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Responses to abomasal infusion of casein, hydrolyzed casein or methionine-lysine and dietary protein degradability in lactating cows.

by

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(ABSTRACT)

Responses to daily abomasal infusions of 400 g sodium caseinate, 400 g hydrolyzed casein or 11.3 g L-methionine-30.1 g L-lysine and rumen degradability of dietary protein were studied in eight Holstein cows during mid-lactation. Basal diets contained corn silage, corn and either soybean meal or 60:40 soybean meal:corn gluten meal, and had estimated rumen degradabilities of 70 and 60.5%. Duodenal cannulas were installed in four of the cows to allow measurement of digesta composition and flow.

Methionine-lysine infusion increased milk protein percentage on both diets and milk fat percentage and yield on the soybean meal diet. Sodium caseinate increased milk and milk protein production, bodyweight gain and decreased milk fat percentage, but not yield. Hydrolyzed casein did not produce the same responses, suggesting differences in amino acid absorption and utilization between the sources. Basal diet affected the responses to abomasal infusions demonstrating that amino acid nutrition of the cow was affected by dietary protein degradability.

Concentration of total essential amino acids, branched chain amino acids and urea cycle amino acids were increased by the infusion of the caseins. There were differences between the caseins in their effects on individual plasma free amino acids. Methionine-lysine infusion increased plasma lysine and taurine, a metabolite of methionine, suggesting that infused methionine was extensively metabolized.

Total duodenal nitrogen flow and non-microbial nitrogen flow tended to be increased by inclusion of corn gluten meal in the diet. Rumen degradation of crude protein was greater for the soybean meal diet. Both diets had lower rumen degradability than predicted from previous experiments.

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Review of Literature

Introduction

Although protein nutrition of dairy cattle has been studied extensively in terms of response to dietary crude protein level and source (76,85), the precise amino acid requirements for lactation are not well defined. However, newer methods of ration formulation (76) require estimates of absorbed amino acids and daily amino acid requirements. Pregastric fermentation of feed in ruminants necessitates the sampling of postruminal digesta in order to quantitate the flow of amino acids available for digestion. Postruminal infusion of amino acids can help delineate limiting amino acids for milk production.

Abomasal infusion of sodium caseinate (25,26) or protein concentrates (92) increases milk and milk protein yield 10 to 15% in cows producing 30 or more kg/d of milk and fed to meet crude protein requirements. However the specific amino acids responsible for the response have not been clearly identified. In one series of experiments, infusion of methionine and lysine accounted for 47% of the milk protein response obtained with casein (101). Some experiments with rumen protected methionine plus lysine or methionine have increased milk protein yield in lactating cows (93) and

yields of milk, fat-corrected milk and protein in primiparous heifers (50,56). However the relative effect of methionine-lysine supplementation versus that of casein is not clear.

Recently the National Research Council published a method of formulating dairy cattle rations based on the rumen degradability of dietary crude protein (76,100). This system attempts to maximize the postruminal protein supply by manipulating the amount of crude protein degraded in the rumen. The objective is to provide enough rumen degradable crude protein to meet microbial requirements, while allowing any additional dietary nitrogen (protein) to escape the rumen and be digested directly in the stomach and intestine. Diets formulated according to this system should more adequately meet the amino acid requirements of the cow as compared to diets based solely on crude protein content.

The first objective of this study was to compare abomasal infusions of sodium caseinate, casein hydrolysate, methionine-lysine or water for effects on lactational performance and plasma free amino acid concentrations in lactating cows. Casein hydrolysate was included to test the hypothesis that an intact protein will be more efficiently utilized than the corresponding mixture of free amino acids (116). The second objective was to test the effects of rumen degradability of dietary protein on the response to postruminal infusions. A diet formulated based on crude protein was compared to one formulated based on rumen degradable nitrogen (76), designed to increase postruminal protein supply. Thirdly, four duodenally cannulated cows were included in the study in order to test the effects of treatments on the flow and chemical composition of postruminal digesta.

Nitrogen Utilization in Dairy Cattle

Nitrogen utilization by dairy cattle has been the subject of numerous studies, but the exact amino acid requirements for lactation and the means of meeting these requirements are unclear. The

complexity of ruminant digestive physiology and its varying responses to feeds of different origin and quality has so far defied any precise modeling or predictive system for nitrogen requirements. However, the discovery that the supply and amino acid compositon of postruminal protein is affected by the extent of rumen degradation of feed protein and by other dietary factors such as forage quality (70), quantity of readily degradable carbohydrate (97) and level of intake (65,88), raised the possibility that amino acid requirements could be balanced in ruminants as they have been in monogastric species. In swine and poultry, balancing dietary amino acid levels to match requirements has resulted in significant increases in feed efficiency. This includes the elimination of excess amino acids from the diet (5). It has been estimated that up to 40 percent of ingested amino acids may be lost during metabolism due to imbalances in supply of individual amino acids (5). Clearly there is great potential to improve feed efficiency in ruminants by more exactly meeting amino acid requirements, thus conserving feed protein and reducing operating costs for the producer.

In order to implement ration balancing for amino acids in ruminants, two major sources of information are required (16):

1. Exact amino acid requirements for lactation

2. The quantity of digestible amino acids supplied to the small intestine by a given diet

Neither has been easy to determine or predict.

Amino Acid Requirements for Lactation

Amino acid requirements are generally defined in terms of the essential or nonessential amino acids (5,106). These are the amino acids which cannot be synthesized by the body at rates sufficient to meet requirements and must therefore be present in the diet or, in the case of the ruminant animal,

synthesized by rumen organisms. This is not to say that the balance of nonessential amino acids absorbed is not important. Growth rates and efficiency are reduced when a single precursor amino acid such as glutamic acid is fed in place of a mixture of nonessential amino acids (5).

Research has shown that in terms of growth and maintenance, ruminants require the same 10 essential amino acids as the rat; phenylalanine, valine, leucine, isoleucine, threonine, tryptophan, methionine, lysine, arginine and histidine (69,76). It was also demonstrated that rumen bacteria synthesize all of these amino acids from nonprotein nitrogen (6,30). However, other experiments have shown that animal performance is enhanced by postruminal infusion of protein or amino acids (14,24,101), which led many to conclude that microbial protein supply was not sufficient to meet the amino acid requirements of high producing dairy cows or rapidly growing stock (100). Intact feed protein also contributes to the postruminal supply, so emphasis has been placed on feeding proteins which resist ruminal degradation in hopes of increasing the net protein supply to the animal (25,72,100). This is theoretically possible providing that microbial protein synthesis is not reduced due to a lack of nitrogen in the rumen and providing that the escaping feed protein contains the proper balance of digestible amino acids relative to animal requirements. This will be discussed later in relation to meeting amino acid requirements of dairy cattle.

Several experimental approaches have been employed in attempts to identify limiting amino acids for milk production in dairy cattle. The limiting amino acid is that amino acid which is in shortest supply relative to demand. Addition of the limiting amino acid to the diet results in increased growth (or milk production) until either the requirement is met or another amino acid becomes limiting (5). The latter occurs when other amino acids are not being absorbed in amounts equal to their requirement. The animal's genetic potential, energy status, stage of lactation etc. will determine the upper limit on milk production (75) and therefore will determine the maximum utilization rate for amino acids. When an amino acid is presented to the mammary gland in amounts equal to its maximum utilization rate then its requirement has been met and milk production is not limited by that amino acid.

Some of the approaches used to determine limiting amino acids for milk production in lactating ruminants include:

1. Postruminal infusion of protein and/amino acids

2. Plasma amino acid analysis and uptake by the mammary gland

3. In vitro culture of mammary tissue

4. Calculation based on amino acid composition of postruminal digesta and milk

Postruminal infusion of sodium caseinate increases milk and milk protein production 10 to 15% depending on initial level of production and stage of lactation (24,91,92,117). The response is greater when cows are held in negative energy balance by limiting intake, and is greater than would be predicted based on the energy content of the casein infused (117). These findings support the hypothesis that specific amino acids present in casein are limiting for milk and milk protein synthesis. Other possible explanations for the stimulating effect of casein on milk production are:

1. increased glucose supply via gluconeogenesis from nonessential amino acids.

2. stimulation of growth hormone secretion by certain individual amino acids (26).

With regard to the first effect, it was reported that casein infusion resulted in higher plasma concentrations of glucagon (29), suggesting an increase in gluconeogenic activity by the liver. Growth hormone was not significantly increased by casein infusion (29). Excess amino acids can be oxidized to provide energy directly to the mammary gland (27,69) or to the liver, thus sparing glucose for use in lactose synthesis. Extensive interconversion of glucose, acetate and amino acids have been demonstrated in lactating cows (36). A greater proportion of labeled glutamic acid was recovered in lactose than in milk protein (36).

Another possible explanation for the increase in milk volume observed with casein infusion could be a stimulation of alpha-lactalbumin synthesis by the mammary gland. Alpha-lactalbumin is a major milk secretory protein and is also a subunit of the enzyme lactose synthetase (60). Lactose is the major osmoregulatory molecule in milk (60) so an increase in its synthesis generally leads to increased milk volume.

Analysis of plasma amino acid profiles, aterio-venous differences for plasma amino acids across the mammary gland and in vitro incubation of mammary tissue have been used in attempts to identify which specific amino acids may be most limiting for milk production in dairy cattle. Comparisons of the amino acid composition of rumen microorganisms or postruminal digesta with that of milk have also been employed.

The amino acid(s) whose concentration in arterial plasma or digesta is lowest relative to its concentration in milk protein can potentially be most limiting for milk protein synthesis (27). Based on this rationale, methionine, lysine and phenylalanine have been designated as most limiting by several workers (15,26,35). Another approach is to supplement animals with increasing increments of postruminal protein and monitor changes in plasma amino acid concentrations. The amino acid which accumulates at the slowest rate in response to supplementation is designated most limiting. Based on this analysis methionine, lysine, phenylalanine, valine and threonine were found to be most limiting (15). Recently the keto analog of leucine was shown to increase fat and fat corrected milk yield in lactating cows (113). Leucine has a stimulatory effect on muscle protein synthesis in non-ruminants (11) and further studies of its effect on mammary protein synthesis would seem appropriate. Generally the branched chain amino acids and arginine are not considered to be limiting because they are taken up by the mammary gland in excess of their output in milk (13,69,81). However these amino acids act as precursors of certain nonessential amino acids which are not taken up in quantities equal to their output in milk, and could therefore play key roles in the intermediary metabolism of the mammary gland (27,69). Mammary arterio-venous differences for plasma amino acids have shown that cystine, phenylalanine, methionine and lysine are extracted in marginal amounts relative to their output in milk (13,27,81). In vitro culture of mammary tissue

also identified cystine as most limiting for milk protein synthesis, followed by threonine and methionine (28). A major source of cystine for the lactating mammary gland may be the tripeptide glutathione (69). Methionine in turn is a precursor of both cystine (38) and glutathione in ruminants (108). The results of in vivo and in vitro analysis suggest that methionine may be first limiting and phenylalanine, lysine and threonine next limiting for milk protein synthesis in lactating cows.

