to 71% of diets fed to cannulated sheep and reported a drop in rumen pH from 6.71 to 5.80 with inclusion of grain mix. Microbial population shifts also occurred. As grain level increased, average numbers of amylolytic or lactate producing bacteria increased peaking after 7 days on high

grain. Lactate utilizing bacteria did not peak until day 54 on high grain rations. Russell and Hino (1985) incubated *S. bovis*, an amylolytic bacteria, at pH 6.6 and 4.7 with equal concentrations of glucose available. At a more neutral pH, acetate, formate, and ethanol were the primary products of fermentation. Acidic pH favored formation of lactate which was the major product at pH 4.7. In concluding, Russell and Hino presented a possible pathway of fermentation control involving lactate production and rumen acidosis (Figure 1).

With shifts in substrate availability and microbial fermentation, changes in volatile fatty acid concentrations occur. Esdale and Satter (1972) found at low rumen pH, acetate:propionate was 1.1 while a more neutral pH was conducive to a higher ratio of 2.8. Davis (1967) fed high grain, low fiber diets to investigate changes associated with lowered acetate concentrations. Although there were no differences in acetate production, acetate:propionate ratio was greater in control animals versus treatment animals. Jenny et al. (1974) reported similar shifts in rumen VFA concentrations and ratios in cows fed low fiber diets. Bauman et al. (1971) reported high grain diets doubled propionate production leading to lowered acetate:propionate ratios. This shift in VFA production is attributed to two factors. First, substrate availability favors proliferation of propionate producing bacteria in the rumen. Second, cellulolytic activity is inhibited by acidic rumen pH therefore depressing acetate production. Stewart (1977) found in vitro disappearance of cotton fiber incubated with excess starch was significantly higher at pH above 6.0 versus pH below 5.5. Acidic rumen pH also enhances propionate production in the rumen.

Diet Physical Form Physical form of diet also influences rumen environment and fermentation patterns. Miller et al. (1969) fed rechopped corn silage, silage with extra ears added, and normal field chop silage. Rechopped and ear supplemented silages significantly reduced fat corrected milk yield in contrast to normal silage. Milk fat depression has also occurred on finely chopped and pelleted forage based diets (Rock et al., 1974). Finely chopped forages induce changes in rumen pH and fermentation similar to high grain feeding. Sudweeks et al. (1975) measured chewing time of steers fed varying levels of concentrate to grain. Increasing concentrate intake decreased chewing time. Long hay and coarsely chopped sorghum silage stimulated chewing activity compared with

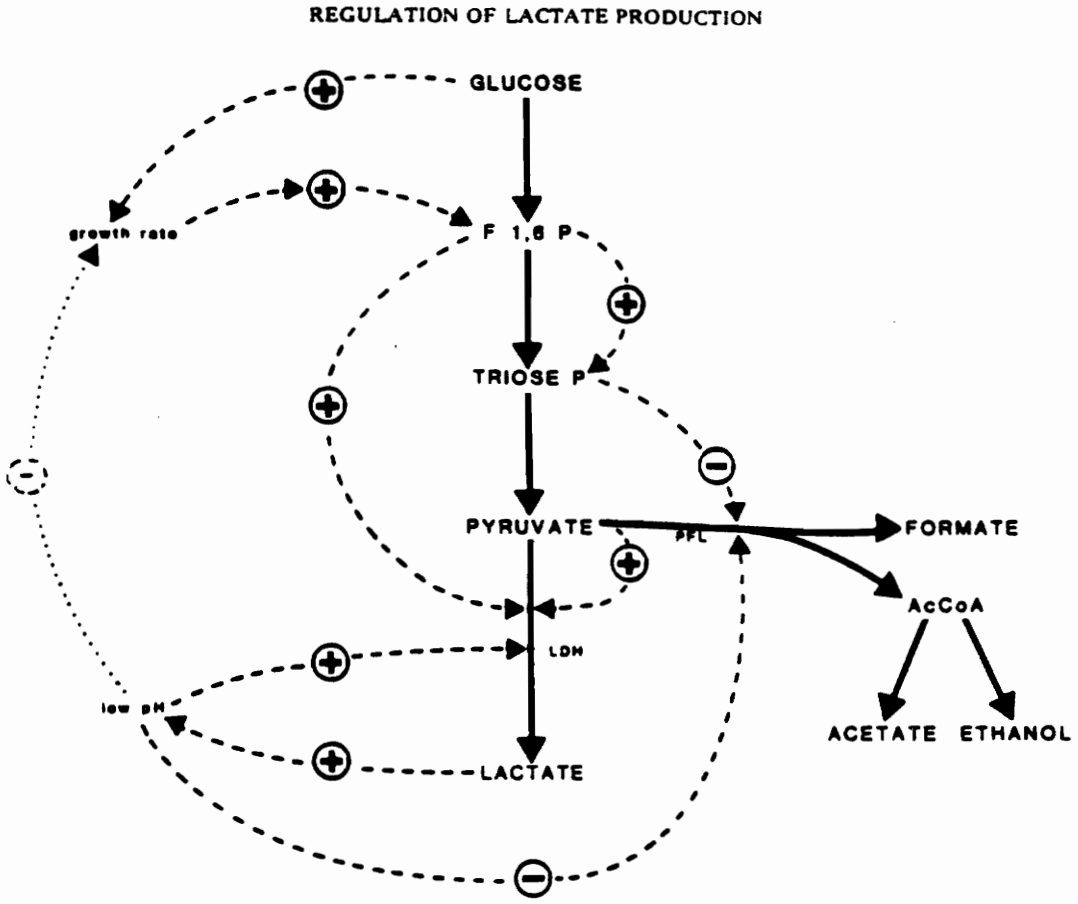

Figure 1. Proposed mechanism of control of lactate production from Russell and Hino (1986).

finely cut corn or sorghum silage. These changes contribute to decreased saliva flow and increased rates of fermentation which depress buffering capacity of the rumen.

Metabolic Shifts and Influencing Mechanisms Metabolic changes result from shifts in rumen VFA concentrations. These changes are due to differences in substrate availability at the tissue level as well as changes in blood hormone levels. Numerous workers have established a high correlation between rumen VFA levels and decreased milk fat percent (Davis,1967; Jenny et al.,1974; Jorgensen and Schultz,1965). Actual metabolic mechanisms involved in milk fat depression have not been fully explained. Frobish and Davis (1977) proposed excessive levels of propionate coupled with an inadequate supply of vitamin B_{12} might be involved. They suggested excess methylmalonic acid inhibited milk fat synthesis. Several other studies have failed to show a relationship between milk fat depression and vitamin B_{12} status of dairy cattle (Croom et al., 1981; Croom et al.,1981A; Elliot et al.,1979).

Milk fat depression seems to occur due to an inhibition of milk fat synthesis or a repartitioning of nutrients away from the mammary gland. Inclusion of fishmeal in the diet has reportedly depressed milk fat yields (Zerbini,1986). Storry et al. (1977) suggest long chain fatty acids of fish oil might inhibit mammary uptake of plasma fatty acids and fatty acid precursors. Depressed availability would lower rates of triglyceride synthesis resulting in milk fat depression. Davis (1967) found no changes in amount of acetate produced which is the primary substrate for de novo milk triglyceride synthesis. Rumen and blood acetate levels are highly correlated with milk fat percent (Jenny et al.,1974; Croom et al.,1981). Jenny et al (1974) used radioisotopic methods to measure utilization of short and long chain fatty acids in lactating cows fed high grain diets. High grain diets increased levels of radioactivity in liver, adipose, and milk while it decreased total milk triglycerides. This would indicate a shifting of nutrients away from the mammary gland and towards other tissue level anabolic processes.

Jenny and Polan (1975) measured post prandial changes of blood glucose and insulin in cows fed high grain diets in order to determine the relationship of insulin and milk fat synthesis. At 2 to 4

h post - feeding, animals receiving high grain diets had increased blood glucose and insulin with milk fat depression. Prior and Smith (1982) reviewed insulin's role in nutrient partitioning in ruminants. Several studies report increased lipogenesis at adipose sites in animals infused with glucose or propionate. Both glucose and propionate stimulate insulin release. Insulin has been shown to increase utilization of fatty acids for lipogenesis in adipose tissue. Furthermore, they propose glucose is a stronger stimulus of lipogenesis since it provides the glycerol backbone required for triglyceride synthesis. In the case of Jenny and Polan's data (1975), both stimuli are present to increase lipogenesis at adipose tissue and decrease substrate availability for de novo synthesis of milk fat at the mammary gland.

While increased milk production is economically favorable, depressed milk fat yields are not. But to reach maximum potential, dairy cows are often fed high energy dense diets which contain low fiber or low effective fiber levels resulting in milk fat depression.

Rumen Nitrogen Metabolism Protein digestion begins in the reticulorumen by the microbial population. Protein digestion proceeds actively in the small intestine and digestion of surviving protein continues by microbial action in the hindgut (Figure 2, from Ruminant Nitrogen Usage, NRC,1985) . Rumen microorganisms are highly proteolytic and can significantly alter ingested protein. Extent of proteolysis in the rumen is related to rumen biomass size (Huber and Kung,1981) with proteolysis resulting in release of amino acids and peptides from dietary protein. Craig and Broderick (1984) used rumen inoculums to measure in vitro amino acid release from ten protein sources. Results indicated differences in amino acid release between proteins. Similarly, Nocek et al. (1983) used commercial protease (*Streptococcus griseus*) to measure differences in amino acid release between protein supplements. Significant differences in amino acid release were evident between sources. Differences also existed between soluble and insoluble protein fractions with soluble fractions having a higher rate of degradation.

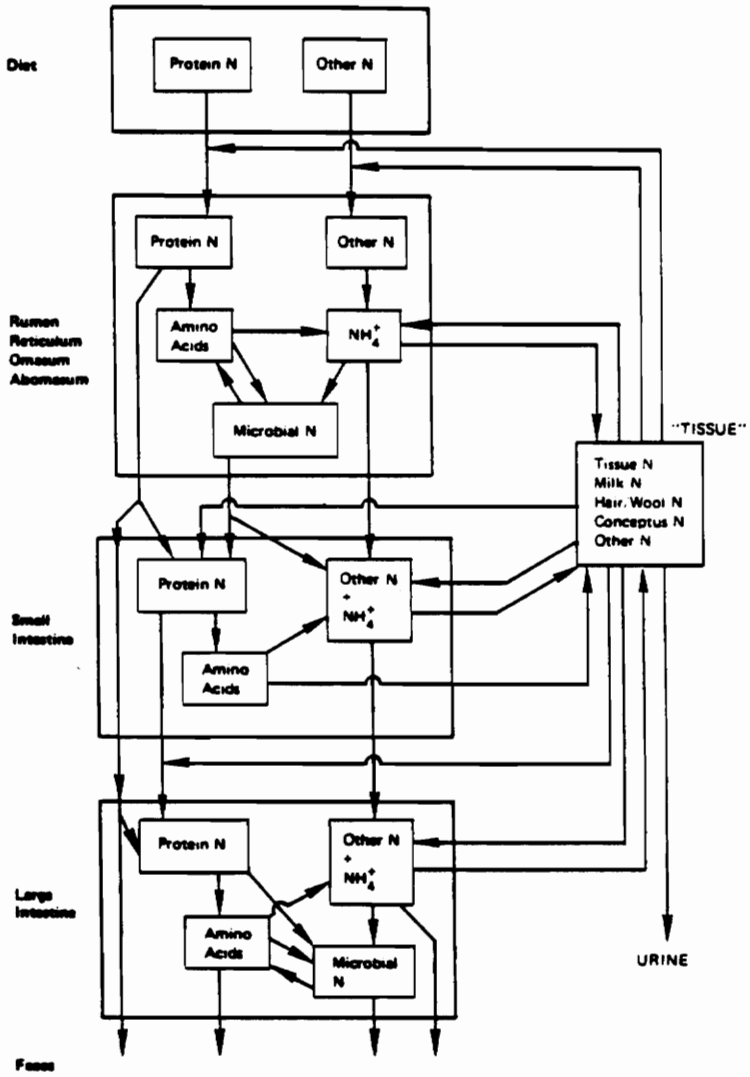

Figure 2. Illustration of nitrogen kinetics through gastrointestinal tract from Ruminant Nitrogen Usage (1985).

In vivo, proteolysis is achieved via bacterial membrane bound proteolytic enzymes. Cleaved amino acids and peptides are absorbed across cell membranes (Huber and Kung,1981). In contrast, protozoa engulf feed particles and bacteria with proteolysis occurring internally (Tamminga,1979). Differences in amino acid release between sources and fractions could be a result of primary, secondary, and tertiary structure differences. Nonetheless, rumen microorganisms are highly proteolytic in providing amino acids and peptides for microbial growth.

Degradation of Amino Acids Protein digestion results in release of free amino acids and peptides into rumen fluid. Normal rumen amino acid concentrations are relatively low. There can be an increase in amino acid concentration approaching 300 mM following feeding (Hogan,1975). This suggests proteolysis occurs faster than microbial amino acid uptake. Chalupa (1979) incubated amino acid mixtures in strained rumen fluid and determined rates of amino acid disappearance. Differences existed in rates of amino acid degradation. In vivo studies resulted in higher but similar rates of amino acid degradation ranging from 0.37 mM to 0.63 mM per h. Scheifinger et al. (1976) found differences in utilization rate of specific amino acids when amino acid mixtures were incubated with pure cultures of selected rumen bacteria. These data support the possible presence of requirements or affinities of amino acids by rumen bacteria.