In order to test the above hypothesis some investigators have infused individual amino acids postruminally or fed methionine and lysine in a form which protects them from rumen degradation. Schwab et al.. (101) found that methionine plus lysine infusion accounted for 47% of the milk protein response observed with a complete mixture of essential amino acids. Methionine, lysine and threonine increased milk and milk protein production relative to control infusion (ammonium citrate) and accounted for 60.7 and 58.6% of the increases in milk and milk protein yields obtained from infusion of 425 g/d of sodium casein (101).

A number of experiments have tested the effects of rumen protected methionine products on lactational performance of dairy cattle. Some studies involving the hydroxy analog of methionine found improvement in fat percentage and fat corrected milk yield in cows fed conventional silage based diets (53,84). However, methionine hydroxy analog was found to have significant effects on rumen microbial metabolism, so the true effect of methionine on the host animal metabolism is not clear (19). Rumen protected methionine or postruminal methionine infusion have in some cases increased milk protein percentage and yield (44,56). A mixture of rumen protected methionine and lysine increased milk protein percentage in 18 midlactation cows (93). Methionine supplementation has had more dramatic effects when nutrient intake was marginal. Milk protein percentage and yield was increased by methionine infusion in low producing cows fed only pasture or pasture silage (91). In that study methionine also enhanced the effect of casein infusion on milk production and fat yield (91). Intravenous methionine increased milk fat yield in cows fed a grass silage based diet calculated to be deficient in methionine delivery to the intestine (18), and increased milk protein yield in cows fed a diet with urea as the major source of supplemental nitrogen (44).

Both protected and unprotected D-L methionine have increased yields of milk, fat and protein during early lactation in mixed groups of mature cows and first-calf heifers fed conventional corn silage/soybean meal and grass silage/corn silage/concentrate diets (50,56). In the latter case analysis was done separately for cows and heifers. The results show that the responses in milk and milk component yield were almost totally confined to the heifers. The beneficial effect of methionine in lactating heifers is probably related to the methionine requirement for growth. Methionine and lysine have been shown to be first and second limiting for growth in ruminants (106).

To summarize, several essential amino acids have been identified as potentially limiting for milk protein synthesis and perhaps for milk yield in lactating cows. Of these only methionine has been tested to any extent via infusion or feeding in protected form. Some benefit has been derived especially in lactating heifers. Lysine has been tested only a few times, once in combination with methionine. The other candidates; phenylalanine, threonine and the branch chain amino acids have been tested to a limited extent in just one experiment.

Metabolic Roles of Methionine and Lysine

Methionine and lysine are in the group of essential amino acids whose uptake by the lactating mammary gland just equals their output in milk protein (69). These two amino acids are also intermediates in key metabolic functions outside the mammary gland which constitutes part of their overall requirement.

Methionine is a major source of methyl groups and the key intermediate in nearly all biological methylation reactions (68). Methionine plus adenosine triphosphate (ATP) combine to form S-adenosyl methionine, which in turn acts as methyl group donor to some forty acceptor molecules (68). A wide variety of compounds including choline, creatinine and cystine are formed in this way. N-formyl-methionine is the universal initiator of protein synthesis. Methionine is also used in the

synthesis of spermidine (as are arginine and ornithine) which stimulates protein synthesis in mammary tissue in vitro, mimicking the effects of glucocorticoids (27,69).

Of particular interest in ruminants is the role of methionine as precursor of choline and cystine. Choline supply may be limited in ruminants due to ruminal destruction and limited re-synthesis (34,39). Choline is required for synthesis of phosphatidylcholine, a phospholipid required for normal lipoprotein structure and which is present in large amounts in bile (68). Radiotracer data from goats showed that 28% of the methionine pool may be converted to choline (38). In the 1960's methionine deficiency was implicated as the cause of low milk fat syndrome and ketosis (66,67). Abnormal lipoprotein structure and a decrease in the ratio of cholesterol esters to triglycerides in the beta lipoprotein fraction were reported during low milk fat syndrome, along with an increase in the ratio of unsaturated to saturated C:18 fatty acids in milk (19). Intravenous methionine apparently corrected these abnormalities while increasing milk fat percentage (67). Abnormal lipoprotein structure, resulting from a shortage of methionine, and hence phosphatidylcholine, was hypothesized to have decreased transfer of triglycerides to the mammary gland (66,67). This is a plausible explanation, as phosphatidylcholine is required for the formation of cholesterol esters of long chain fatty acids (118), which are integral components of lipoproteins. However little supporting evidence for the theory has been found since. As mentioned earlier, methionine increased milk fat yield in cows fed a grass silage based diet which was likely to be deficient in methionine (18). More detailed studies of this area are warranted.

Methionine can be major source of cystine in ruminant animals. Up to 60% of infused methionine was converted to cystine in sheep (108), presumably via hepatic transulphuration reactions (45). Duodenal infusion of methionine increased the concentration of reduced glutathione in sheep (108). Glutathione, a cystine containing tripeptide (68), has been shown to be taken up in significant amounts by the mammary gland of lactating cows (69). In view of these findings and the low rate of cystine synthesis by rumen microorganisms (106), conversion of methionine to cystine may be quantitatively important in lactating cows. No follow-up studies have been conducted concerning glutathione metabolism in lactating cows.

Metabolic roles of lysine are primarily related to formation of connective tissues and fiberous proteins (68). N-N-N-trimethyl-lysine is component of connective tissue and precursor of carnitine (68). Other lysine derivatives include desmosine used in elastin formation, e-N-methyl-lysine and 5-hydroxy-lysine in collagen (68).

Factors Affecting Postruminal Amino Acid Supply, Composition and Digestibility in Ruminants

The shortcomings of crude protein (N x 6.25) as a system for formulating ruminant diets is clear. Newer formulation systems proposed emphasize the quantity of amino acid nitrogen reaching the small intestine as a critical factor in meeting the protein requirements of ruminants (16,76,94). The amount of amino acid nitrogen (AAN) leaving the rumen is determined by the relative rates of:

1. protein and non-protein nitrogen hydrolysis within the rumen

2. microbial incorporation of nitrogen (N) containing compounds from the soluble phase of rumen digesta

3. solid and liquid phase outflow.

Rumen hydrolysis of dietary proteins can proceed from peptides to free amino acids to ammonia (6,23), but the proportion of peptides, amino acids and ammonia at any given time postfeeding are not necessarily the same for every protein source (22). The chemical composition and hydrolytic properties of the non-protein fraction of dietary N may vary as well, although it is assumed that these compounds are very rapidly converted to ammonia (34).

Peptides accumulate in rumen fluid after feeding (22,23) and are rapidly utilized by rumen bacteria (6). Some evidence suggests that production and bacterial uptake of peptides may be the rate limiting step in proteolysis, and a crucial step in rumen microbial protein synthesis (23). Free amino acids and ammonia are readily utilized, and in some cases required, by rumen bacteria. About 80% of rumen isolates can grow on ammonia as their sole source of nitrogen (6,34). However, addition of amino acids to a purified urea diet stimulates microbial growth yields (63). The three major cellulolytic species all require ammonia and branched chain volatile fatty acids (BCVFA) (5,34). Among the four major starch digesting species there are species requiring amino acids, ammonia and BCVFA (6). The saccharolytic species, which feed on digestion products of the first two groups, have similar requirements to the starch digesting species (6). The major proteolytic species are members of the saccharolytic group (6). In view of the adaptability of this group, it is not surprising that rumen proteolytic activity remains high across a variety of diets (100).

Assuming the availability and right proportion of N containing compounds in the rumen, the major factor affecting the synthesis of microbial protein is the quantity of fermentable energy in the diet (94). In fact it is the quantity of energy liberated from feed and converted to ATP by the microbes which sets the requirement for nitrogen (34). In addition to the total quantity available, the timing of energy release from feeds must be synchronized with release of N compounds in order to maximize microbial protein synthesis (34,104). Little attention has been paid to this area. For example, microbial protein synthesis is often greater with barley than with corn grain because corn is relatively resistant to ruminal degradation (31,94). Rumen microbial protein yields are also dependent on high quality forage in the diet needed to promote a favorable rumen environment without limiting intake of metabolizable energy (34,70,76,104).

Another consideration is the recycling of blood urea back into the rumen. Blood urea is hydrolyzed by a subgroup of bacteria adhering to to rumen wall (34). Diffusion of urea across the wall appears linked to hydrolysis (58) and is inversely proportional to rumen ammonia concentration (58). Thus there is a mechanism by which blood urea derived from a previous meal can recycle back into the rumen to meet N requirements of bacteria when rumen ammonia concentration falls.

A third interrelated factor affecting rumen microbial protein synthesis is the rate of rumen digesta outflow. Rumen contents are usually separated into at least two phases; solid-particulate, associated with the fiberous portion of the diet, and liquid-small particle, which is thought to include most of the dietary concentrates (34,37). Both phases contain large numbers of bacteria and protozoa (34,104). Outflow rates determine the residence time of both feeds and bacteria. In theory, increased rumen turnover will increase microbial protein yield, but also decreases ruminal digestion of organic matter and N compounds (34). This in turn could result in shortages of either energy or soluble N compounds between feedings and result in reductions in microbial protein synthesis (34,96,104).

In cattle, solids turnover rate is negatively related to dietary energy density (41). Roughage stimulates rumination and saliva flow promoting a more favorable rumen environment for fiber digesting bacteria (34). Faster rates of digestion and particle size reduction will increase rate of passage of particulate matter from the rumen (34). The amount of indigestible fiber fed will also be a factor. Poorer quality forage will digest more slowly and decrease rumen solids turnover (41). Solids turnover will also increase with increasing levels of intake (41).

Liquid turnover rate is increased by increased saliva flow (40). (Saliva flow in cattle averages 125 L/d, while water intake averages 30 L/d.) (34). Rumen liquid phase turnover increases with level of intake and with the amount of roughage in the diet when fed at a constant intake (40). In general liquid outflow is increased by the same factors as solid outflow rate, with intake having the most effect.

Increased liquid and solids outflow from the rumen will increase the outflow of bacteria from the rumen, thus increasing the yield of bacterial protein (104). Efficiency of bacterial protein production may increase as the higher washout rate allows fewer bacteria to live beyond the initial growth phase (104).

Increased rumen outflow rates also increase the amount of intact feed protein and soluble nitrogen leaving the rumen. The outflow or escape of feed protein has received considerable attention from the standpoint of increasing the supply of amino acid nitrogen to the host (37,100). This will be true providing that microbial protein synthesis is not reduced and that the escaping feed protein is mostly digestible in the intestines. The outflow of soluble N compounds such as ammonia may also be important, as it was recently shown that ammonia accounts for about 50% of the total nitrogen absorbed from the G.I. tract of dairy cows (55). Thus the cow's capacity to synthesize amino acids from ammonia and carbon skeletons would be of interest.

Recent Studies of Amino Acid Outflow from the Rumen of Cattle

Robinson et al.. (88) studied ruminal digestion, bacterial yields and flow of non-ammonia nitrogen (NAN) to the duodenum in dairy cows consuming 6.0, 10.6, 14.8 or 17.4 kg of organic matter (OGM) per day. Apparent ruminal digestion of OGM, N, acid and neutral detergent fibers (ADF and NDF) decreased as intake increased, while duodenal NAN flow increased linearly from 97.4 to 402.8 g/d. NAN as a percent of N intake also increased significantly. Bacterial N flow to the duodenum increased from 64.0 to 271.0 g/d, and the efficiency of bacterial N synthesis increased from 17.4 to 38.2 g/kg apparently digested OGM. Similar trends were found by McAllan and Smith (65) using steers.

Rode et al.. (89) examined the effects of percent forage in the diet (24, 38, 58 or 80% alfalfa hay) and forage particle size on ruminal digestion and microbial protein synthesis in lactating dairy cows. Efficiency of microbial protein synthesis (MPS) was decreased by the higher concentrate diets and was positively correlated ($p < .05$) with rumen turnover rate. Grinding the 80% alfalfa diet increased MPS 15%. Efficiency of MPS was positively related to rumen solids turnover but negatively related to liquid dilution rate. Therefore the rate of solids turnover may be the more accurate index of MPS.

Another area of interest is the effect of feeding frequency on rumen microbial protein synthesis. The few studies undertaken so far have not shown any significant effect of increased feeding frequency on MPS (76). However these studies did not record the actual number and size of meals for comparison between the different feeding regimines.

Increasing dietary N concentration has sometimes increased MPS, often in a quadratic manner (95) as one particular level of dietary N best matches the energy supply from the basal diet. In cattle fed silage, which contains a significant proportion of soluble non-protein nitrogen compounds, MPS was stimulated by continuous intraruminal infusion of glucose or glucose plus casein (97). MPS was not increased by the infusion of water, urea or casein alone, suggesting that the primary deficiency was that of energy required for microbial incorporation of ammonia,amino acids or peptides (97).

Source of nitrogen in the diet has been shown to affect rumen microbial protein synthesis. Zinn et al..(122) compared several protein sources for effects on NAN and bacterial N flow to the duodenum of calves. Dietary crude protein levels were high (23%). Neither microbial nitrogen nor NAN flows were significantly different between protein sources, but microbial N flow was numerically highest with the more soluble/degradable protein sources; soybean meal, linseed meal and urea. Total flow of NAN was ranked as follows:

- experiment 1; corn gluten meal, soybean meal, cottonseed meal, urea, casein;
- experiment 2; meat and bone meal, linseed meal, corn gluten meal, cottonseed meal, soybean meal, urea.

Therefore, rumen escape protein did impact total NAN flow.

In lactating cows, soybean meal promoted greater yields of microbial protein at the duodenum when compared to fishmeal, while total flows of protein were not different between the two supplements (21,120). These two studies encompassed four ratios of soybean meal to fishmeal (100:0,

66:33, 33:66, 0:100) in corn silage-corn diets fed from early through mid-lactation. Santos et al. (99) compared soybean meal, corn gluten meal, dried distillers grains and wet brewers grains in corn silage-hay-corn based diets fed to lactating cows at 16% crude protein. No significant difference in duodenal bacterial N flow was found. Influx of blood urea N into the rumen, reflected by a net negative apparent rumen N digestibility may have supplied ammonia for rumen microbial protein synthesis when the less degradable proteins were fed. Total duodenal NAN flow was higher for corn gluten meal and dried distillers grains due to undegraded feed protein.

In steers fed corn-oat straw diets at 12% crude protein, linseed meal and soybean meal supplementation produced the highest flows of bacterial N to the duodenum versus corn gluten meal or urea (47). Efficiency of bacterial synthesis of N containing compounds (g/g organic matter truly digested in the rumen) was lowest for the corn gluten meal diet. Flow of NAN (g/d) to the duodenum was higher with soybean meal (144.7) than for urea (127.2). Values for linseed meal (143.5) and corn gluten meal (131.7) were not different from either soybean meal or urea. In this study the two more soluble protein sources produced superior flows of NAN to the duodenum. In this case the diet was fairly low in total nitrogen, and the basal portion of the diet would supply little readily soluble nitrogen for rumen hydrolysis. Therefore the the faster degrading protein supplements would be beneficial, providing the necessary N compounds for microbial growth. Rumen ammonia concentrations were lower for corn gluten meal than for the other three N sources. These results show the importance of meeting microbial N requirements when feeding less degradable N sources.

The effect of moisture content of alfalfa haylage at harvesting on protein digestion was examined in cows during early lactation (70). Diets consisted of alfalfa haylage harvested at 29, 40 or 66% dry matter or alfalfa hay plus a corn-urea concentrate mix. Non-ammonia nitrogen flow to the duodenum as a percent of N intake was greater for alfalfa haylage harvested at 66% dry matter versus the other three forages. Bacterial N flow was not different between forages, but was numerically highest for the 40% dry matter haylage.

Although the rumen fermentation is very complex, with many interrelated factors affecting the outflow of protein, a recent study has shown that duodenal flow of non-ammonia nitrogen (NAN) in dairy cows can be predicted based on intake of metabolizable energy and rumen undegraded protein (94). Regression analysis of 84 experiments conducted by the authors were used as the data base. The best prediction equation (smallest coefficient of variation) was applicable to the results of 69 other experiments from the literature (U.S.A. and U.K.). It was also found that amino acid nitrogen comprised a relatively constant proportion (0.7) of duodenal NAN. Thus the flow of amino acid nitrogen to the duodenum could be predicted fairly precisely from dietary parameters. These results re-emphasize the importance of fermentable energy supply in determining rumen microbial protein yield.

Effects of Diet on Duodenal Flow and Digestibility of Amino Acids

In the previous section, the effects of diet on the flow of non-ammonia nitrogen to the duodenum and on the proportion of NAN accounted for by bacterial nitrogen were discussed. However, in order to delineate amino acid requirements of the ruminant, it is necessary to know the amino acid composition of postruminal digesta and its digestibility in the small intestine (16).

In the study of Santos et al..(99) total duodenal flow of amino acid nitrogen was highest when the diet contained corn gluten meal or dried distillers grains versus soybean meal or wet brewers grains. However the quantity of absorbed amino acids (determined from ileal cannulae) averaged 2198, 1750, 1507 and 1545 g/d for corn gluten meal, dried distillers grains, soybean meal and wet brewers grains. Therefore, merely increasing the flow of amino acids to the duodenum does not automatically increase the amount of amino acids absorbed by the host animal. Differences in intestinal digestibility between the protein sources were evenly distributed across individual amino acids. In general the essential amino acids had a higher digestibility. Leucine supplied and absorbed was particularily high for corn gluten meal.

Garrett et al.. (47) found no significant differences between urea, soybean meal, linseed meal or corn gluten meal in the total amino acid supply to the duodenum and digestibility in the small intestine. As discussed earlier, the duodenal flow of NAN was highest for soybean meal and nearly equaled by linseed meal. Total amino acid flows reflected NAN flow, but were not significantly different, perhaps due to fewer observations.

In a study with sheep (61) fed corn-soy diets of 8, 11, 14 or 17% crude protein, duodenal flows of essential amino acids showed a significant positive correlation with crude protein percent in the diet. Nitrogen retention was also increased by increasing dietary crude protein. Microbial nitrogen contributed to the increase in postruminal amino acid supply.

Zerbini (121), using lactating cows at two stages of lactation, found that duodenal recovery of both nonessential and essential amino acids was higher when corn based diets were supplemented with fishmeal as compared to soybean meal. Amino acid composition was not markedly affected by protein supplement except that duodenal methionine was higher, and cystine lower, for fishmeal versus soybean meal. Microbial protein yield and efficiency was higher for soybean supplemented diets.

Formaldehyde treatment of soybean meal increased duodenal flows of total essential and nonessential amino acids versus untreated soybean meal in steers fed grass silage diets (96). Individual flows of arginine, lysine, valine, aspartic and glutamic acids and serine were increased by formaldehyde treatment. Formaldehyde treatment of rapeseed meal did not significantly increase amino acid supply, perhaps because this source is less degradable in the untreated form, and contains less amino acid nitrogen than soybean meal. Soybean meal supported higher yields of bacterial protein.

Merchen and Satter (70) reported that duodenal flow of total amino acids and nonessential amino acids were higher in lactating cows fed alfalfa haylage harvested at 66% dry matter versus that harvested at 29% DM or as hay. Haylage harvested at 40% dry matter gave intermediate values

for amino acid flow. Digestibility of individual amino acids in the small intestine were generally similar although higher valine and phenylalanine absorption occured with 66 versus 40% DM haylage. Therefore total absorbed amino acids tended to increase for the high dry matter haylage. This silage contained less buffer-soluble nitrogen and ammonia nitrogen than the lower dry matter silages.