Tamminga (1979) proposes deamination of amino acids with subsequent decarboxylation of the alpha -ketoacid is the most prevalent pathway for amino acid degradation. This pathway would provide ammonia and carbon skeletons for assimilation of amino acids required for microbial protein synthesis. Amino acids could also be utilized as substrates for catabolic energy producing pathways. Therefore, different amino acid requirements between bacterial species could be due to differences in amino acid composition of microbial protein or differences in capabilities for amino acid catabolism.

Microbial Protein Synthesis Microbial protein is highly digestible with a high biological value and is a significant source of protein for dairy cattle. Bacterial growth rate is affected by numerous factors including pH and rumen turnover rates (Hespell,1979). For optimal growth to occur, required

nutrients must be available at adequate levels for uptake and utilization. Potentially more important is that these nutrients are available at the same time. Sniffen and Robinson (1987) suggest balanced flow of nutrients would provide maximum bacterial growth similar to levels achieved in chemostat culture systems. They also note that 40 to 80% of daily amino acid requirement is met by microbial amino acids.

Owens and Isaacson (1977) suggest that low quality feedstuffs should be used to take advantage of the ruminants pregastric fermentation and microbial protein synthesis. For example, rumen bacteria can convert nonprotein nitrogen (NPN) into high quality microbial protein. Hume (1970A) measured nitrogen flow out of the rumen of sheep fed different levels of urea in a purified diet. As nitrogen intake increased from 2 to 9 g per d, flow of protein increased from 32.5 to 50.0 g per d. These data indicate the importance of supplying rumen microbes with adequate levels of nitrogen. Pilgram et al. (1970) used sheep and radiolabeled ammonia sulfate to measure ammonia incorporation into bacterial and protozoal protein. Ammonia supplied approximately 38 and 63% of nitrogen incorporated into protozoal and bacterial protein, respectively. Ibrahim and Ingalls (1972) fed conventional or semipurified diets to four fistulated dairy cows and found rumen microorganisms accounted for 54.4 or 62.4% of rumen digesta amino acids.

Cotta and Russell (1982) found amino acid supplementation improved energy efficiency with respect to microbial protein synthesis and stimulated microbial growth. A ratio of amino acid to glucose of 12.5% was conducive to highest microbial yields. Hume (1970) fed purified diets with NPN and achieved 71 g microbial protein synthesized per d in the rumen of mature wethers. Addition of branched chain VFAs increased protein production to 81 g per d. Hume also reported a negative correlation ($r = -0.62, P < 0.025$) between rumen acetate concentrations and protein synthesis. Similarly, Cummins and Papas (1985) found addition of isocarbon 4 and isocarbon 5 salts to batch culture fermentors significantly increased microbial protein yield. These results are similar to those of Russell and Sniffen (1984) who reported increased cell protein yields with addition of isovalerate, valerate, isobutyrate, and 2 methyl-butyrate to bacterial cultures. These data support

the limiting nutrient theory with respect to supplying adequate carbon and energy for microbial protein synthesis.

Rumen turnover rate also affects bacterial growth and efficiency. Sniffen and Robinson (1987) report increased rumen liquid turnover rate is positively correlated with increased bacterial yields. Owens and Isaacson (1977) suggest increased flow of bacteria decreases microbial recycling in the rumen and maintenance energy requirements. Addition of forages to concentrate diets stimulate microbial protein yields. Van Soest (1982) reports inclusion of forage stimulates salivary flow and liquid turnover. These results illustrate the relationship of microbial protein production, energy, and carbon availability and physical environment.

Rumen Nitrogen Cycle Rumen microorganisms are highly proteolytic with amino acids and peptides released from proteolysis of ingested and endogenous proteins. Leng and Nolan (1984) found 59% of dietary N was digested in the rumen. Similarly, Satter and Roffler (1975) suggest 60% of dietary protein would be degraded in the reticulorumen under typical feeding regimes. However, of protein degraded in the rumen, 29% was utilized as amino acids while 71% was degraded to ammonia (Leng and Nolan,1984). Tamminga (1979) theorized that proteolysis supplies ammonia and ketoacids.

Ammonia metabolism within the rumen is a complex system involving influxes and outflows of nitrogen. A major nitrogen source is dietary protein. Dietary NPN, salivary N, and endogenous N also contribute to the rumen ammonia pool (Figure 2) Ammonia, as discussed previously, can be combined to carbon skeletons providing amino acids for microbial growth. However, energy availability can limit ammonia utilization. Rumen ammonia concentrations can reach levels that exceed bacterial utilization rates. Pilgrim et al. (1970) found low N diet stimulated ammonia utilization over high N diets. Erfle et al. (1977) found glutamine synthetase activity increased 10 fold at low NH_4+ concentrations less than 0.5 mM. This enzyme is important in proposed flow of nonprotein nitrogen into amino acids that can be used by the microbe. No relationship was reported between NH_4^+ and glutamate dehydrogenase activity which suggests enzyme activity would

not limit ammonia assimilation at high concentrations of NH_4^+ Satter and Roffler (1975) discuss the importance of determining the point of ammonia overflow. Ammonia overflow is defined as rumen ammonia concentration at which microbial growth is maximized while no net loss of ammonia occurs from the rumen. They suggest 5 mg NH_3 - N per dl rumen fluid is optimal. Hogan (1975) reported unpublished data in which 2 mg nitrogen per dl rumen fluid was associated with maximum feed intake. Microbial protein synthesis and animal performance were not reported. Inadequate nitrogen levels limit microbial growth and fermentation which can limit dry matter intake and animal performance.

Low rumen ammonia concentrations can stimulate nitrogen recycling to the rumen. Hume (1970A) fed diets with varying protein solubility. Diets of low solubility resulted in lower rumen ammonia and a lower ammonia outflow in rumen digesta while stimulating a net gain of 7.5 g N per d. This gain would be from salivary nitrogen and possibly nitrogen transfer from the blood into the rumen. Thorton (1970) replaced rumen contents with physiological saline during intravenous urea infusions. Nitrogen content of saline was measured as urea and ammonia. Plasma urea concentrations increased as urea infusate increased. Rumen fluid ammonia increased in a similar fashion. Rumen ammonia and urea concentrations were not different between mid and high infusate concentrations while salivary flow and rumen fluid outflow were not considered. Leng and Nolan (1984) fed animals diets of low quality roughage and reported recycling of 11 to 14 g N per d. Sucrose supplementation increased N recycling to 23 g per d. They concluded urea recycling across the rumen epithelium is possible. Simple diffusion of urea across rumen epithelium would theoretically supply ammonia during periods of low rumen ammonia concentrations. This influx of ammonia would maintain minimum rumen ammonia concentrations as discussed by Satter and Roffler (1975).

Salivary nitrogen can contribute significant levels of nitrogen. Hogan (1975) reports sheep saliva contained 28 mg N per 100 ml. Urea nitrogen supplied 24% and amino acid nitrogen, either free or in mucoproteins, accounted for 68%. Bailey and Balch (1961) found dairy cows fed hay and

dairy cubes secreted 15 kg saliva of 12% nitrogen (1.8kg N) per kg dry matter intake. These data suggest the importance of nitrogen recycling in rumen ammonia supply.

Satter and Roffler (1975) proposed the following regression equation for predicting ruminal ammonia concentrations:

NH_3 - N (mg/dl) = 38.73 - 3.04(%CP) + .171 (%CP^2) -.49(%TDN) + .0024(%TDN^2). This equation was highly correlated (r^2 = .92) accounting for 92% of the variability of the variation in rumen ammonia. If rates of ammonia release are significantly higher than rate of microbial use, rumen ammonia concentrations can increase. Diffusion of ammonia across rumen epithelium into blood can occur at high levels. Bartley et al. (1976) fed diets of ground corn and urea with different degrees of extrusion. Diets were administered via rumen cannula at levels to supply 0.5 g urea per kg body weight. Animals which exhibited signs of ammonia toxicity had significantly higher blood ammonia than non - toxic animals. Rumen pH was also significantly different. Toxic animals averaged rumen pH 7.41 versus 7.16 for non -toxic animals. They propose these results could be associated with ratio of ammonia (NH_3) and ammonium (NH_4^+) at high pH which would affect movement of ammonia into the blood. Data of Thorton (1970B) support this theory. Cattle were fed a basal diet and urea was infused intravenously. As plasma urea increased, rumen ammonia concentrations increased as defined by:

$y = -0.596 + 0.896x - 0.028x^2$

where: y = ammonia N mg/dl rumen fluid

x = urea N mg/dl plasma.

Urinary urea loss was significantly correlated with plasma urea nitrogen concentration ($r^2 = 0.947$) as defined by:

$y = -273.66 + 167.637x$

where: y = urinary urea mg N/ h

x = plasma urea concentration mg N/dl.

Excess protein in the diet will result in urinary nitrogen loss.

Ammonia is not absorbed solely from the reticulorumen. Thorton et al. (1970C) investigated nitrogen kinetics in the hindgut. Infusion of glucose increased fecal nitrogen content with a concurrent decrease in urinary urea nitrogen loss. They concluded urea transfer to the rumen or hindgut is the preferred excretory pathway given adequate energy. Determining practical methods of manipulating nitrogen metabolism could be used to improve efficiency of protein nutrition by minimizing large nitrogen fluxes and urinary nitrogen losses .

Intestinal Protein Digestion Protein reaching the small intestine includes microbial, dietary, and endogenous protein. Digestion of these proteins produce amino acids and peptides for absorption and utilization by the animal. Tamminga and Oldham (1980) classify amino acid requirements of dairy cows into four classes: maintenance, deposition, gluconeogenesis, and milk protein synthesis. Microbial protein typically provides most of the amino acids available for absorption . As Clark (1975) noted, high producing cows might fall short of genetic potential due to amino acid deficiencies with microbial protein the primary protein source. Storm and Orskov (1984) investigated a theory involving limiting amino acids with respect to rumen microbial protein. Freeze dried rumen microorganisms (RMO) were infused in four treatment combinations: 0.9 g RMO - N per kg.^{75} BW, basal treatment plus amino acid mix similar to RMO, basal treatment plus amino acid mix with nonessential amino acids deleted, or 1.3 g RMO per kg.^{75} BW. Nitrogen retention was highest on the high RMO treatment. Amino acid supplementation improved nitrogen retention over RMO alone with deletion of nonessential amino acids having no affect. Further work found limiting amino acids of RMO to be arginine, histidine, lysine, and methionine. Therefore, post -ruminal supplementation of amino acids might enhance animal performance by enriching amino acid quality and quantity.

Clark (1975) reviewed post - ruminal supplementation of proteins and amino acids. Post - ruminal supplementation was found to increase milk yields 1 to 4 kg per d with a concurrent increase in milk protein yield. Milk fat yields also tended to be increased as well. Lough et al. (1983) used lactating goats to investigate response to ruminal acetate or propionate infusions with or without abomasal casein infusions. Casein significantly increased milk yields with a nonsignificant but apparent increase in milk protein yield. Milk fat depression occurred with propionate infusions while acetate infusions increased fat yields. Milk fat and fat corrected milk yields were higher on acetate - casein combination versus acetate infusion alone. Ranwana and Kellaway (1977) abomasally infused casein or glucose and reported increased milk production with milk fat depressed by glucose infusion. Plasma free dispensable amino acids were increased by glucose but decreased by casein. Using radiolabelled glucose, they report a nonsignificant increase in glucose pool size with casein and glucose infusions. Konig et al. (1984) also reported increased milk and milk protein yield following abomasal casein infusion. Plasma glucose, acetate, palmitate, and growth hormone were not affected by casein infusion. These data disagree with the theory of gluconeogenic affect of amino acids supplied by casein. Post - ruminal protein supplementation is beneficial but the mechanism is not known.

Dietary Protein Level Protein is required for production of milk and is supplied by dietary, microbial, and tissue protein (Edwards et al.,1980). Supplying adequate levels of dietary protein is essential to maximize milk production in lactating dairy cattle. Edwards et al. (1980) fed cows 13, 15, and 17% crude protein (CP) diets over three consecutive lactations. Milk, fat, and protein yields were higher on 15 and 17% diets versus the low protein diet. Burgess and Nicholson (1984) fed 10, 13, and 16% CP to midlactation cows. Cows fed 10% CP had lower dry matter intake, but cows fed 10% CP still had gains in body weight during the first trial. In the second experiment, cows fed 10% CP had lowered 4% FCM yield. This indicates inadequate nitrogen was supplied for optimal rumen fermentation which could limit intake and production. Huber and Boman (1965) supplemented grazing lactating cows with 8.3 or 22.6% CP at three levels of grain per kg milk produced. Milk production was significantly increased on 22.6% supplement. Milk fat depression

occurred when 1 kg concentrate was fed per 1.75 kg milk and was not affected by protein level, although ruminal acetate was higher on the high protein treatment. Actual crude protein intake was not reported. Barney et al. (1981) fed corn silage based diets of 12, 14, 16 and 18% CP to multiparous midlactation cows. They reported no differences in milk or solids - corrected - milk yield but a linear increase in milk protein percent as diet protein level increased. Foldager and Huber (1979) found addition of soybean or nonprotein nitrogen above 12% CP did not improve performance of early lactation cows. Evidence is non -conclusive regarding response to additional protein. In many instances, microbial fermentation and growth are improved by additional protein. Improved fermentation can stimulate DMI which increases nutrient availability to the animal resulting in improved performance. Therefore, protein level affect on performance could be secondary to increased dry matter intake.

Dietary Protein Source Due to microbial proteolysis, protein which is available for animal utilization can differ significantly from intake protein. Urea and anhydrous ammonia have been used extensively as nitrogen supplements in dairy cattle rations. Huber et al. (1967) replaced up to 48% of protein nitrogen with urea on corn silage based diets. Milk production was depressed at urea contents higher than 11%. Diets containing urea as primary nitrogen source had decreased milk production and solids - not -fat. Decreases in performance was attributed to lower energy intake. In a follow - up experiment, these same workers reported increased protein yields with depressed milk fat percent as concentrate feeding increased. Van Horn and Jacobson (1971) fed diets with increased protein or NPN. A basal diet (18.8% urea pellets,11.4% CP) was compared to diets containing two levels of additional soybean meal or urea. Supplemented diets had higher DMI. SBM supplementation stimulated an increase in milk and solids -corrected milk over basal diet. Failure of urea to give similar responses has been related to inadequate carbon availability. Data of Felix et al. (1980) support this theory as lactating cows performed better when fed urea with isoacids (isobutyrate, isovalerate, 2 -methylbutyrate, and valerate). Soy protein diets supported best performance, however. Isoacid supplementation did decrease rumen ammonia and plasma urea levels, suggesting improved nitrogen utilization by rumen microorganisms. This is probably due to

enhanced microbial amino acid synthesis in the presence of adequate carbon skeletons. Thus, urea can be utilized, but a proper balance of required nutrients must be provided.

Protein Degradability Nitrogen flow from the rumen is important due its influence on protein flow to the small intestine. Microbial protein has a high biological value, but as previously discussed may support only moderate levels of production because of limited amino acid composition . By - passing rumen degradation could be beneficial in providing limiting amino acids. Chalupa (1975) reviewed theories and data of postruminal protein supplementation via by - pass and protected amino acids and proteins. Techniques of altering ruminal degradation of a protein source included different feed processing and chemical treatments. Stern et al. (1985) fed diets differing in soybean protein, including SBM, whole soybeans, and soybeans extruded at 132° and 149°C. Extruded soybeans had decreased ruminal nitrogen disappearance compared to SBM. There were no differences in milk production or composition although extruded soybeans had higher absorption of nonammonia nitrogen in the small intestine as flow of total amino acids were increased by 10% relative to raw soybeans. This would indicate that amino acids absorbed over raw soybeans were not limiting production. Crooker et al. (1986) investigated differences in rumen degradation and amino acid flow to the small intestine of heat treated, formaldehyde treated, and untreated SBM fed to Holstein steers. Formaldehyde treated SBM had higher dry matter and crude protein remaining in polyester bags after 12 h rumen incubation. Formaldehyde treatment maintained SBM amino acid composition in contrast to heat treatment. Protection of amino acid content of SBM would compliment amino acid quality reaching the small intestine. Folman et al. (1981) fed formaldehyde treated SBM to high producing dairy cows in early lactation. Total VFA's produced in the rumen were significantly lower on treated supplement although propionate was higher. Milk yields were not affected, but protected SBM produced lower milk fat and protein. Reduced VFA concentrations could be attributed to inadequate ruminal nitrogen levels which would depress microbial fermentation. Ashes et al. (1984) infused formaldehye, glutaraldehyde, or glyoxal treated radiolabelled casein into the abomasum of cannulated sheep. Formaldehyde treatment decreased in vitro degradability using strained rumen fluid versus treatment by the other aldehydes. Increasing

formaldehyde and glutaraldehdye treatment of casein increased concentrations of unabsorbed lysine, tyrosine and cystine. Muller et al. (1975) fed formaldehyde treated whey to lactating cows increasing milk and 4% FCM yields. Thus, data show inclusion of protected protein in the diet has potential to improve performance, but can also decrease quality by lowering digestibility of the treated feed.

Techniques and processes used to protect proteins can be expensive. In some cases, processing can actually decrease nutritive value of the feed by heat damage or cross - links formed which decrease digestibility as results of Ashes et al. (1984) indicate. Therefore, variable results can be the result of protein damage. Other alternatives such as by - product protein sources which are resistant to rumen degradation are being used to compliment microbial amino acid supplied to the small intestine. Use of by - product protein sources has been studied to determine animal performance and digestive characteristics. Fishmeal is a by -product protein source which by - passes rumen degradation. Oldham et al. (1985) replaced urea with fishmeal and reported a significant increase in milk protein and fat yields with a slight increase in milk yield during early lactation. High levels of fishmeal also decreased body weight gains. Milk fat percentage was significantly lower in midlactation cows fed fishmeal with protein yields significantly higher. Milk and milk fat yield tended to be higher on fishmeal but were not significant. Zerbini (1986) reported fishmeal increased the flow of methionine to the small intestine of lactating cows. Therefore, responses to fishmeal inclusion could be a result of amino acid differences. Polan et al. (1985) reported dried and wet brewers grains compared to soybean meal increased milk, milk protein yields, and milk fat percent. Holter et al. (1985) reported lower N solubility of dietary nitrogen was conducive to higher milk production. Kung and Huber (1983) reported improved milk yield and profitability by feeding heated SBM in combination with ammoniated corn silage. Heated SBM had 36.3% N disappearance versus 76.9% for normal SBM. Janicki et al. (1985) found nitrogen solubility did not improve performance in lactating Holstein cows nor did it change N partitioning. Cummins et al. (1982) found low degradable N diets enhanced efficiency of nitrogen utilization in growing male calves.

It seems as if physiological status of the animal can affect nitrogen partitioning and utilization. With changes in physiological status, amino acid requirements can change and amino acids required over microbial supply could also change. Considerable work needs to be directed towards better understanding this area of research, including differences in status of the animal, protein sources used, amino acids supplied, amino acid utilization, and responses under different management systems.

Materials and Methods

Animal Selection and Care Thirty primiparous Holstein cows were selected from available animals in the University herd. Average days post - partum was 114.5 (range 66 to 147). Average milk production was 24.3 kg per d. Animals were randomly assigned space in a tie stall barn and allowed to adapt for 6 d to the new environment. During adaptation, all cows were fed a diet balanced to contain 16% acid detergent fiber (ADF), 16% crude protein (CP), and 71% total digestible nutrients (TDN). The diet contained corn silage, high moisture corn, soybean meal and minerals - vitamin supplement. Each animal was fed ad libitum. Animals were fed individually and feed was available except when animals were out for exercise and/or to be milked. Water was available at all times. Animals were bedded with sawdust over rubber mats.

Cows were allowed to exercise from 0900 to 1300 daily in a eathern and gravel - covered lot where they were observed for heat detection. A commercial mineral mix was available ad libitum. Animals were milked twice daily at 0100 and 1300. Animals were fed at 0600 and 1400 each day. Orts were removed daily between 0900 and 1100.

Experimental Design The study was divided into two 32 d periods. During first 7 d, animals were allowed to adjust to experimental diets. The last 25 d constituted the experimental period. Feeding,

milking, and general management practices were the same as those described in animal selection and care section.

Following 6 d environmental adjustment, cows were randomly assigned as a block in an incomplete block design (Table 1). Each animal received two diets. Diets were assigned such that each diet occurred with all other diets twice in reverse order between cows receiving the diet combination. Each diet occurred five times per period for a total of ten observations per diet.

Experimental Diets Animals were offered one of 6 diets, which were balanced to provide similar energy and fiber levels (Table 2). Low soybean meal diet (LSB) contained 13% CP and high soybean meal diet (HSB) contained 20% CP. All other diets were balanced to contain 20% CP. Fishmeal was used as protein supplement in diet FM. Diet CG contained corn gluten meal as protein supplement. Diets CG:SB and FM:SB were balanced to provide equal amounts of protein from corn gluten and soybean meal or fishmeal and soybean meal, respectively. All diets were balanced to contain inadequate fiber levels in order to predispose animals to milk fat depression. Diets were mixed in a Uebler mix cart and fed as total mixed rations.

SAMPLING AND MEASUREMENTS

Intake and Production Intake was measured 4 consecutive days per week during experimental periods. Milk production was recorded at each milking. Body weights were measured weekly following Monday afternoon milking throughout the study. Milk samples were taken at 4 consecutive milkings on days 19, 20, or 21 and again on days 26, 27, or 28 during each period. Samples were preserved with potassium dichromate and submitted to the Virginia Regional DHIA Laboratory for analysis of milk fat, protein and lactose content by infrared spectophotometry. Fat was determined by number of ester bonds, protein by peptide linkages, and lactose by hydroxy groups present on lactose ring structure.

Table 1. Assignment of treatments to cows in incomplete block design.

Cow	Period 1	Period 2
1	LSB	HSB
2	LSB	FM
3	LSB	CG
4	LSB	CG:SB
5	LSB	FM:SB
6	HSB	FM
7	HSB	CG
8	HSB	CG:SB
9	HSB	FM:SB
10	FM	CG
11	FM	CG:SB
12	FM	FM:SB
13	CG	CG:SB
14	CG	FM:SB
15	CG:SB	FM:SB
16	HSB	LSB
17	FM	LSB
18	CG	LSB
19	CG:SB	LSB
20	FM:SB	LSB
21	FM	HSB
22	CG	HSB
23	CG:SB	HSB
24	FM:SB	HSB
25	CG	FM
26	CG:SB	FM
27	FM:SB	FM
28	CG:SB	CG
29	FM:SB	FM
30	FM:SB	CG:SB

Table 2. Composition of diet shown by diet component percentage of dry matter.

	Diet					
	LSB[1]	HSB	FM	CG	CG:SB	FM:SB
Ingredients			% dry matter			
Corn silage	50.0	50.0	50.0	49.0	50.0	50.0
High moisture corn	40.8	20.2	28.8	26.2	23.6	25.3
Soybean meal	6.7	27.3	--	--	13.1	13.2
Fishmeal	--	--	18.7	--	--	9.6
Corn gluten	--	--	--	21.3	10.8	--
Mineral and vitamin mix[2]	2.5	2.5	2.5	4.2	2.5	2.5

[1] LSB = Low soybean meal
HSB = High soybean meal
FM = Fishmeal
CG = Corn gluten meal
FM:SB = Fishmeal:soybean meal
CG:SB = Corn gluten:soybean meal

[2] For mineral mix composition, see Table 3.

Table 3. Mineral and vitamin mix composition.

Ingredient	%
Sodium Chloride	15.0
Mineral	**%**
Calcium	20.0
Phosphorus	5.0
Magnesium	2.0
Sulfur	1.0
Mineral	**ppm**
Copper	100
Cobalt	3
Manganese	1200
Zinc	1500
Iodine	25
Selenium	5
Vitamins	**IU/kg**
Vitamin D	45
Vitamin A	454

Rumen Fluid and Blood Rumen fluid was sampled via stomach tube 2 to 4 h after feeding on days 21,22,or 23 and again on days 30,31, or 32 of experimental periods. Cows sampled on day 22 were sampled on day 30 in reverse order of day 22 sampling with a similar schedule used for cows sampled on days 23 and 31. Approximately 100 ml of rumen fluid were collected, mixed by swirling, and a subsample of 10 ml mixed with 3 drops of concentrated sulfuric acid in a plastic tube. Acidified rumen fluid was stored on ice until transferred to lab. Remaining rumen fluid sample was placed in plastic cups and stored on ice until transferred to lab. Acidified rumen fluid samples were centrifuged at 3220 x g for 20 minutes at 10&degC. Rumen fluid samples were stored at - 20°C until analyzed.

Twenty ml blood samples were taken by jugular puncture and divided into two 10 ml aliquots in plastic tubes containing 20 µg heparin. Samples were stored on ice until returned to the laboratory for processing. Blood samples were centrifuged as described for rumen fluid to separate red blood cells from plasma. Plasma was transferred into plastic test tubes and stored at -20°C until analyzed.

Diet Sampling and Analysis Rations were sampled twice at 1300 h during each period. Ration samples were stored in sealed plastic bags at -20° C until analyzed. Individual feed ingredients were taken on the last day of the first period. Corn silage and high moisture corn samples were handled like ration samples.

All feed samples were wet ground through a 6 mm screen using dry ice . Ground samples were returned to freezer and dry ice allowed to dissipate. Samples were sealed and remained frozen until analyzed. Samples were composited by experimental period and representative sub - samples were submitted to University Forage Testing laboratory for dry matter, crude protein, and acid detergent fiber determination. Other sub - samples were subjected to wet analysis for determination of crude protein by Kjeldahl procedure to compare crude protein content if samples were analyzed before or after drying. No differences were measured and Forage Testing laboratory values are used in this manuscript. Diet protein degradability for soybean meal and fishmeal were reported by Zerbini (1986). These values and NRC (1986) degradability estimates for all other diet components were

used to estimate diet protein degradability. These values were compared to revised NRC requirements for degradable and non - degradable protein.