Comparisons of soybean meal to dried brewers grains, a less degradable protein source, revealed that soybean meal supported greater microbial protein synthesis, while dried brewers grains escaped the rumen in larger amounts tending to increase the postruminal flow of certain essential amino acids (3).

Mathers et al. (64) evaluated the biological value of fish meal (FM) and sunflower meal (SFM) in rats before and after a six hour in situ rumen incubation. The protein efficiency ratio (weight gain/weight of protein consumed) for FM, FM + residue, SFM and SFM + residue were: 2.91, 2.93, 2.41 and 2.06. (Casein + methionine = 3.01). Apparent N digestibilities were higher for FM than SFM, but not significantly decreased by rumen incubation, suggesting that the lower nutritive value of SFM residue was caused by a change in amino acid composition.

In conclusion, postruminal supply of amino acids can be increased by inclusion of proteins which resist ruminal degradation in the diet. This may be of benefit to the animal providing that a deficiency exists for the amino acids in question and providing that neither microbial protein yield nor intestinal digestibility of amino acids is reduced. The more soluble/degradable protein sources such as soybean and linseed meals support higher levels of microbial growth. Partial replacement of these sources with less degradable proteins may be beneficial at higher levels of intake and dietary crude protein, situations where the microbial requirement for soluble nitrogen (i.e. ammonia, peptides and free amino acids) is being exceeded. More accurate descriptions of rumen microbial N requirements and predictions of postruminal digestibility of proteins are needed.

Digestibility and Nutritive Value of Rumen Microbial Protein

The digestibility and nutritive value of rumen microbial protein is of great importance in the scheme of ruminant amino acid nutrition. At least 50% (and probably more) of the amino acids absorbed by the host are of microbial origin (34,76,94). Some recent studies have addressed this issue.

Salter and Smith (98) reported the intestinal digestibility of &sup15.N labeled rumen bacterial protein (minus nucleic acids) to be 74%. Isotope dilution studies using the labeled protein and &sup14.C urea showed the overall efficiency of utilization (digestibility * N retention) to be 52%. Storm and Orskov (106) estimated the nutritive value of rumen microbial protein in lambs maintained solely on intraruminal infusion of volatile fatty acids and abomasal infusion of microbial protein or test mixtures of amino acids. Digestibility of microbial protein was 84.7%. In previous studies the authors calculated the efficiency of use of absorbed amino acids from rumen microbial protein as being 80% (107). This would indicate a net efficiency of utilization of 67.4%. Efficiency of utilization calculated by difference between the low and high levels of infusion of microbial protein is 51%, similar to Salter and Smith. Methionine was the amino acid most limiting for growth of the lambs (106). Exclusion of lysine, arginine and histidine from the test mixture of amino acids also reduced growth, and these were also limiting in rumen microbial protein (106). The conservative figure of 70% digestibility of rumen microbial protein adapted by most protein formulation systems seems appropriate (76).

Postruminal Digestion and Amino Acid Absorption in Ruminants

Gastric and Pancreatic Digestion of Protein

Protein and polypeptides stimulate abomasal secretion of HCl and pepsin (as pepsinogen), an endopeptidase active at pH 2 to 3 (10,102). Pepsin preferentially cleaves internal peptide bonds at the carboxy terminal of hydrophobic amino acids (10,68).

Continuous infusion of soy protein into the abomasum of sheep resulted in an initial increase in retention of digesta (111). However, 52 hours after the start of infusion, duodenal flow rate and abomasal volume had both increased, partially due to an increase in abomasal secretions (111). There was a tendency toward increased retention of digesta in the proximal duodenum. The authors conclude that despite a considerable increase in protein entering the abomasum (100 g/d), the retention of digesta was not markedly altered such that peptic digestion would not be decreased.

Pancreatic secretions of ruminants contain a typical array of mammalian proteolytic enzymes including the endopeptidases trypsin, chymotrypsin and pancreopeptidase and the exopeptidases carboxypeptidase A and B (10,102), as well as elastase (103). Pancreatic secretions of the ruminant also contain a high level of nuclease activity (10), consistent with the large input of microbial nucleic acids (11% of total N; 34). The majority of pancreatic enzymes display a pH optimum of 7.5 to 8.0 (8,10,102). Some evidence suggests that in the ruminant the pH of intestinal digesta does not exceed 7.0 until the mid-ileum (10), but a recent study showed considerable absorption of nitrogen prior to the ileum in sheep (103). Some of this nitrogen could have been in the form of ammonia, which was shown to constitute upwards of 50% of total N absorbed from the gut of ruminants (54,55).

Digestion and absorption of N compounds was studied in sheep by sampling at several sites along the gastrointestinal tract after slaughter with &sup41.Ce used as a non-absorbable marker (103).

Results from five sheep showed that pancreatic secretions nearly doubled the flow of total N through the duodenum. This input far outweighs the contribution of sloughed cells and other endogenous secretions reported for cattle maintained on intragastric infusion, 6.2 g/d for a 600 kg cow (78). Activities of the proteolytic enzymes were highest in the first 4 meters of the small intestine (103). Initially the activities of carboxypeptidase A and B exceeded that of trypsin and chymotrypsin. Elastin had the lowest total activity. At 4 M from the pylorus, the four major proteolytic enzymes showed similar activities. These values were determined at pH 7.5 (optimum) and therefore reflect total amount of intact enzyme. Other studies suggest that intestinal pH of ruminants does not reach 7.5 until the mid-jejunem or ileum (9,76).

The largest disappearance of nitrogen occurred in the duodenum and jejunum of the sheep (103). Small peptides and amino acids accumulated in this region however, suggesting that rate of hydrolysis exceeded rate of absorption. Because peptides of molecular weight 7,000 to 14,000 accumulated in all regions of the small intestine, the authors concluded that hydrolysis of these peptides was the rate limiting step in digestion. These peptides may be remnants of the proteolytic enzymes.

Ben-Ghedalia et al. (8) examined protein digestion in sheep cannulated at several sites along the small intestine. Maximal activity of proteolytic enzymes measured at in situ pH was 7 meters (28%) along the small intestine. Peptide and free amino acid N accumulated between 1 and 3 meters. The peptide fraction disappeared between 3 and 15 M, with maximal disappearance from 7 to 15 M. Free amino acids were absorbed from 7 to 25 M.

In a later study (9) the same author compared intestinal protein digestion and absorption in sheep fed a basal diet of barley, citrus pulp, cottonseed meal and soybean oil, with and without duodenal infusion of 100 g/d sodium caseinate. Sheep were cannulated .05, 11 and 25 M from the pylorus. Doudenal flows of amino acid N were 85.1 and 177 g/d for control and infusion. Apparent digestibilities were 66.6 and 80.9% respectively. Residual amino acid N at the terminal ileum was 28.4 and 33.8 g/d, a relatively constant value which may reflect endogenous secretions. It might

be expected that digestive enzymes and other compounds would be resistant to digestion so that their activity would not be destroyed. A much larger proportion of amino acid N was absorbed distal to 11 M when casein was infused, demonstrating that although the middle third of the intestine is usually the region of greatest N absorption, there is considerable capacity for amino acid absorption available in the ileum if needed. Absorption of individual amino acids were quite similar for the basal diet. During casein infusion isoleucine, valine, threonine, proline, glutamic and aspartic acids tended to be absorbed to a greater extent than the other amino acids. The pH of digesta at 11 M was 7.5 to 7.6. Total activities of trypsin, chymotrypsin and carboxypeptidase (11 M) were increased by casein infusion. Therefore the ruminant intestine was capable of adapting to an increased protein load, actually increasing digestibility.

Peptide Hydrolysis at the Brush Border Membrane

A major phase of intestinal protein digestion occurs at the brush border membrane. Both the brush border and the cytosol of enterocytes contain an array of peptidases (10). Recently a study was made of peptidase activity in the intestinal mucosa of sheep (87). Mucosal homogenates from several sites along the small intestine were tested for activity towards six dipeptides. Activity for all peptides tested was lowest in the proximal duodenum, increased through the jejunum and peaked in the mid-ileum (87). Activity towards two tripeptides followed a similar pattern. Inhibitor studies revealed that dipeptidase activity was primarily localized in the cytosol, while tripeptidases were about evenly distributed between cytosol and the brush border. Tetrapeptidase activity was confined mainly to the brush border. These results agree with findings in non-ruminants (1). Thus peptidase activity coincided with the region of greatest absorption of amino acid N as reported by others (10,103). The relative distribution of peptidase activity suggests that dipeptides and some tripeptides may be absorbed intact into the enterocyte but that intracellular hydrolysis is likely, (other studies show that intracellular dipeptidases are capable of hydrolyzing a wide array of

peptides (1). However, peptides could be resynthesized by the cell and released into the bloodstream.

Amino Acid and Peptide Transport Across the Intestinal Mucosa

Soluble nitrogen compounds may cross the intestinal mucosa by several mechanisms including; 1) diffusion, as is certainly the case with ammonia, and which accounts for part of the amino acid transport (33,73). 2) facilitated diffusion and 3) active transport where energy (ATP) is expended to create an ion gradient across the cell membrane the dissipation of which drives transport of amino acids or peptides through a specific membrane macromolecule (68). Conceivably, part of the energy liberated from peptide hydrolysis could be used for transport processes.

Ammonia may account for over 50% of the total nitrogen absorbed in the ruminant (54,55). Most of the ammonia crosses the rumen wall by diffusion (58). The reverse process is significant when rumen ammonia concentrations are low, i.e. between feedings (58). Some ammonia is absorbed from the omasum, abomasum and intestines as well (54). Part of this ammonia may be derived from deamination of amino acids or hydrolysis of nucleic acids. The extent to which the deaminated carbon skeletons of amino acids are absorbed has not been determined. Ammonia entering the portal blood must be converted to urea by the liver. Hepatic capacity to synthesize amino acids from ammonia and carbon skeletons should also be important.

Mechanisms of amino acid transfer across the intestinal mucosa of ruminants have only recently been investigated. Studies using brush border membrane vesicles prepared from the small intestine of steers have shown that transport of methionine, lysine, phenylalanine, alanine and proline occur via both diffusion and active transport (73). Methionine was transported by several active routes sometimes in combination with alanine and phenylalanine (73). Methionine and phenylalanine inhibited transport of the other amino acids tested (33,73). Similar results were found using isolated strips or loops of sheep intestine (49). Methionine transport has generally been most efficient and

tends to inhibit the transport of other amino acids. The apparent Km for methionine transport was quite low is quite low, indicating a high affinity between methionine and its transporter (49). The transport of essential amino acids appears to take precedence over the nonessential.