Rumen Fluid and Plasma Analysis Plasma samples were used to measure plasma ammonia, urea, and insulin. Plasma samples were allowed to thaw at room temperature. A 1 ml aliquot was deproteinized using tungstic acid. Following deproteinization, samples were analyzed for plasma ammonia using a phenol reagent assay described by McCullough (1967). Deproteinized plasma samples were used to determine plasma urea as described by Coulombe and Favreau (1963). Plasma insulin was quantitated using a standard radioimmunoassay procedure outlined by Barnes et al. (1985).

Rumen fluid pH was determined after samples were stored on ice and returned to lab. Rumen fluid was analyzed for ammonia using the phenol based assay described for plasma samples. Acidified rumen fluid samples were recentrifuged as described earlier to remove sediment which had become resuspended during thawing. Supernatant was saved and used for ammonia analysis. Non - acidified rumen fluid was analyzed for rumen volatile fatty acids. After mixing, a 10 ml aliquot was taken and centrifuged at 3220 x g for 20 minutes at 10° C. Supernatants were mixed with metaphosphoric acid, filtered through 40 µm microfilters and analyzed by gas -liquid chromatography (GLC). The GLC used a hydrogen flame ionizer with nitrogen serving as inert carrier and oven temp held constant at 125° C.

Statistical Analysis Data were analyzed using general linear model procedure of SAS (SAS Users Guide,1985). There are missing data points due to sample contamination or inadequate sample size and therefore means listed in this manuscript are least squares means. Contrasts used for comparing treatments were nonorthogonal and were tested using Bonferonni F test values (Gill,1978). Statistical model and contrasts are shown in Table 4. Contrast for VFA data were calculated using the model given in Table 4 without period by treatment interaction as no significant differences existed in the interaction with regard to VFA data and was deleted to generate least square means using GLM procedure of SAS.

Table 4. Experimental model and nonorthogonal contrasts.

Model

$Y = \mu + cow + period + treatment + per*trt + error$

Contrasts

Low soybean meal	vs.	High soybean meal
High soybean meal	vs.	Fishmeal
High soybean meal	vs.	Corn gluten meal
Fishmeal	vs.	Corn gluten meal
Soybean:Fishmeal	vs.	Soybean:Corn gluten

Results and Discussion

Dry Matter Intake Dry matter intake (DMI) was not significantly different ($p < .05$) across treatments with average intake 14.2 kg dry matter per day (Table 5). Using NRC prediction equation (Ruminant Nitrogen Usage ,1986), DMI for these animals based on pre - experimental production and weight was predicted to be 14.7 kg per day. The prediction equation also estimated CP, rumen dgraded CP, and rumen undegraded CP intake at 2.26, 1.44, and 0.82 kg/d, respectively. On all other diets, DMI prediction seemed to be accurate. Depressed intake of CG has two possible explanations. First, dietary protein degradability estimated using literature values (Zerbini,1986; NRC, 1986) indicated CG failed to provide adequate ruminally degraded crude protein (Table 6). Inadequate rumen nitrogen can inhibit rates of microbial growth and fermentation. Depressed microbial fermentation has been shown to depress dry matter turnover rates which would limit intake level. Inclusion of soybean meal in CG:SB provided adequate ruminally degraded protein and subsequently higher intakes. Second, corn gluten meal is an unpalatable ingredient and high levels of corn gluten meal feeding could cause feed refusal due to palatability problems (Etgen and Reaves,1978).

Milk Yield Differences in milk yields were also not significant ($p < .05$) with means ranging from 24.6 to 27.7 kg milk per d. (Table 5). Animals receiving CG had a trend towards lower milk yield (24.6 kg per d) versus other 20% CP diets (mean 27.2 kg/d). Depressed production may be attributed to lower DMI. Depressed intake has been found to depress production as flow of VFAs and

Table 5. Effect of diet on milk yield, dry matter intake, feed conversion, and body weight changes.

Item	Diet					
	LSB	HSB	FM	CG	CG:SB	FM:SB
Dry Matter Intake (kg/d)	14.8	14.2	14.5	12.4	14.6	14.4
Milk Yield (kg/d)	25.1	26.8	27.1	24.6	27.0	27.7
Milk / DMI	1.7	1.9	1.9	2.0	1.9	1.9
Body Weight Changes (kg/d)	.28	.02	.30	.17	.37	.38

Contrasts				
1	HSB	vs	LSB	NS, $p < .05$
2	HSB	vs	FM	NS, $p < .05$
3	HSB	vs	CG	NS, $p < .05$
4	FM	vs	CG	NS, $p < .05$
5	CG:SB	vs	FM:SB	NS, $p < .05$

Table 6. Diet dry matter, acid detergent fiber, and crude protein degradability, including protein and intake.

	Diet					
Composition	LSB	HSB	FM	CG	CG:SB	FM:SB
			%			
Dry matter	52.0	45.0	55.0	50.0	48.0	47.0
Acid detergent fiber	15.9	17.6	15.9	13.8	15.5	14.1
Crude protein	12.0	20.2	19.8	21.7	20.7	20.0
Rumen degraded CP	54.6	61.4	43.8	39.5	50.0	50.0
Rumen undegraded CP	45.4	38.6	56.2	60.5	50.0	50.0
Intake			kg/d			
Crude protein	1.78	2.88	2.87	2.69	3.02	2.88
Rumen degraded CP	0.97	1.77	1.26	1.06	1.51	1.44
Rumen undegraded CP	0.81	1.11	1.61	1.63	1.51	1.44

a NRC recommended intake (NRC,1986)

Crude protein	2.26 kg/d
Rumen degraded CP	1.44 kg/d
Rumen undegraded CP	0.82 kg/d

microbial protein from the rumen is lower. LSB supported .5 kg/d more milk than CG, but 1.7 to 2.6 kg milk per d less than other 20% CP diets (Table 5). LSB did not provide required levels of crude protein intake with ruminally degraded protein inadequate as estimated using revised recommendations of NRC (Table 6). Edwards et al. (1980) found cows fed 15 and 17% CP diets produced significantly more milk versus 13% CP diets. Burgess and Nicholson (1984) had variable results including no significant differences in production in the first study but significantly less 4% fat corrected milk on 10 versus 13% CP diets during a second study. Protein intake did not seem to affect fermentation as indicated by intake in the present experiment . Burgess and Nicholson (1984) reported significantly lower DMI on low protein versus high protein diets. Their data indicated shifts in rumen fermentation with significantly higher acetate but lower propionate on low protein versus high protein rations. Inadequate dietary protein in LSB could have resulted in lower microbial growth and subsequently depressed microbial protein flow rate from rumen to small intestine. This in turn could result in amino acid deficiencies and lowered levels of production.

HSB failed to increase milk yields compared with LSB. Several reports indicated increased dietary protein can improve production. Excess protein intake can result in elevated levels of ammonia nitrogen absorption which can become toxic. In order to prevent ammonia toxicity, ammonia can be converted to urea, a less toxic molecule. This conversion, however, involves energy expenditure which could be otherwise used in productive, anabolic pathways such as milk component synthesis.

Body Weight Change Least square means body weight changes ranged from 0.02 to 0.38 kg per d, but there were no significant differences by treatments ($p < .05$) (Table 5). All animals were fed excess energy based on NRC recommendations. Excess energy intake would stimulate increases in plasma insulin which would in turn stimulate lipogenesis and acquisition of adipose tissue (Prior and Smith, 1982). This should lead to increased body weight. In addition, cows used were all first calf heifers and would still be growing.

Milk Composition and Component Yield Milk composition differed significantly across diets (Table 7). Milk fat % was significantly higher on CG and HSB versus FM (Figure 3). These results were

Table 7. Least square means of milk composition and component yields.

	Diet					
	LSB	HSB	FM	CG	CG:SB	FM:SB
Component			%			
Fat[2,4,5]	3.02	2.89	2.11	3.13	2.82	2.37
Protein	3.10	3.20	3.04	2.95	2.99	3.01
Lactose[2,3,4,5]	5.21	5.16	5.00	5.30	5.22	5.09
			kg/d			
FCM[2,4]	23.2	24.2	20.5	23.0	24.0	22.3
Fat[2,4]	0.75	0.78	0.54	0.75	0.76	0.64
Protein[2,4]	0.75	0.85	0.81	0.73	0.80	0.85
Lactose	1.31	1.35	1.30	1.30	1.41	1.40

Contrasts:

1	HSB	vs	LSB	Significant,	$p < .05$
2	HSB	vs	FM	Significant,	$p < .05$
3	HSB	vs	CG	Significant,	$p < .05$
4	FM	vs	CG	Significant,	$p < .05$
5	CG:SB	vs	FM:SB	Significant,	$p < .05$

similar to those reported by Oldham et al.(1985) and Zerbini (1986). Storry (1977) suggested two possible interactions of residual fish oil present in fishmeal and mammary de novo triglyceride synthesis. First, fatty acids of fish oil may inhibit lipoprotein lipase activity and decrease uptake of fatty acids by the mammary gland. Storry also reported fish oils shift rumen fermentation to lower acetate:propionate ratio which would also lower availability of milk fat precursor acetate. Furthermore, fishmeal protein contains a high level of quickly degraded nitrogen (fraction A) but low levels of slowly degraded nitrogen as reported by Zerbini and Polan (1986). Animals in this study were fed twice daily and although rumen ammonia was adequate as defined by Satter and Roffler for microbial growth and activity after feeding, ammonia availability throughout the day might have limited microbial activity. This theory is similar to that proposed by Sniffen and Russell (1987) who suggested a constant supply of nutrients to rumen microbes would optimize microbial growth and fermentation. Residual fish oils in fishmeal might also inhibit fiber digestion by inhibiting microbial attachment and digestion. Stewart (1977) reported addition of tallow to rumen fluid decreased in vitro cellulolytic activity as measured by cotton fiber disappearance. Milk fat depression response to fishmeal was further substantiated by lowered milk fat with inclusion of fishmeal in place of soybean meal in contrast to CG:SB combination diet. Low fiber diets stimulate increased blood insulin levels which stimulate lipogenesis and decrease acetate availability for milk fat synthesis. Parker (University New Castle, unpublished data) reported inclusion of fishmeal in grass silage diets increased blood propionate and insulin levels in sheep portal blood. Thus, two stimuli proposed by Smith and Prior (1984) to affect lipogenesis are increased by inclusion of fishmeal in the diet. Therefore, low fiber diets probably enhanced the depressing effect of fishmeal and residual oils in FM and FM:SB.

Milk fat percentage was not different ($p < .05$) between LSB and HSB (Figure 3). These data contradict results of Jaquette et al. (1986) who reported increased milk fat content on 23 % CP diets versus 12 % CP diets. Diets used by these workers contained chopped alfalfa as the sole forage. Rakes (unpublished data) has been unable to attain a milk fat response to protein when corn silage was the only forage. Differences between forages might be responsible for different responses to

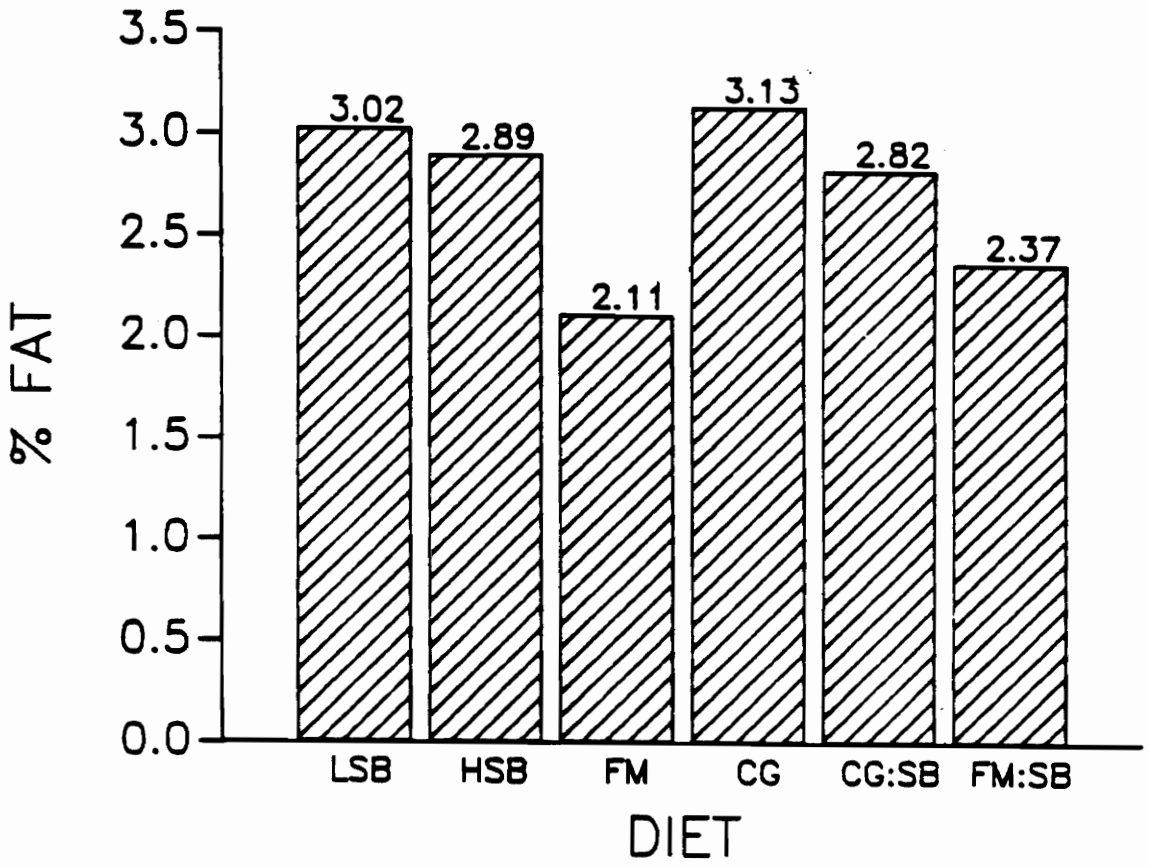

Figure 3. Effect of diet on milk fat percentage.