In two recent studies, methionine and lysine uptake was most active in mucosal strips taken from the mid-ileum of beef steers and heifers. Transport was least in the proximal jejunum (49). These findings agree with previous in vivo studies (8,9,83). Saturating concentrations were 1.0 mM for methionine and 0.5 to 1.0 mM for lysine. The authors concluded that these systems would probably not be saturated in vivo. Lysine transport was sodium-independent while methionine transport had both sodium stimulated and sodium independent components. This is at variance with results with membrane vesicles where sodium stimulation was detected for lysine transport (73). Lysine transport was inhibited by ornithine and arginine (structurally similar) and by methionine. Methionine transport was inhibited by leucine. In non-ruminants a wide array of competitive interactions between free amino acids for transport have been found. This may partially explain why intact protein is absorbed more efficiently than a corresponding mixture of free amino acids (116). Intact protein would allow for peptide transport, thus avoiding some of the competition between free amino acids. Free amino acids appear to inhibit peptidase activity in the brush border (46). Based on the distribution of peptidases between brush border and cytosol cited earlier (87) this could lead to inhibition of absorption.

Peptide Absorption

Although there is indirect and direct evidence for peptide absorption from the small intestine (46,86,116), the nutritional significance of this route is not clear. Increases in plasma free amino acids across the sphlanchnic bed account for 20 to 70% of the amino acids disappearing from the lumen of the intestine (109). Some of these may be used by the gut tissue itself, which is highly active (1) both in terms of metabolism and protein synthesis (1,11). Some of the proteins synthe-

sized by enterocytes enter the bloodstream, including apoproteins (1). Some amino acids may be deaminated enter the blood as the keto acids.

However there is evidence that a significant percentage of the amino acids absorbed from the intestine enter the blood in the form of peptides. Studies with Holstein steer calves (116), showed a significant flux of peptide nitrogen from the splanchnic bed to the portal circulation. Peptide amino acids in blood were measured as the difference in free amino acid content between intact plasma and plasma hydrolyzed with methanesulfonic acid. Red blood cell amino acids were also determined. Up to 70% of amino acid nitrogen entering the portal blood appeared in the peptide fraction. Red blood cells appeared to release amino acids during passage through the portal circulation.

Other studies with non-ruminant animals have reported that a smaller percentage of amino acids enter the blood as peptides. Gardner (46) measured peptide absorption in perfused rat jejunum and ileum, using pancreatic digests of casein, soybean protein and muscle protein or specific dipeptides. From 15 to 27% of total non-protein amino nitrogen was absorbed in the peptide form from the protein digests. No appreciable peptide absorption occured when the muscle digest was used, less in fact that with the negative control (no protein infused). Thus there is some question as to effect of animal versus vegetable protein, which would be interesting to test in the ruminant in vivo.

Approximately 6% of glycine-L-phenylalanine and 2% of L-leucine- glycine were absorbed intact from 0.1 M solutions in the intestine. Adding a mixture of free amino acids (hydrolyzed casein) did not seem to reduce absorption of glycine-L-phenylalanine, although this would not neccesarily be true in vivo. The author concluded that peptide absorption was not of major nutritional significance in the rat.

A recent study examined peptide absorption from three plant and three animal proteins using isolated intestinal loops of rats (86). The absorption of diglycine was also studied. Absorption was estimated by the disappearance of peptides from the intestinal lumen.

It was found that in vitro digestion of animal proteins yields a larger proportion of small peptides than plant proteins. Protein quality was correlated with the release of small peptides during in vitro digestion. Although more peptide N tended to be absorbed from digests of plant protein, there was no difference in the disappearance of small versus large peptides. From 15 to 42% of the injected peptide solutions were absorbed. However hydrolysis of peptides by peptidases located in the brush border could have confounded peptide absorption with that of free amino acids.

In conclusion it appears that a significant percentage of amino acids may be absorbed in the peptide form. In the case of ruminants much more data from different laboratories is required in order to assess this process. Some discrepancies may be due to methods of blood analysis. Analysis of whole blood and plasma free amino acids must be reconciled with the fractionation of blood into plasma, peptide fraction and red blood cells. Also the actual extent of amino acid metabolism by gut tissue must be known in order to assess amino acid requirements.

Postabsorptive Metabolism of Amino Acids

Briefly, alanine and glycine, gluconeogenic amino acids, are removed in large amounts by the ruminant liver (11,12). Given the dependence of the ruminant on gluconeogenesis the flux may be greater than in non-ruminants. Peripheral tissues release these two amino acids (11). The origin of the carbon skeleton of alanine is probably glucose (11). Thus glucose can be released by the liver, partially oxidized to pyruvate in muscle, then transaminated to form alanine which is released and converted back to glucose by the liver (68).

The urea cycle enzymes, arginine, citrulline and ornithine, also show cooperative metabolism. The liver extracts arginine in excess of that absorbed from the gut, but releases most of this as ornithine and citrulline (11) plus urea. The kidneys and hindquarters take up ornithine and citrulline and release arginine. Arginine may therefore act as a shuttle for nitrogen between muscle and the liver

(11,12), where arginase activity is present. The lactating mammary gland also possesses arginase activity (119). These findings support the concept of a "whole body " urea cycle.

The branched chain amino acids, leucine, isoleucine and valine, are removed from blood by the liver (40%) and muscle (60%) for protein synthesis and catabolism respectively (11). Oxidation of the keto analogs of the branched chain amino acids also occurs in the liver (68). Leucine stimulates protein synthesis in muscle (11).

Glutamine is removed by the gut and liver and released by the kidneys and peripheral tissues. Glutamic acid is released by the liver and taken up by peripheral tissues (11,12). Glutamine taken up by gut tissue is oxidized as the major respiratory fuel (1) and also transaminated to alanine (1). Glutamic acid accepts an amino group in muscle tissue to form glutamine, which acts as a nitrogen carrier to the liver (1).

Mammary Gland Metabolism of Amino Acids

In ruminants the majority of milk protein is thought to synthesized from plasma free amino acids (69), although extraction of peptides by the gland could be significant. The tripeptide glutathione (gamma-glutamyl-cystenyl-glycine) has been shown to be taken up in significant amount by the lactating mammary gland of cows (27), thus explaining the small net output of free cystine observed in other studies (13). Interestingly, recent studies with lactating rats have shown that 5-oxoproline, an intermediate in the gamma-glutamyl cycle, markedly stimulates uptake of free amino acids from plasma (115). This may have implications for intestinal transport of amino acids.

In general the essential amino acids are extracted by the lactating mammary gland in amounts sufficient to account for their appearance in milk protein (13,81). The ratio of uptake to output is around one for methionine, phenylalanine, histidine, lysine and threonine, suggesting that these are potentially limiting for milk protein synthesis (13,81). In contrast arginine and the branched chain

amino acids are taken up in considerable excess of requirement for milk protein and have been shown to be converted to nonessential amino acids, whose uptake is generally less than their output in milk (27). The concept of limiting amino acids for milk production were discussed earlier. Clearly there is need for further study of amino acid metabolism by the lactating mammary gland.

Experimental Procedures

Animals

Eight multiparous Holstein cows averaging 133 (± 16) d of lactation were used. Average age was 50 months and average bodyweight was 570 kg. Average mature equivilent milk production was 7531 kg. All cows had been fitted with rumen cannulae and four cows were also fitted with soft, T-type duodenal cannulae located just distal to the pyloric sphincter. The animals were housed in comfort stalls, fed twice daily at 0600 (35%) and 1400 (65%) h and milked at 1200 and 2400 h.

Diets

Total mixed diets were based on corn silage and high moisture corn and supplemented with either soybean meal (S) or soybean meal and corn gluten meal in a 60:40 ratio (SG), plus a vitamin-mineral premix (Table 1).

Trace mineralized salt was available free choice during the daily exercise periods. The diets were fed ad libitum (10% refusals). Both diets were balanced according to NRC requirements for energy, acid detergent fiber, crude protein and trace nutrients. The diet containing corn gluten meal (SG)

Table 1. Composition of Experimental Diets.

Ingredient (kg DM)	Diets S		Diets SG	
Corn silage	74.3		74.3	
High moisture corn	8.4		9.6	
Soybean meal	14.5		8.6	
Corn gluten meal	0.0		4.3	
Vitamin mineral mix[1]	2.8		3.2	
	100.0		100.0	
Analysis	$\bar{x}$	SD	$\bar{x}$	SD
Dry matter %	45.2	3.1	45.0	3.1
Crude protein %	14.2	1.2	13.8	1.5
ADF %[2]	21.1	1.4	20.9	1.5
ADIA %[3]	2.2	1.5	1.8	1.6
Organic matter %	91.0	2.2	90.3	2.4

[1] As fed: Ca 15.9%, P 5.7%, K 6.2%, Mg 2.2%, S 3.2%, $NaHCO_3$ 19.4, Cl 10.6, Fe 265 ppm, Cu 132 ppm, Co 3 ppm, Mn 1100 ppm, Zn 1323, I 44 ppm, Se 5 ppm, Vit. A 50,000 IU/lb, Vit. D 25,000 IU/lb, Vit. E 500 IU/lb.

[2] Acid detergent fiber.
[3] Acid detergent insoluble ash.

was formulated to meet the new NRC recommendations for rumen degradable crude protein (76), 60.5%.

Infusions

Infusions consisted of:

1. 5 liters of tap water

2. 400 g sodium caseinate in 5 liters of water

3. 400 g enzymatically hydrolyzed casein in 5 liters of water

4. 11.3 g L-methionine and 30.1 g L-lysine in 5 liters of water

Infusions were administered over 22 h/d via a peristaltic pump (Buchler Multistaltic, Haake-Buchler, Inc.) Abomasal infusion was done by the method of Clark et al. (26). Solutions were mixed daily, chilled to $4^{0}C$ and kept cool during infusion by means of ice chests and ice packs.