Contrast:

LSB	vs	HSB	Nonsignificant	$p < .05$
HSB	vs	FM	Significant	$p < .05$
HSB	vs	CG	Nonsignificant	$p < .05$
FM	vs	CG	Significant	$p < .05$
CG:SB	vs	FM:SB	Significant	$p < .05$

protein level. Schingoethe et al.(1976) found hay shifted rumen VFA concentrations to a higher acetate:propionate and higher rumen fluid pH versus corn silage diets. Cows fed alfalfa, brome hay and haylage compared to corn silage based diets produced significantly less short chain fatty acids. Fermented forage diets had more 18:1 fatty acid secreted in milk than hay diets. This indicates forage selection may be important in ration design for feeding cows when animals border on milk fat depression.

Hypothesis of milk fat synthesis inhibition is further supported by differences in actual milk fat yields. FM gave significantly lower milk fat yields in contrast to HSB and CG (Figure 4). Depressed milk fat percent was not soley due to dilution, but actual fat yield was depressed. Inclusion of fishmeal in FM:SB depressed fat versus fat yield on CG:SB, but differences were not significant (Figure 4).

Four percent fat-corrected-milk yields were significantly different ($p < .05$) between diets (Figure 5). Due to depressed milk fat yields coupled with no significant differences in milk yields, CG and HSB had higher FCM yields in contrast to FM. This would be expected since FCM is a function of milk and milk fat yields. These results are economically important since dairy farmers are paid on the basis of volume and fat content of their milk.

Milk protein content did not differ significantly by diet ($p < .05$) with mean range of 2.95 to 3.20% (Table 7). Burgess and Nicholson (1984) reported significant increases in % milk protein with increased dietary protein level. In a second experiment, however, these workers found no differences evident. Barney et al. (1981) found a linear response in milk protein percent with increased protein intake. Literature varies in responses reported with respect to increased protein content of diet and milk protein percent.

Diet did significantly affect protein yields (Figure 6). HSB had significantly higher milk protein yield versus LSB. These results were similar to those reported in the literature which show increased protein yields with increased protein intake as Jaquette et al. (1986) reported for cows fed low fiber

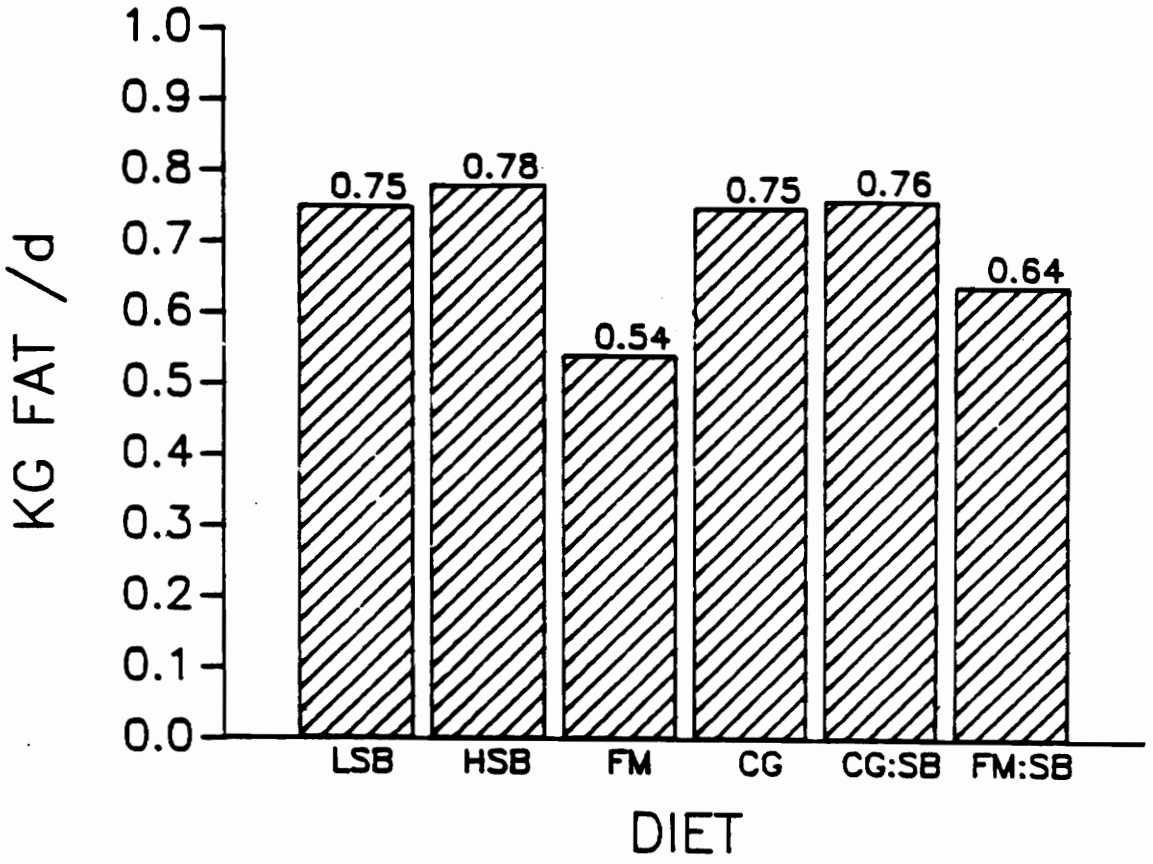

Figure 4. Effect of diet on milk fat yield.

Contrast:

LSB	vs	HSB	Nonsignificant	$p < .05$
HSB	vs	FM	Significant	$p < .05$
HSB	vs	CG	Nonsignificant	$p < .05$
FM	vs	CG	Significant	$p < .05$
CG:SB	vs	FM:SB	Nonsignificant	$p < .05$

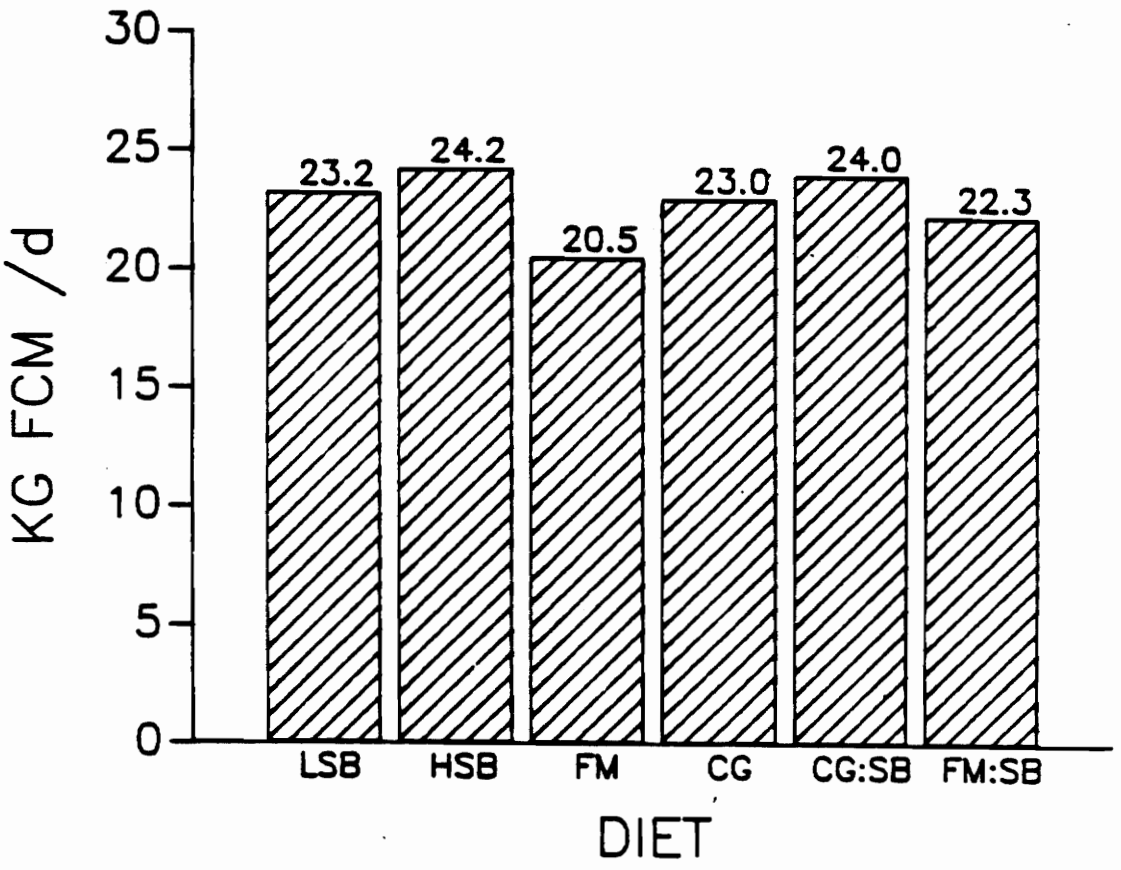

Figure 5. Effect of diet on fat corrected milk yield.

Contrast:

LSB	vs	HSB	Nonsignificant	$p < .05$
HSB	vs	FM	Significant	$p < .05$
HSB	vs	CG	Nonsignificant	$p < .05$
FM	vs	CG	Significant	$p < .05$
CG:SB	vs	FM:SB	Nonsignificant	$p < .05$

diets. In contrast, Van Horn and Jacobson (1971) reported significantly higher solids-corrected-milk with increased protein intake, but no differences in milk nitrogen secretion. HSB and FM also yielded significantly more milk protein than CG. Although these diets had a range in %CP, CPI was similar across diets (Table 5). Differences in protein production might be due to differences in protein quality which is actually available for use by the animal. Broderick et al. (1967) reported limiting amino acids for milk production were methionine, valine, and lysine. Orskov (1982) showed several amino acid levels in microbial protein which were inadequate with respect to levels in milk protein. Therefore, HSB and FM could be enhancing protein quality by supplementing amino acids that were limiting milk protein synthesis and yield. Zerbini (1986) reported fishmeal increased flow of methionine to the small intestine. However, presence in duodenal digesta does not equal availability as amino acid must be made available at the tissue level for utilization.

Based on DMI, microbial activity and growth might have been limited on CG, which could theoretically limit flow of microbial protein to the small intestine. Addition of soybean to CG for CG:SB diet improved intake possibly by stimulating microbial activity which would increase availability of energetic substrates as well as microbial protein.

Lactose content of milk was significantly ($p < .05$) affected by diet with means ranging from 5.00 to 5.30% (Figure 7). CG and HSB had significantly higher lactose percent than FM. Percent lactose was significantly higher on CG:SB than FM:SB. Lactose is produced via de novo synthesis by secretory epithelial cells of the mammary gland from glucose. Generally, little variation occurs in lactose percent and is usually unaffected by diet. Jenness (1971) cites two studies which found underfeeding decreased milk lactose percent while overfeeding increased lactose percent. Under normal nutritional status, lactose percent varies little in dairy cattle. At present, no explanation can be offered to explain these differences. No differences existed in lactose yields.

Rumen and Blood Metabolites Average rumen pH ranged from 6.3 to 6.6 across diets but no significant differences were measured (Table 8). Normal rumen pH is near neutral (approximately 6.8). Low fiber diets have been reported to cause significant decreases in rumen pH, sometimes

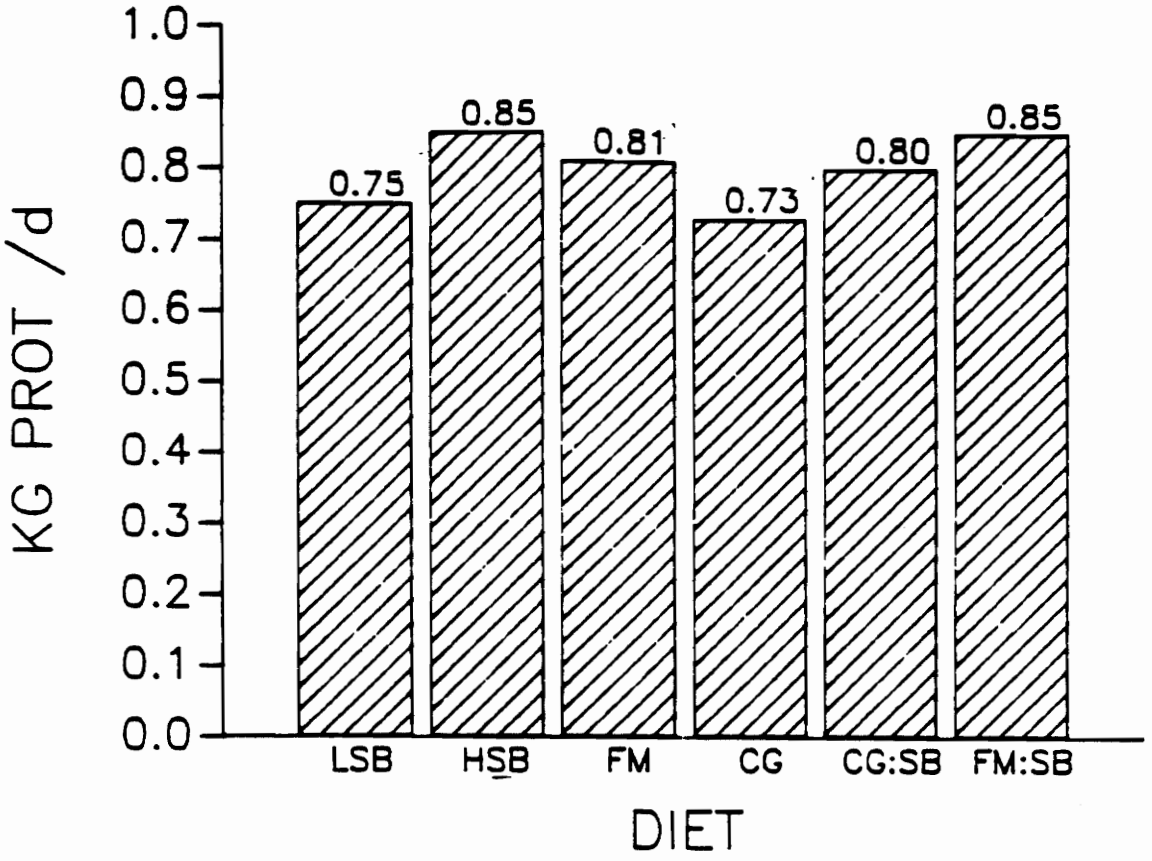

Figure 6. Effect of diet on milk protein yield.

Contrast:

LSB	vs	HSB	Significant	$p < .05$
HSB	vs	FM	Nonsignificant	$p < .05$
HSB	vs	CG	Significant	$p < .05$
FM	vs	CG	Significant	$p < .05$
CG:SB	vs	FM:SB	Nonsignificant	$p < .05$

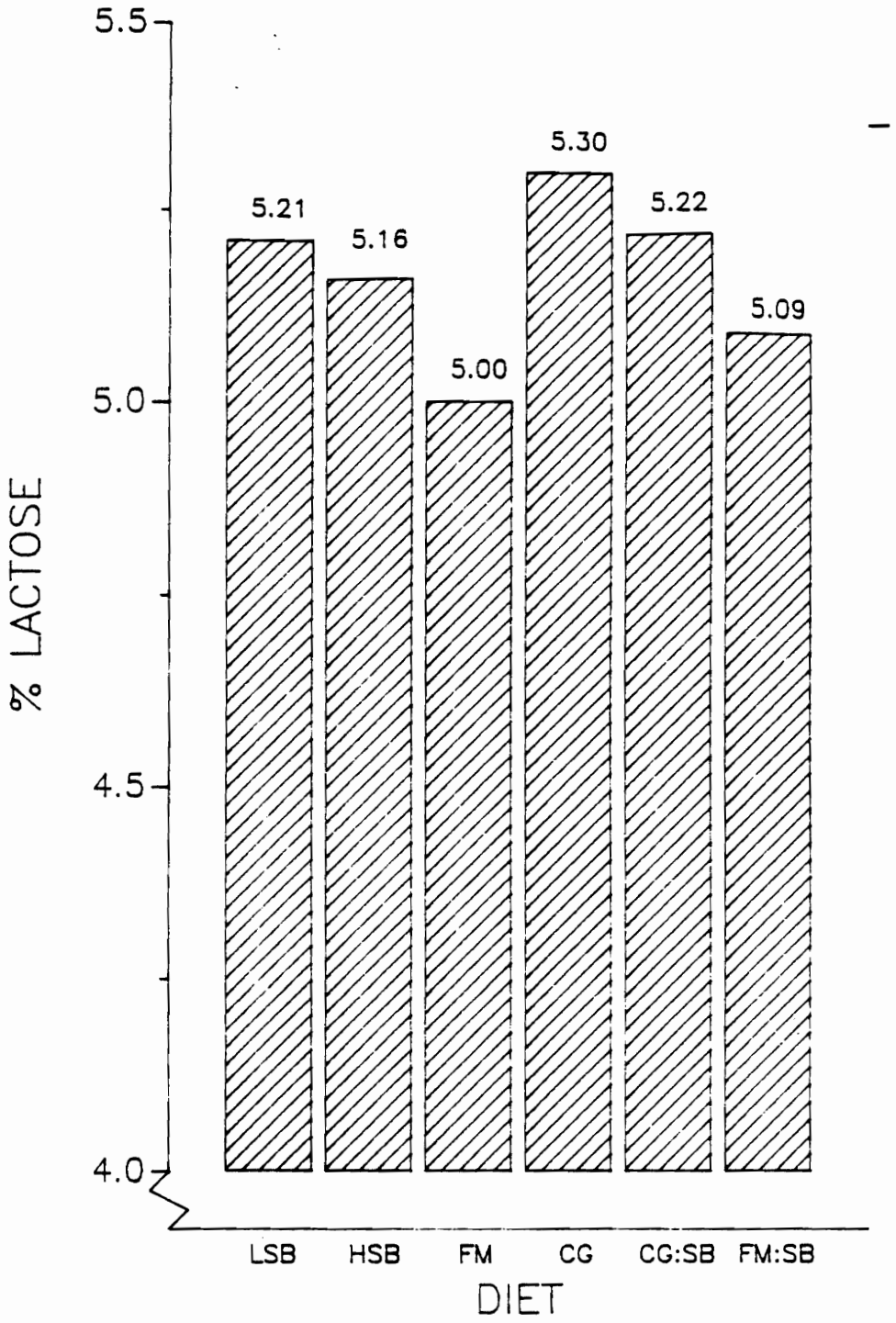

Figure 7. Effect of diet on milk lactose percentage.

Contrast:

LSB	vs	HSB	Nonsignificant	$p < .05$
HSB	vs	FM	Significant	$p < .05$
HSB	vs	CG	Significant	$p < .05$
FM	vs	CG	Significant	$p < .05$
CG:SB	vs	FM:SB	Significant	$p < .05$

below 5.7 (Davis,1967; Slyter,1976). Rumen fluid samples were taken by stomach tube and contamination by saliva is highly possible. Some studies indicate a difference can exist across regions of the rumen. High protein levels have also been found to act as a buffering agent in the rumen. Although mineral mix fed in the ration did not contain buffers, mineral mix that was available in salt boxes did contain buffers which could have possibly affected pH measurements.

Rumen ammonia nitrogen means ranged from 7.3 to 13.6 mg per dl and were significantly affected by diet (Table 8). LSB had significantly less rumen ammonia than HSB (Figure 8). These data agree with data of several workers who report that increasing degradable protein levels in the diet results in increased rumen ammonia concentrations. Increased ammonia indicates ammonia release is occurring faster than microbial utilization. Levels of ammonia on LSB (7.3 mg/dl) meet minimum requirements set forth by Satter and Roffler of 5 mg/dl. Although adequate levels were present at sampling, nitrogen might have become limiting between meals. Furthermore, low fiber diets decrease rumination and therefore salivary nitrogen flow to the rumen. Rumen ammonia was also significantly lower on CG than HSB (Figure 8). Both diets were balanced to contain 20% CP. CG contained higher %CP but intake of degradable CP was lower on CG than HSB. CG, as indicated by degradability values in literature, has a lower level of ruminally -degradable protein versus soybean meal (Zerbini,1986, NRC,1985). Therefore less CG protein is degraded in the rumen which decreases level of ammonia. Furthermore, a slower release of ammonia allows for better microbial utilization given adequate energy and carbon available. Lower DMI of diet CG might have limited the availability of these required nutrients.

Plasma ammonia means ranged form 6.1 to 8.1 μg per dl but were not affected by diet (Table 8). LSB diet tended to have lower plasma ammonia than diets containing higher crude protein. Possible explanations may be, first, total nitrogen entering the circulatory system was elevated by high protein feeding. Secondly, a small range of plasma ammonia indicates animals were converting excess ammonia to urea, a less toxic compound while maintaining a steady plasma ammonia level. HSB and CG:SB had highest plasma ammonia. Similar ammonia levels in the plasma indicated proper maintenance of potential toxic metabolites by the animals.

Table 8. Least square means of rumen pH, ammonia, and plasma ammonia, urea, and insulin.

Metabolite	Diet					
	LSB	HSB	FM	CG	CG:SB	FM:SB
Rumen pH	6.4	6.3	6.6	6.6	6.5	6.5
Rumen ammonia[1,3] (mg/dl)	7.3	13.5	12.1	9.8	13.6	12.1
Plasma ammonia (μg/dl)	6.1	7.9	7.1	7.6	8.1	7.8
Plasma urea [1] (mg/dl)	6.4	16.2	13.7	13.7	17.0	14.1
Plasma insulin (ng/ml)	1.4	1.2	1.2	1.4	1.4	1.2

Contrasts:

1	HSB	vs	LSB	Significant,	$p < .05$
2	HSB	vs	FM	Significant,	$p < .05$
3	HSB	vs	CG	Significant,	$p < .05$
4	FM	vs	CG	Significant,	$p < .05$
5	CG:SB	vs	FM:SB	Significant,	$p < .05$

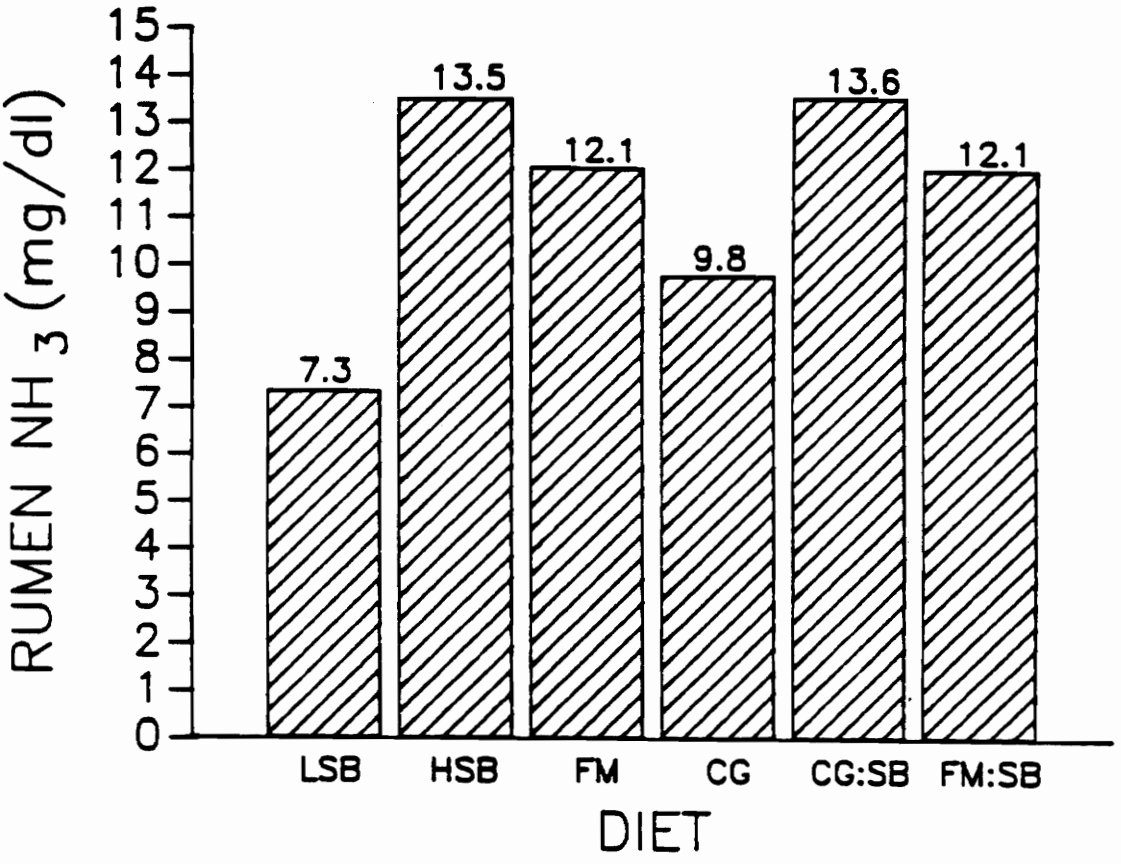

Figure 8. Effect of diet on rumen ammonia.