Design and Application of Treatments

An 8*6 incomplete block design was used in order to complete the study while cows were still in mid-lactation. Each cow received a different combination of 6 of the 8 treatments (diet * infusion). However, each treatment was given during each period. Six 11 d periods were used with the first 3 d allowed for adjustment to infusions. Treatments were arranged such that each cow remained on the same diet (either soybean meal or soybean meal:corn gluten meal supplemented) during periods 1 through 3 then switched to the other diet during periods 4 through 6. A 10 d interim period between periods 3 and 4 was used to make all diet changes.

Measurements and Sampling Procedures

Milk production and feed refusals were measured daily. Milk samples were taken at both the AM and PM milking on days 8 and 9 of each period and submited to the Virginia Federation DHIA Laboratory for analysis of protein, fat, lactose, solids-not-fat and somatic cell counts. Bodyweights were taken on days 1 and 11 of each period. Feed ingredients and total mixed diets were sampled on days 1, 5, 9, 10 and 11 of each period. Orts were sampled for days 9 and 10. One set of feed samples was analyzed immediately by the Virginia Tech Forage Testing Laboratory. The second set along with orts was stored at -20^{0}C. Blood samples from the coccygeal vein and mammary vein were taken 2 to 3 h postmilking in day 11 of each period from the four dual cannulated cows. Heparin was added and the samples were kept in ice during transport to the laboratory where they were centrifuged at 3000 * g. One aliquot of plasma was frozen at -20^{0}C and a second deproteinized with sulphosalicylic acid and frozen at -80^{0}C for subsequent amino acid analysis.

Digesta Markers and Sampling

Acid detergent insoluble ash was used as the solid phase marker and cobalt ethylenediaminetetraacetic acid (CoEDTA) (110) was used as the liquid phase marker. CoEDTA (18 g/d) was administered into the rumen in gelatin capsules as three equal doses at 0900, 1700 and 0100 h days 4 through 11 of each period. During the last 48 h of each period, six samples of rumen fluid, duodenal digesta and feces were taken at 8 hour intervals representing 0200, 0600, 1000, 1400, 1800 and 2200 h.

Rumen fluid (150 ml) was obtained from digesta from several areas of the rumen by squeezing through four layers of cheesecloth. The pH was determined on site and two 5 ml aliquots, preserved with either two drops of concentrated sulfuric acid or 1 ml of 60% phosphoric acid, were frozen at -20^{0}C for analysis of ammonia and volatile fatty acid concentrations, respectively.

Duodenal digesta (750 ml) was collected in a plastic beaker after discarding the initial surge of digesta. The sample was then stirred, the pH determined and two samples of 26 and 150 ml taken into plastic cups. At each sampling, 26 ml was added to the first cup, producing a composite sample for each cow * period. The larger samples (150 ml) representing each sampling time were kept in reserve. Samples were frozen at -20°C. Fecal grab samples of approximately 500 g were mixed, subsampled (150 g), then frozen at -20°C.

On the last day of each period 1.0 to 1.5 liters of rumen fluid was taken by siphon from each of the dual cannulated cows and preserved with 50 ml of 37% formaldehyde solution. A bacterial pellet isolated as described (71). The pellet was frozen at -80°C for later analysis of dry matter, nitrogen and cystosine.

Laboratory Analysis

All analyses were done in duplicate unless otherwise noted. Samples of feeds and orts were thawed, dried at 50°C for 48 h and ground through a 1 mm screen in a Wiley mill. Duodenal digesta samples were thawed and centrifuged at 3000 * g for 15 min to separate liquid (LPD) and solid (SPD) phases. The supernatant (LPD) was decanted and frozen at -20°C. The pellet (SPD) and all fecal samples were lyophylized to 40°C and ground through a 1 mm screen in a Cyclone mill.

Absolute dry matter, organic matter, total nitrogen, and acid detergent fiber were determined in feeds, orts, LPD, SPD and feces as described (2,48). Acid detergent insoluble ash was the residue obtained after ashing acid detergent fiber at 500°C for 4 h. Precipitable protein was measured in LPD and SPD using trichloroacetic acid (21). Cytosine was measured in rumen bacteria, LPD and SPD by high performance liquid chromatography (59,121). Dry matter and nitrogen were also analyzed in rumen bacteria. Cobalt was measured in LPD, SPD and feces by a modification of the method used by Hart and Polan (52), in that 0.1 M disodium EDTA was used in place of ammonium EDTA. Rumen ammonia (74) and plasma urea nitrogen (32) were determined by

colorimetric assays. Rumen volatile fatty acids were measured by gas chromatography (21). Plasma free amino acid concentrations were analyzed by the Health and Nutrition Laboratories, Eastman Kodak Co., Kingsport, TN. Samples of deproteinized plasma are thawed, mixed 1:1 with Beckman diluting buffer containing an internal standard (S-2-aminoethyl-L-cysteine*HCL; Sigma). The sample was filtered through a 0.22 um Millex-GS filter (Millipore). Analysis was conducted on a Beckman 6300 amino acid analyzer. Standards were analyzed after every tenth unknown.

Calculations

Duodenal flows of dry matter and constituents were calculated by the method of Armentano and Russell (3). Apparent digestibilities were calculated by marker ratio technique (112). Contribution of microbial protein to duodenal digesta was estimated from the ratio of nitrogen to cytosine in mixed rumen bacteria and that in duodenal digesta (34).

Statistical Analysis

Data were analyzed using the general linear model (GLM) procedure of SAS (Statistical Analysis System, Cary, NC).

Y = &mu. + diet + infusion + diet*infusion + cow + period + error.

Treatment effects were tested by the error mean square. Least squares means for each treatment (infusion, diet*infusion) were compared to the appropriate control mean using Dunnet's comparison (120).

Details of Animal Preparation and Care

Duodenal cannulae were installed in two of the four cows used in the study. Cannulae were manufactured by A. D. McGilliard of Iowa State University, Ames, Iowa. The cannulae were made of flexible plastisol with an inner diameter of 25.4 mm. Cows were allowed several weeks of recovery time after surgery before rumen fistulas were installed. Rumen fistulas were installed in five of the eight cows used in the study (three cows had been fistulated during the previous lactation). Two of the these cows had received duodenal cannulae. Recovery of the animals was good, based on recovery of milk production (1 wk) and maintenance of bodyweight. Rumen fistulae were plexiglass with an inner diameter of 100 mm and were installed by personnel of the Virginia-Maryland College of Veterinary Medicine. The study began approximately 5 weeks after the last surgery had been performed.

Abomasal Infusions

Abomasal infusions were done by the method of Clark et al. (26). A 60 ml plastic bottle was perforated with about sixteen 1 mm holes, including one each in the bottom and cap. Hard plastic tubing was inserted through the cap and held in place with plastic ties and cyanoacrylate glue. A one-way plastic valve was attached inside the bottle. A 17 cm rubber flange was cut from flexible, rubber dishmats (Rubbermaid,Co.), and secured against the outside of the cap with plastic ties and glue. The bottle cap was glued in place. About 1 to 2.5 m of flexible tubing (Tygon R1000, I.D. 1/8 ", wall thickness 1/16 ") was attached to the hard plastic tubing in the bottle cap with plastic ties. The bottle assembly was tested to insure that flow was not impeded, then inserted in the abomasum as follows. Enough digesta must be removed from the rumen to be able to reach the reticulum easily. With the left hand the reticulum and reticulo-omasal orifice were located. The operator then reached through the omasum, down and to the right until the smooth mucosa of the abomasum could be felt. (The digesta in the abomasum is much more liquid allowing more free-

dom of movement). The procedure in then repeated with the bottle assembly in hand. The closure of the reticulo-omasal orifice and movement of digesta kept the bottle in place very well. Excess tubing was allowed in the rumen to prevent the movement of solid digesta from stretching the tubing and retracting the bottle and flange from the abomasum. For this reason the location of the bottle assembly was checked daily to insure that the bottle assembly was retained in the abomasum. This was done by tracing the tubing back from the rumen fistula to the omasum. The location of the bottle was checked to insure that it had not been retracted to the omasal orifice. If the tubing in the rumen was wound around the solid digesta mass, it was freed, and pushed down and forward towards the reticulum. The tubing was exteriorized through a hole in the rumen cannula and plastic connecters attached to prevent retraction of excess tubing into the rumen. Tubing was suspended from the overhead beams above the cows with strings made by joining several rubber bands. This allowed the tubing to stretch when the cow laid down and retraction of tubing when the cow was standing.

Infusates were mixed daily in 5 liters of water, and refrigerated in collapsible, plastic water jugs. Infusates were changed at 1200 h daily, and the residual of the previous days infusate recorded. During infusion the jugs were placed in styrofoam ice chests with several refreezable ice packs. Ice packs were changed twice daily. Two holes were punched in the bottom of each ice chest so that the jugs could be turned upside down, allowing infusate to run out into the infusion tube. Peristaltic pumps were used for infusion. Infusions were stopped for 1.5 to 2 h per day when the cows were moved to and from the milking parlor.

Details of Laboratory Analysis

Absolute dry matter was determined by drying 1 g of sample to constant weight at 105°C. Organic matter was measured as the material lost from 1 to 2 g of sample after ashing at 500°C for 4 h. Acid detergent fiber was analyzed by the methods of Goering and Van Soest (48). Total nitrogen

was analyzed by the Kjeldahl procedure (2). Acid detergent insoluble ash was the residue obtained after ashing acid detergent fiber at 500^{0}C for 4 h.

Duodenal digesta composites representing each cow * period, were thawed and centrifuged at 3000 * g to separate liquid (LPD) and solid (SPD) phases. For chemical analysis, approximately 1 ml of LPD was pipetted from LPD stirring on a magnetic stir plate and the exact weight recorded on an electronic balance.

Trichloroacetic acid precipitable nitrogen was measured in duodenal digesta as follows: For SPD about 200 mg of sample was placed in a 50 ml screwcap centrifuge tube and 10 ml of 5% (w/v) TCA added. The tubes were vortexed and placed in a 90^{0}C water bath for 10 min. The tubes were then shaken on a wrist action shaker for 10 min, then the contents were filtered through Whatman No. 42 filter paper and the filtrand washed thoroughly with hot, distilled water. The residue and filter paper were then analyzed for total nitrogen, which was expressed on a dry matter basis. Approximately 5 g of LPD was pipetted into a 50 ml screw-cap centrifuge tube (exact weight recorded) and 5 ml of 10% TCA added. Tubes were heated, shaken, filtered and analyzed for total nitrogen as described for SPD.