Contrast:

LSB	vs	HSB	Significant	$p < .05$
HSB	vs	FM	Nonsignificant	$p < .05$
HSB	vs	CG	Significant	$p < .05$
FM	vs	CG	Nonsignificant	$p < .05$
CG:SB	vs	FM:SB	Nonsignificant	$p < .05$

Diets also differed significantly with regard to plasma urea (Table 8). LSB had significantly lower plasma urea levels than HSB (Figure 9). These data are similar to those of others (Zerbini,1986; Jaquette,1986). There were no differences between high protein diets ranging from 13.7 to 17.0 mg/dl. Dietary protein can be degraded at primarily three different locations. First, ruminal degradation provides nitrogen for microbial growth with excess ammonia absorbed. Secondly, undegraded protein and microbial protein can be deaminated by microbes in the small intestine and hindgut. Finally, excess absorbed amino acids are deaminated at the liver and ketoacids can be utilized. In total, this ammonia can be converted to urea if plasma ammonia levels rise above normal. Protein sources fed in this study differ in site of digestion. However, providing equal amounts of dietary nitrogen can result in similar levels of plasma urea as found in this study and reported by others (Zerbini, 1986).

Rumen Volatile Fatty Acids Rumen volatile fatty acid values are shown in Table 9. No significant differences occurred with respect to total volatile fatty acids (VFA) although cows fed CG tended to have lower VFA compared to other 20% CP diets. This would indicate a lower rumen fermentation on diet CG which could limit DMI as discussed earlier. Lowered VFA production would also lower energy flow from the rumen limiting energy available for milk production. This would explain the lower milk production on diet CG.

Diet did not affect acetate, propionate, isobutyrate, butyrate, or valerate molar percent. Molar percentage (M %) acetate means ranged from 62.0 to 70.0 but were not different across treatments. Davis (1967) found high grain diets decreased acetate to less than 60.0 % molar basis. Jaquette et al. (1986) reported decreased acetate M % in cows fed low fiber diets (8-10% ADF). In this study, high levels of protein were fed which can buffer the rumen in contrast to diets fed by Davis. Jaquette and co - workers fed chopped alfalfa hay at lower fiber levels compared to the corn silage diets at 16 % ADF in this trial.

Propionate means ranged from 18.2 to 22.2 M % but was not significantly affected by diet. Bauman et al. (1971) reported high grain diets doubled propionate production in the rumen of dairy

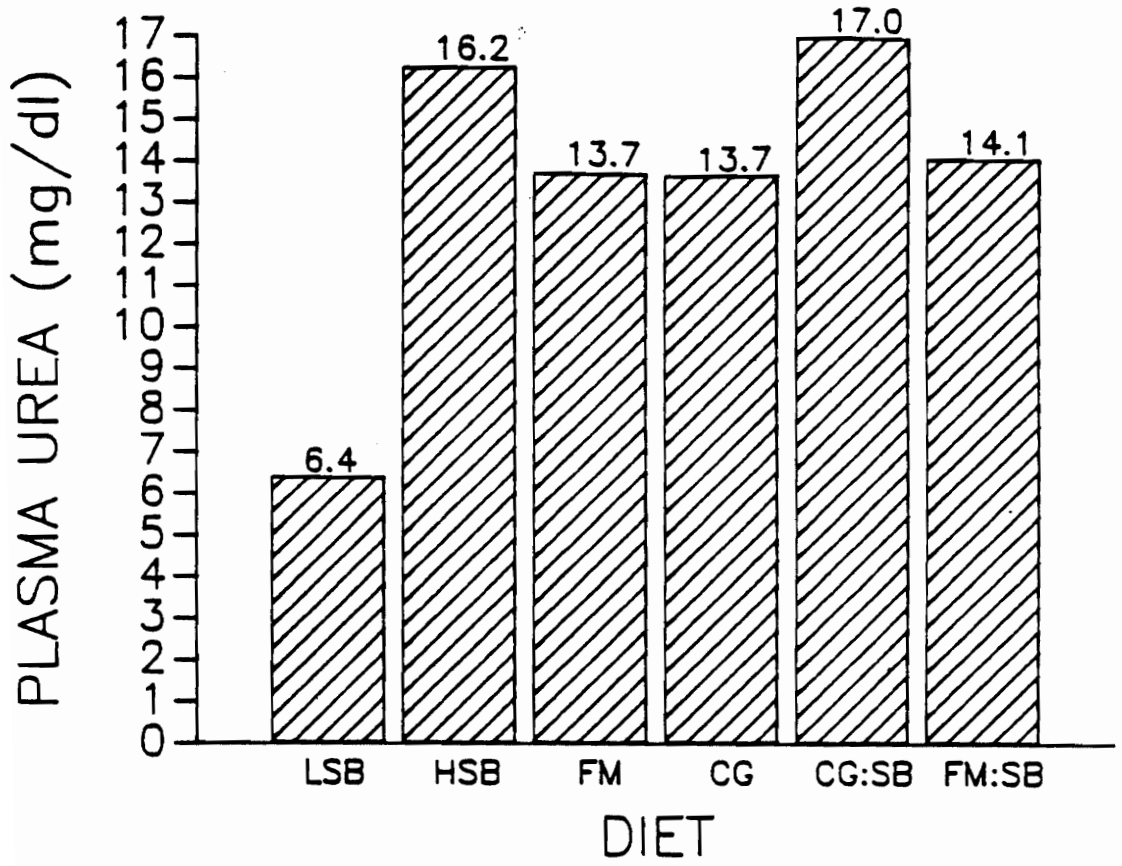

Figure 9. Effect of diet on plasma urea.

Contrast:

LSB	vs	HSB	Significant	$p < .05$
HSB	vs	FM	Nonsignificant	$p < .05$
HSB	vs	CG	Nonsignificant	$p < .05$
FM	vs	CG	Nonsignificant	$p < .05$
CG:SB	vs	FM:SB	Nonsignificant	$p < .05$

Table 9. Least square means of rumen volatile fatty acid concentrations.

	Diet					
	LSB	HSB	FM	CG	CG:SB	FM:SB
Total VFA (mmol/dl)	120.3	154.8	131.3	117.5	122.2	138.0
Acetate (M%)	64.4	70.0	62.9	66.7	62.5	62.0
Propionate (M%)	22.2	18.4	21.6	18.2	22.2	19.4
Isobutyrate (M%)	1.1	1.2	1.3	1.1	1.2	1.3
Butyrate (M%)	9.7	10.1	10.2	11.3	10.8	12.7
Isovalerate[4] (M%)	1.5	1.4	1.9	1.3	1.7	1.8
Valerate (M%)	0.97	0.97	1.60	0.96	1.30	1.47

Contrasts:

1	HSB	vs	LSB	Significant,	$p < .05$
2	HSB	vs	FM	Significant,	$p < .05$
3	HSB	vs	CG	Significant,	$p < .05$
4	FM	vs	CG	Significant,	$p < .05$
5	CG:SB	vs	FM:SB	Significant,	$p < .05$

cows. This study involved high protein diets which can buffer rumen fluid promoting cellulolytic actvivity. Although no buffer was added to the ration, buffer was available in a mineral mix on the exercise lot.

Average butyrate ranged from 9.7 to 12.7 M % but was not significantly affected by treatment. Isobutyrate and valerate were not different between diets averaging 1.1 -1.3 M % and 0.96 - 1.6 M %, respectively. Tamminga (1979) suggested deamination of amino acids occur in the rumen for the production of ammonia and ketoacids. Production of these volatile fatty acids are not different across protein sources added to these diets.

Molar % isovalerate was affected significantly by treatment. Molar % of 1.9 of cows fed FM was significantly higher than 1.3 of cows fed CG. Branch chain volatile fatty acids have been shown to stimulate growth and activity of cellolytic bacteria (Cummins and Papas,1985) and therefore increased VFA production. This could explain higher VFA production on FM diet compared to CG. Higher fermentation also stimulates dry matter digestion and subsequently DMI. This agrees with intake data and also explains numerical differences with respect to milk production between these two diets.

Differences in milk fat % and fat production were not explained by VFA differences. Acetate is slightly lower and propionate higher on FM, but these differences are not of the magnitude to explain differences seen between diets with regard to milk fat production.

Animals did not differ with respect to plasma insulin (Table 8). Diets did not differ in energy level with all diets providing excess energy. Differences did exist with respect to lipid metabolism as indicated by differences in milk triglyceride synthesis. Jenny et al.(1974) found elevated insulin levels were associated with high grain diets and milk fat depression. Associated with increased insulin levels, Jenny and co-workers reported increased plasma propionate. VFA data of the present study did not indicate elevated propionate in the rumen of cows fed high grain. Blood samples were taken at the same time each sampling and only two samples were used. Plasma insulin is better measured

by numerous samples distributed throughout the day as data reviewed by Trenkle (1978) show plasma insulin increase 4 to 6 h after feeding.

Epilogue

Protein source significantly affected milk composition of dairy cows fed high-grain, low-fiber diets. Inclusion of corn gluten or soybean meal yielded significantly more milk fat and fat-corrected-milk compared to inclusion of fishmeal. Previous work with fishmeal indicated this protein source may increase propionate production in the rumen. Increased propionate stimulates deposition of fat in adipose which limits fatty acid availability for milk triglyceride synthesis. Feeding fishmeal, however, had no affect on rumen fermentation but depressed milk fat yields, suggesting a component of fish oil was inhibiting milk fat synthesis. Future work should be directed at identifying this agent and determining the level of this agent which inhibits fat synthesis.

Soybean meal and fishmeal stimulated higher milk protein production than corn gluten meal. Data in the literature indicates amino acid composition differences of small intestine digesta btween protein sources. Amino acid absorption and utilization data could indicate amino acid affect on milk protein synthesis. Hepatic arteriovenous differences would be a logical step in answering this question.

Finally, differences in fat yield and composition across forage types deserves further investigation. Hay, haylage, and corn silage affects fat yield and compostion. Understanding mechanisms in-

volved would allow management of diets with respect to forage composition and physiological status of the animal.

Bibliography

Arambel, M.J. and C.N. Coon.1981. Effect of dietary protein on amino acids and microbial protein of duodenal digesta. J. Dairy Sci. 64:2201.

Ashes, J.R., J.L. Mangan, and G.S. Sidhu.1984. Nutritional availability of amino acids from protein cross -linked to protect against degradation in the rumen. Brit. J. Nutr. 52:239.

Bailey, E.E. and C.G. Balch. 1961. Saliva secretion and its relation to feeding in cattle. 1. the composition and rate of secretion of parotid saliva in a small steer. Brit. J. Nutr. 15:371.

Barnes, M.A., G.W. Kazmer, R.M. Akers, and R.E. Pearson. 1985. Influence of selection for milk yields on endogenous hormones and metabolites in Holstein heifers and cows. J. Anim. Sci. 60:271.

Barney, D.J., D.G. Grieve, G.K. Macleod, and L.G. Young.1981. Response of cows to dietary crude protein during midlactation. J. Dairy Sci.64:655.

Bartley, E.E., A.D. Davidovich, G.W. Barr, G.W. Griffel, A.D. Dayton, C.W. Deyse, and R.M. Bechtle.1974. Ammonia toxicity in cattle I. Rumen and blood changes associated with toxicity and treatment methods. J. Anim. Sci. 43:835.

Bauman, D.E., C.L. Davis, and H.F. Bucholtz. 1971. Propionate production in the rumen of cows fed either a control or high-grain, low-fiber diet. J. Dairy Sci. 54:1282.

Burgess, P.L. and J.W.G. Nicholson.1984. Protein levels in grass silage - based mixed rations for dairy cows in midlactation. Can. J. Anim. Sci. 64:435.

Chalupa, W. 1975. Rumen by - pass and protection of proteins and amino acdis. J. Dairy Sci. 58:1198.

Chalupa, W. 1976. Degradation of amino acids by the mixed rumen microbial population. J. Anim. Sci. 43:828.

Clark J.H.1975. Lactational responses to postruminal adiminstration of proteins and amino acids. J. Dairy Sci. 58:1178.

Cotta, M.A. and J.B. Russell.1982. Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture. J. Dairy Sci. 65:226.

Craig, W.M. and G.A. Broderick.1984. Amino acids released during protein degradation by rumen microbes. J. Anim. Sci. 58:436.

Crooker, B.A., J.H. Clark, R.D. Shanks, and E.E. Hatfield.1986. Effects of ruminal exposure on the amino acid profile of heated and formaldehyde -treated soybean meal. J. Dairy Sci. 69:2648.

Croom, W.J., D.E. Bauman, and C.L. Davis.1981. Methylmalonic acid in low - fat milk syndrome. J. Dairy Sci. 64:649.

Croom, W.J., A.H. Rakes, A.C. Linnerud, G.A. Ducharme, and J.M. Elliot.1981A. Vitamin B_{12} administration for milk fat synthesis in lactaing dairy cows fed a low fiber diet. J. Dairy Sci. 64:1555.

Cummins, K.A., and A.M. Papas.1985. Effect of isocarbon-4 and isocarbon-5 volatile fatty acids on microbial protein synthesis and dry matter digestibility in *vitro*. J. Dairy Sci. 68:2588.

Cummins, K.A., J.E. Nocek, and C.E. Polan.1982. Growth and nitrogen balance of calves fed rations of varying nitrogen degradability and physical form. J. Dairy Sci. 65:773.