Cobalt was extracted from LPD, SPD and feces as described (52). For SPD and feces, 200 mg of sample was weighed into a 50 ml screw-top centrifuge tube and 20 ml of 0.1 M disodium EDTA added. The tubes were shaken for 30 min on a wrist action shaker, and the contents filtered through Whatman No. 1 filter paper. The filtrate was refrigerated until analysis. For LPD, approximately 5 g of liquid was lyophilized then mixed with 20 ml of 0.1 M disodium EDTA, shaken and filtered as for the solid samples.

Analysis of cobalt was carried out with a Perkin-Elmer model 370 atomic absorption spectrophotometer (82). Cobalt standards of 1 to 5 micromolar concentration were prepared in 0.1 M disodium EDTA. An air-acetylene flame was used and the wavelength set at 241 nm. Standards were run every 5 to 10 samples, and the absorbance of the standards reset if neccesary. The un-

knowns were then re-run in reverse order to insure an accurate reading. The purity of Co-EDTA used in the experiment was determined (> 97%) in order to calculate exact dosages of cobalt.

Cytosine was analyzed by HPLC according to the method of Koenig (59) as modified in our laboratory (121). For LPD, 10 g were weighed into 15 ml screw-cap glass extraction tubes and dried in a forced air oven at 90^{0}C for not more than 12 h, then hydrolyzed and handled as described for solid samples (121). LPD samples were diluted to 100 ml instead of 500 ml after hydrolysis. Microbial samples of .25 g were hydrolyzed and diluted as SPD samples but the volume injected was reduced to 10 ul and the 5, 10, 15, 20 and 30 uM standards used. Standards made up in .35% perchloric acid ranged from 1 to 30 micromolar cytosine (1-5, 10, 15, 20, 30). Standards of guanine, adenine, uracil and thymidine were also tested. Bases were separated on a Whatman Partasil 10 SCX column at room temperature with a flow rate of 0.67 ml/min. The buffer used was 0.2 M ammonium phosphate (HPLC grade) made up in HPLC grade water and adjusted to pH 3.5 with concentrated HCl. Detector settings were: wavelength, 254 nm; absorbance range 0.02 and voltage, 10 mV. Under these conditions cytosine eluted at 10.5 min. The standard curve r value (simple regression) averaged 0.999.

Volatile fatty acid and ammonia concentrations in rumen fluid and plasma urea nitrogen were determined as described (20,32,74). Volatile fatty acids were measured using a Varian Vista 6000 gas chromatograph with a glass column packed with 10% SP-1200, 1% H&sub3.P0&sub4. liquid phase on 80/100 chromasorb WAW packing. Isocaproic acid was the internal standard.

Milk samples were analyzed within 72 h of collection on a Multispec Infared Analyzer (Berwind Intruments, Ltd., York, U.K.). Samples were preserved with potassium dichromate.

Results and Discussion

Diets

Diets S and SG (Table 1) were similar in formulation except for the partial substitution of corn gluten meal for soybean meal in diet SG. Percent dry matter, crude protein, acid detergent fiber, acid detergent insoluble ash and organic matter were similar between the diets. Therefore the primary difference between diets was in the estimated rumen degradability of supplemental protein, 70% and 60.5% for S and SG, respectively, and in the amino acid composition of the supplemental protein (25). The amino acid composition of dietary protein escaping the rumen will reflect in part the amino acid composition of the original protein fed (25). Lowering rumen degradation of feed crude protein increases the escape of feed protein from the rumen but decreases the nitrogen available to rumen microbes. Therefore amino acid availabilty to both rumen microbes and the host animal may be affected. As shown, the content of acid detergent insoluble ash in the diet was quite variable. Most of this material was found in the corn silage. The variation reflects the fact that silage source was changed several times during the study.

Production Parameters

Effects of diet, infusion and diet*infusion on production parameters are shown in Tables 2 and 3. Dry matter intake was reduced ($p < .10$) during infusion of methionine-lysine (ML). Most of the effect was expressed on the soybean meal diet (Table 3) where control cows had higher and more typical dry matter intakes. Intake was not significantly lower for casein (C) or hydrolyzed casein (HC) infusions. Previous studies have shown that dry matter intake can be depressed by feeding high levels of methionine-hydroxy-analog (84). Therefore the level of methionine administered in the present study may have exceeded requirements for the level of milk production. Theoretical values for methionine requirement suggest this amino acid may be limiting when cows produce more than 15 to 20 kg milk/day (21). More recent estimates suggest a figure of 30 kg/d (50). Alternatively, the ratio of methionine to lysine may have deviated from optimal, the value of which is not known.

Infusion of sodium caseinate resulted in greater bodyweight gain versus control ($p < .05$) and the effect was distributed evenly across diets (Tables 2 and 3). However, hydrolyzed casein did not significantly increase bodyweight gain, suggesting that HC was not utilized as efficiently as was C. Cows tended to lose weight during control (W) and ML infusions. Weight gain tended to be greater with the SG diet.

Milk Yield

Milk yield was increased 5.2 % ($p < .10$) by abomasal infusion of sodium caseinate (Table 2). The increase of 1.2 kg/d is less than the average of 1.5 kg milk/d observed with corn silage-corn based diets (24). This is probably because, in the present study, initial milk production was lower, and the cows studied were in mid-lactation. During mid-lactation nutrient requirements are usually met by the diet. The effect of C on milk yield was relatively evenly distributed across diets.

Table 2. Effect of basal diet and abomasal infusion on lactational parameters of cows in mid-lactation.

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
Intake (kg DM/d)	17.2	17.1	.20	17.5	17.2	17.3	16.8*	.30
Bodyweight (kg/p)[1]	0.3	4.9	3.40	-3.1	13.2**	3.4	-3.1	5.00
Milk (kg/d)	23.6	23.9	.30	23.3	24.5*	24.0	23.2	.40
Fat %	3.55	3.45	.05	3.51	3.30*	3.59	3.60	.07
Fat (kg/d)	0.83	0.81	.02	0.81	0.80	0.84	0.83	.02
Protein %	3.27**	3.22	.01	3.21	3.26	3.21	3.29*	.03
Protein (kg/d)	0.76	0.75	.01	0.74	0.79*	0.76	0.75	.02
SNF %	8.77	8.75	.03	8.75	8.79	8.77	8.73	.04
Lactose %	4.78	4.81	.03	4.78	4.81	4.82	4.76	.04
3.5% FCM (kg/d)	23.5	23.4	.30	23.1	23.5	24.0	23.4	.50
SCC (x1000)	94	73	26	143	85	64	42	38

* $p<.10$ comparing diets; or C, HC, ML versus control infusion.
** $p<.05$ " " " " " "
[1] bodyweight change, kilograms per experimental period (11 d).

Table 3. Combined effects of diet and abomasal infusion on lactational parameters of cows in mid-lactation.

	Diet * Infusion								
Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
Intake (kg DM/d)	17.9	17.1	17.2	16.8 *	17.1	17.2	17.4	16.8	.4
Bodyweight (kg/p)[1]	-5.2	10.8	3.0	-7.4	-0.9	15.6	3.9	1.1	7.0
Milk (kg/d)	23.3	24.4	23.6	22.9	23.4	24.6	24.3	23.5	.6
Fat %[2]	3.48	3.44	3.51	3.79 *	3.55	3.16 **	3.67	3.42	.1
Fat (kg/d)	0.80	0.83	0.81	0.87 *	0.81	0.77	0.87	0.79	.03
Protein %	3.24	3.29	3.20	3.35	3.18	3.23	3.22	3.23	.04
Protein (kg/d)	0.75	0.80	0.75	0.76	0.72	0.77	0.77	0.74	.03
SNF %	8.73	8.79	8.77	8.80	8.77	8.79	8.77	8.66	.06
Lactose %	4.75	4.78	4.79	4.77	4.81	4.84	4.85	4.74	.06
3.5% FCM	23.0	23.9	23.4	23.9	23.2	23.1	24.5	23.0	.70
SCC (x1000)	227	51	89	10 *	59	119	39	74	54

[1] bodyweight change, kg per experimental period (11 d).
[2] diet * infusion interaction, $p < .05$
Differs from control infusion; * $p < .10$; ** $p < .05$.

Milk yield was not significantly increased by infusion of either methionine-lysine or hydrolyzed casein (Table 2 and 3). Milk yield responses to methionine-lysine or rumen protected methionine have been observed in first-lactation heifers (50,56), but generally not in multiparous cows (52). The response of primiparous cows to methionine may be related to the growth requirement of these animals, a process for which methionine and lysine have been shown to be limiting in ruminant animals (106).

Lack of significant milk production response to hydrolyzed casein suggests that this protein source was not absorbed and/or utilized as efficiently as sodium casein. The milk production data suggest that HC was more beneficial on the SG diet (soybean-corn gluten meal), as compared to diet S (Table 3). On diet S, infusion of HC increased milk production 0.3 kg/d over the control infusion W, while on diet SG infusion of HC increased milk production 0.9 kg/d. Neither was significantly greater than the control infusion ($p > .10$). Milk production was similar between the basal diets (Table 2).

Milk Fat Yield

Milk fat percentage was decreased ($p < .10$) during infusion of sodium casein (Table 2). Similar results have been found in previous studies (24). Clark et al. (26) suggested that glucose synthesized from excess nonessential amino acids in casein could stimulate lactose synthesis. Lactose is major determinant of milk volume, therefore milk fat percentage may be decreased by dilution (24). Consistent with this hypothesis, Cohick et al. (29) have reported that casein infusion increases plasma glucagon concentrations and the glucagon-to-insulin ratio. Glucagon enhances gluconeogenic activity in the liver. Milk fat yield (kg/d) was not decreased by C infusion, supporting the dilution theory.