Davis, C.L. 1967. Acetate production in the rumen of cows fed either control or low fiber, high grain diets. J. Dairy Sci. 50:1621.

Edwards, J.S., E.E. Bartley, and A.D. Dayton.1980. Effects of dietary protein concentration on lactating cows. J. Dairy Sci. 63:243.

Elliot, J.M., E.P. Barton, and J.A. Williams.1979. Milk fat as related to vitamin B_{12}status. J. Dairy Sci. 62:642.

Erfle, J.D., F.D. Sauer, and S. Mahadevan. 1977. Effect of ammonia cocnetration on activity of enzymes of ammonia assimilation and on synthesis of amino acids by mixed rumen bacteria in continuous culture. J. Dairy Sci. 60:1064.

Esdale, W.J. and L.D. Satter.1972. Manipulation of ruminal fermentation IV. Effect of altering ruminal pH on volatile fatty acid production. J. Dairy Sci. 55:964.

Etgen, W.M. and P.M. Reaves.1978. Dairy Cattle Feeding and Management. Sixth Edition. John Wiley and Sons. N.Y.,N.Y. p. 168.

deFaria, V.P. and J.T. Huber.1984. Effect of dietary protein and energy levels on rumen fermentationin Holstein steers. J. Dairy Sci. 58:452.

Felix, A., R.M.Cook, and J.T. Huber.1980. Isoacids and urea as a protein supplement for lactating cows fed corn silage. J. Dairy Sci. 63:1098.

Folman, Y., H. Neumark, M. Kaim, and W. Kaufman.1981. Performance, rumen and blood metabolites in high -yielding cows fed varying protein percents and protected soybean. J. Dairy Sci. 64:759.

Frobish, R.A. and C.L. Davis.1977. Theory involving propionate and vitamin B_{12}in the low - milk fat syndrome. J. Dairy Sci. 60:268.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. ISU Press, Ames,Iowa.

Grubb, J.A. and B.A. Dehority.1975. Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in sheep. App. Env. Microb. 30:404.

Herbein, J.H., R.J. Aiello, L.I. Eckler, R.E. Pearson, and R.M. Akers.1985. Glucagon, insulin, growth hormone, and glucose concentrations in blood plasma of lactating dairy cows. J. Dairy Sci. 68:320.

Hespell, R.B. 1979. Efficiency of growth of rumen bacteria. Fed Proc. 38:2709.

Hogan, J.P. 1975. Quantitative aspects of nitrogen utilization in ruminants. J. Dairy Sci. 58:1164.

Holter, J.B., W.E. Hylton, and C.K. Bozak.1985. Varying protein content and nitrogen solubility for pluriparous, lactating Holstein cows: lactation performance. J. Dairy Sci. 68:1984.

Huber J.T. and RL Boman.1965. Effect of grain level and protein of the grain for grazing cows on milk composition and yield and certain blood and rumen constituents. J. Dairy Sci. 48:395.

Huber, J.T., R.A. Sandy, C.E. Polan, H.T. Bryant, and R.E. Blaser.1967. Varying levels of urea for dairy cows fed corn silage as the only forage. J. Dairy Sci. 50:1241.

Huber, J.T. and L. Kung.1981. Protein and nonprotein nitrogen utilization in dairy cattle. J. Dairy Sci. J. Dairy Sci. 64:1170.

Hume I.D., R.J. Moir, and M. Somers.1970. Synthesis of microbial protein in the rumen I. Influence of level of nitrogen intake. Aust. J. Agric. Res. 21:283.

Hume, I.D. 1970A. Synthesis of microbial protein in the rumen III. The effect of dietary protein. Aust. J. Agric. Res. 21:305.

Hume, I.D.1970. Synthesis of microbial protein in the rumen II. A response to higher volatile fatty acids. Aust. J. Agric. Res. 21:297.

Ibrahim, E.A. and J. R. Ingalls.1972. Microbial protein biosynthesis in the rumen. J. Dairy Sci. 55:971.

Janicki, F.J., J.B. Holter, and H.H. Hayes.1985. Varying protein content and nitrogen solubility for pluriparous, lactating Holstein cows: digestive performance during early lactation. J. Dairy Sci. 68:1995.

Jaquette, R.D., A.H. Rakes, and C.J. Croom. 1986. Effects of dietary protein on milk, rumen, and blood parameters in dairy cattle. J. Dairy Sci. 69:1026.

Jenny, B.F., C.E. Polan, and P.T. Chandler.1972. Acetate and stearate utilization by lactating cows fed high grain -restricted roughage rations. J. Nutr. 104:379.

Jenny, B.F., C.E. Polan, and F.W. Thye.1974. Effects of high grain feeding and stage of lactation on serum insulin , glucose, and milk fat percentage in lactating cows. J. Nutr. 104:379.

Jenny, B.F. and C.E. Polan.1975. Postprandial blood glucose and insulin in cows fed high grain. J. Dairy Sci. 58:512.

Jorgensen, N.A. and L.H. Schultz.1965. Ration effects on rumen acids, ketogenesis, and milk composition. II. Restricted roughage feeding. J.Dairy Sci. 48:1040.

Konig, B.A., J.D. Oldham, and D.S. Parker.1984. The effect of abomasal infusion of casein on acetate, palmitate, and glucose kinetics in cows during early lactation. Brit. J. Nutr. 52:319.

Kung, L. and J.T. Huber.1983. Performance of high producing cows in early lactation fed protein of varying amount, sources, and degradability. J. Dairy Sci. 66:227.

Leng, R.A. and J.V. Nolan.1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072.

Lough, D.S., E.C. Prigge, W.H.Hoover, and G.A. Varga.1983. Utilization of ruminally infused acetate or propionate and abomasally infused casein by lactating goats. J. Dairy Sci. 66:756.

Mackie, R.I. and F.M.C. Gechrist.1979. Changes in lactate producing and lactate utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high concentrate diet. App. Env. Microb. 38:442.

McCulluogh, H. 1967. The determination of ammonia in whole blood by direct calometric method. Clin. Chem. Acta. 17:297.

Merchen, N., T. Hanson, and T. Klopfenstein.1979. Ruminal by - pass of brewers dried grains protein. J. Dairy Sci. 49:192.

Miller, C.N., C.E. Polan, R.A. Sandy, and J.T. Huber.1969. Effect of altering the physical form of corn silage on utilization by dairy cattle. J. Dairy Sci. 52:1955.

Muller, L.D., D. Rodriguez, and D.J. Schingoethe.1975. Formaldehyde treated whey protein concentrate for lactating dairy cows. J.Dairy Sci. 58:1874.

National Research Council. 1985. Subcommittee on Nitrogen Usage in Ruminants. Ruminant Nitrogen Usage. National Academy Press. Washington, D.C.

Nocek, J.E., J.H. Herbein, and C.E. Polan.1983. Total amino acid release of soluble and insoluble protein fractions of concentrate feedstuffs by *Streptomyces griseus*. J. Dairy Sci. 66:1663.

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

Oldham, J.D., D.J. Napper, T. Smith, and R.J. Fulford.1985. Performance of dairy cows offered isonitrogenous diets containing urea or fishmeal in early and in mid - lactation. Brit. J. Nutr. 53:337.

Orskov, O.R. 1982. Protein nutrition in ruminants. Academic Press. N.Y., N.Y.

Owens, F.N. and H.R. Isaacson. 1977. Ruminal microbial yields: factors influencing synthesis and by -pass. Fed. Proc. 36:198.

Pilgrim, A.F., F.V. Gray, R.A. Weller, and C.B. Belling. 1970. Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. Brit. J. Nutr. 24:589.

Polan, C.E., C.N. Miller, and M.L. McGilliard.1976. Variable dietary protein and urea for intake and production in Holstein cows. J. Dairy Sci. 59:1910.

Polan, C.E., T.A. Herrington, W.A. Wark, and L.E. Armentano.1985. Milk producton response to diets supplemented with dried brewers grains, wet brewers grains, or soybean meal. J. Dairy Sci. 68:2016.

Prior, R.L. and S.B. Smith. 1982. Hormonal effects on partitioning of nutrients for tissue growth: role of insulin. Fed. Proc. 41:2545.

Ranwana, S.S.E. and R.C. Kellaway.1977. Responses to postruminal infusions of glucose and casein in lactating goats. Brit. J. Nutr. 37:395.

Rock, C.G., C.E. Polan, W.M. Etgen, C.N. Miller.1974. Varying dietary fiber for lactating cows fed corn and barley silages. J. Dairy Sci. 57:1474.

Russell, J.B. and R.B. Hespell.1981. Microbial rumen fermentation. J. Dairy Sci. 64:1153.

Russell, J.B. and T. Hino. 1985. Regulation of lactate production in *Streptococcus bovis*: a spiraling effect that contributes to rumen acidosis. J. Dairy Sci. 68:1712.

Russell, J.B. and C.J. Sniffen. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in *vitro*. J. Dairy Sci. 67:987.

Satter, L.D. and R.E. Roffler.1975. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.

Scheiflinger, C., N. Russell and W. Chalupa.1976. Degradation of amino acids by pure cultures of rumen bacteria. J. Anim. Sci. 43:828

Schingoethe, D.J., H.H.Voelker, G.L. Beardsley, and J.G. Parsons. 1976. Rumen volatile fatty acids and milk composition from cows fed hay, haylage, or urea-treated corn silage. J. Dairy Sci. 59:894.

Slyter, L.L. 1976. Influence of acidosis on rumen function. J. Anim. Sci. 43:910.

Sniffen, C.J. and P.H. Robinson. 1987. Microbial growth and flow as influenced by dietary manipulations. J. Dairy Sci. 70:425.

Statistical Analysis System Institute Inc. 1985. SAS User's Guide. SAS Inst., Cary, N.C.

Stern, M.D., K.A. Santos, and L.D. Satter.1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat treated whole soybeans. J. Dairy Sci. 68:45.

Stewart, C.S. 1977. Factors affecting the cellulolytic activity of rumen contents. App. Env. Microb. 33:497.

Storm, E. and E.R. Orskov.1984. The nutritive value of rumen micro - organisms in ruminants IV. The limiting amino acids of microbial protein in growing sheep determined by a new approach. Br. J. Nutr. 52:613.

Storry, J.E., P.E. Brumby, A.J. Hall, and B. Tuckley 1974. Effects of free and protected codliver oil on milk fat secretion in the dairy cow. J. Dairy Sci. 57:1046.

Sudweeks, E.M., M.E. McCullough, L.R. Sisk and S.E. Law. 1975. Effects of concentrate type and level and forage type on chewing time of steers.

Sudweeks, E.M. 1977. Chewing time, rumen fermentation, and their relationship in steers as affected by diet composition. J. Anim. Sci. 44:694.

Tammminga, S. and J.D. Oldham.1980. Amino acid utilization by dairy cows II. Concept of amino acid requirements. Livestock Prod. Sci. 7:453.

Tamminga, S.1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49:1615.

Thorton, R.F. 1970. Urea excretion in ruminants II. Studies in sheep whose rumen contents were replaced with physiological saline. Aust. J. Agric. Res. 21:337.

Thorton, R.F.1970C. Urea excretion in ruminants III. The role of the hindgut. Aust. J. Agric. Res. 21:345.

Thorton, R.F.1970B. Urea excretion in ruminants. I. Studies in sheep and cattle offered the same diet. Aust. J. Agric. Res. 31:323.

Trenkle, A. 1978. Relation of hormonal variations of nutritional studies and metabolism of ruminanats. J. Dairy Sci. 61:281.

Van Horn, H.H. and D.R. Jacobson.1971. Response of lactating cows to added increments of dietary protein and nonprotein nitrogen. J. Dairy Sci. 54:379.

Van Soest, P.J. 1964. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency: A review. J. Anim. Sci. ??:204.

Van Soest, P.J. 1982. Nutritional Ecology of the ruminant. O and B Books, Inc. Corvallis, Oregon.

Wangsness, D.J. and L.D. Muller.1981. Maximum forage for dairy cows: review. J. Dairy Sci. 64:1.

Zanartu, D., C.E. Polan, L.E. Ferreri, and M.L. McGilliard.1983. Effect of stage of lactation and varying available energy intake on milk production, milk compositon, and subsequent tissue enzymic activity. J. Dairy Sci. 66:1644.

Zerbini, E. 1896. Effects of dietary soybean meal and fishmeal on protein digesta flow in Holstein cows during early and midlactation. PhD. Thesis. Virginia Polytechnic Institute and State University.

Zerbini, E. and C.E. Polan. 1986. Protein sources evaluated for ruminating calves. J. Dairy Sci. 68:1416.

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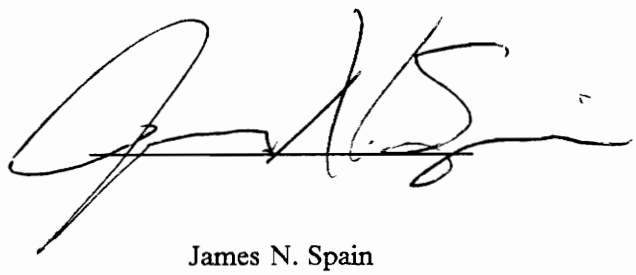

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