The effect of casein on milk fat percentage was most pronounced on the SG diet (Table 3). Fat percentage was slightly, but not significantly lower for diet SG versus diet S (Table 2). Therefore

cows fed diet SG may have been more deficient in milk fat precursors and more sensitive to any increase in glucose supply, which tends to depress milk fat percentage (24). However infusion of hydrolyzed casein tended to increase milk fat percentage and yield, especially on diet SG (Table 3). The increase in milk fat yield approached significance for the SG diet. It appears that the amino acids absorbed from HC interacted with other diet derived nutrients in a manner different from C. Molar percentage of rumen butyrate was lower on the SG diet (Table 13). Beta-hydroxy butyrate, the major splanchnic metabolite of rumen butyrate, is a significant precursor of milk fat in ruminants (19). The non-gluconeogenic ratio, defined by Orskov (77) as the sum of molar percentages acetate, 2*butyrate and valerate divided by the sum of propionate and valerate, was lower for diet SG. Therefore the proportion of gluconeogenic volatile fatty acids was greater for diet SG. The greater potential for glucose supply may explain the tendency toward milk fat depression in response to casein (C) infusion on this diet. Interaction between abomasal casein and ruminally infused acetate with regard to milk fat secretion has been demonstrated in goats (62). There may be an interaction between absorbed butyrate and certain amino acids; perhaps leucine whose keto-analog has been shown to stimulate milk fat yield in lactating cows (113). The proportions of nonessential and essential amino acids absorbed may have differed between C and HC leading to different interactions with absorbed VFA. Fewer gluconeogenic amino acids may have been absorbed from HC.

Infusion of methionine-lysine tended to increase milk fat percentage across diets (Table 2) and significantly increased milk fat percentage and yield on diet S (Table 3). Responses to ML relative to controls were largely responsible for a significant interaction ($p < .05$) of diet*infusion for milk fat percentage. Milk fat percentage and yield was increased on the diet (S) calculated to be most deficient in methionine (25). Methionine supplementation has increased milk fat percentage and yield in cows during mid-lactation fed a diet shown to produce low amounts of postruminal methionine (18). Milk fat percentage was increased by rumen protected methionine and lysine in cows fed corn-soy diets during mid-lactation (93). A milk fat response was detected in primiparous heifers fed rumen-protected methionine during early lactation (56). In these instances the effect of

methionine (and lysine) was on intermediary metabolism, as opposed to effects on rumen microbial lipid synthesis. The latter effect is at least partially responsible for the beneficial effects of methionine hydroxy analog (MHA) on milk fat secretion in cows (53). However, there is evidence for direct effects of methionine on intermediary metabolism when either MHA or unprotected D-L methionine are fed to lactating cows. Huber et al. (53) reported that feeding 25 g/d MHA increased plasma methionine and its extraction by the mammary gland of lactating cows. Feeding D-L methionine to primiparous heifers increases not only fat yield, but milk and milk protein yield as well (50). In the present study, cows fed a corn silage, corn and soybean meal diet responded to methionine-lysine supplementation with a significant increase in milk fat percentage and yield. It appears that under certain conditions methionine and possibly lysine stimulate milk fat secretion in dairy cows.

The biochemical mechanism by which methionine affects milk fat secretion in ruminants appears to involve the synthesis of phospholipids which are integral components of plasma lipoproteins (68). Methionine is a significant precursor of choline in ruminants (108). Choline is a component of the phospholipid, phosphatidyl choline or lecithin (66). Phosphatidyl choline is a major constituent of bile secretions, lipoproteins and many cell membranes (68). Lecithin is required for the formation of certain cholesterol esters or long chain fatty acids via the activity of lecithin-cholesterol acyl transferase (118). Lecithin-cholesterol acyl transferase activity has a significant impact on both lipoprotein structure and cholesterol metabolism (109). Therefore, in the ruminant methionine supply may directly impact lipid transfer between tissues via its effect on choline supply. In the ruminant, choline supply may be low, due to limited synthesis in the rumen (34), which would place demands on the supply of methionine.

Milk Protein Yield

Milk protein percentage was higher ($p < .10$) for diet S versus diet SG (Table 2). However milk protein yield was not different between diets, as actual milk yield (kg/d) was slightly higher for diet SG.

Infusion of sodium caseinate (C) did not significantly increase milk protein percent but did increase daily protein yield ($p < .10$) 6.8%. As in the case of milk yield, the increase is less than typically observed during casein infusion (24,25,29) perhaps because the cows were in mid-lactation and producing less than 30 kg milk/d. The response was comparable to that reported for well fed cows in mid-lactation (80). The effect of casein on milk protein production was evenly distributed across the diets (Table 3) suggesting that the response was to a general increase in total amino acids, rather than particular amino acid interactions.

Hydrolyzed casein (HC) infusion did not significantly increase either milk protein percentage or yield (Table 2). There was apparently more benefit of HC on milk protein yield with diet SG, due to slightly higher milk production for HC versus controls (Table 3). As was the case with milk yield, responses to HC were not equal to those obtained with sodium casein, suggesting poorer utilization of amino acids in HC.

Infusion of methionine-lysine (ML) increased milk protein percentage ($p < .10$) from 3.21 to 3.29% (Table 2). There appeared to be more effect of ML on milk protein percentage on diet S (Table 3). Diet S was calculated to be more deficient in methionine relative to diet SG, while diet SG was relatively more deficient in lysine (25). Milk protein yield was not increased by ML infusion either across or within diets (Tables 2 and 3). A recent study reported an increase in milk protein percentage in 18 mid-lactation cows fed a corn silage-corn-soybean meal diet in response to feeding rumen protected methionine and lysine (93). The magnitude of response, 0.1 %, was comparable to that observed in the present study. Schwab et al. (101) found increases in both milk protein

percent and yield to abomasal methionine-lysine, which accounted for an average of 47% of the milk protein response obtained with casein infusion. Milk protein responses to methionine alone have been reported under more restrictive dietary regimens, such as urea supplemented diets (44) and in cows fed grass silage as their only feed (91). It is clear that methionine and probably lysine can be limiting for milk protein and even milk and fat-corrected milk yield in dairy cows under nutrient limiting conditions, or when fed grass silages (18). Methionine and lysine supply are more adequate in cows fed typical commercial dairy rations in the U.S., but there is potential use for these amino acids during early lactation in high producing cows, especially first lactation cows. In first lactation cows, milk yield and fat-corrected milk yield as well as milk protein yield are increased by protected methionine or unprotected D-L methionine supplementation (56,50). In mature cows the increases in milk protein percentage in response to protected methionine and lysine would be of economic significance in areas where dairy producers receive increased payment based on milk protein content.

Other Milk Components

Percentages of lactose and solids-not-fat (SNF) were not significantly affected by diets or infusions (Tables 2 and 3). Percent SNF tended to be lowest for SG-ML and highest for S-ML, mainly due to the combined effects of protein and lactose percents. Therefore cows appeared to have been less responsive to methionine-lysine supplementation when fed diet SG as compared to diet S.

Yields of 3.5% fat-corrected milk were not significantly different between diets or infusions and controls (Tables 2 and 3). Values tended to be highest for cows fed SG and infused with HC.

Somatic cell counts (SCC) in milk were similar between diets, but tended to be higher for control (W) than for the other infusions. On diet S somatic cell count was higher ($p < .05$) in SW versus SML infused cows (Table 3). There tended to be an interaction ($p < .12$) of diet*infusion for SCC, mainly due to the differential responses to W and C within diet (Table 3). Only one cow contracted

clinical mastitis during the experiment and this was not during milk sampling. Furthermore, there was no statistical effect of cow on SCC. Therefore there may be a nutritional cause for the response of SCC, presumably related to amino acid metabolism. A detailed explanation is not apparent from this experimental data.

Productive Efficiency

Nitrogen consumption, infused nitrogen and conversion to milk and milk components are shown in Tables 4 and 5. Cows fed diet S consumed more nitrogen (383.4 vs 373.5 g/d) ($p < .10$) than cows fed diet SG. Total nitrogen intake (oral plus infused) was also greater for diet S. Yields of fat-corrected milk, milk protein, milk fat and milk energy per kg of crude protein intake were not significantly different between basal diets.

Oral nitrogen consumption was not significantly different among infusions (Table 4). Infused nitrogen was higher for C, HC and ML versus the control (W). Total N intake was higher for C and HC versus control.

Yield of fat-corrected milk per kg crude protein intake was lower for both C ($p < .05$) and HC ($p < .10$) than for control infusion. Efficiency was higher for HC due to a tendency toward higher fat yield with this infusion (Table 2 and 3). Efficiency of fat yield (kg/d) per kg of crude protein intake was lower ($p < .05$) for C but not HC versus control. Milk protein yield per kg crude protein intake was lower than control for HC but not C, demonstrating a more efficient utilization of infused N from sodium caseinate. Therefore there was a difference in the partitioning of nutrients between C and HC, with C promoting greater milk volume and milk protein yield and HC tending to produce higher yields of milk fat. Presumably the differences were due to differences in amino acid absorption between the sources of casein. Total milk energy (29) (Mcal/d) per kg crude protein intake was decreased ($p < .10$) for both C and HC relative to control (W). Thus similar amounts of total energy were transferred from each casein source to milk, but in differing proportions of

Table 4. Effect of diet and infusion on nitrogen intake and efficiency of utilization.

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
N consumed (g/d)	383.4	373.5*	3.70	383.8	376.1	382.0	371.9	5.40
N infused (g/d)	28.5	28.4	.08	0	55.7**	54.2**	3.9**	.10
Total (g/d)	411.8	401.9*	3.80	383.8	431.7**	436.1**	375.8	5.50
FCM/CPI[1]	8.55	8.61	.14	8.93	8.01**	8.16*	9.22	.20
MP/CPI[2]	.30	.30	.01	.31	.29	.28*	.32	.01
MF/CPI[3]	.33	.32	.01	.34	.29**	.31	.35	.01
ME/CPI[4]	6437	6483	110	6716	6084*	6133*	6907	158

[1] 4.0% fat corrected milk / total crude protein intake, kg/d
[2] Milk protein kg/d / "
[3] Milk fat kg/d / "
[4] Milk energy kcal/d / "
(ref. in text)
Differs from control infusion; * p<.10; ** p<.05.

Table 5. Combined effects of diet and infusion on nitrogen intake and efficiency of utilization.

	Diet * Infusion								
Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
N intake (g/d)	396.3	378.8 **	382.6 **	375.8 **	371.3	373.3 **	381.4 **	368.0 **	7.7
N infused (g/d)	0	55.7 **	54.2 **	3.9	0	55.7 **	54.1 **	3.8	.2
Total (g/d)	396.3	434.5	436.8	379.7	371.3	429.0	435.5	371.8	7.8
FCM/CPI[1]	8.66	8.17	7.96 *	9.43 *	9.20	7.84 **	8.35 *	9.02	.3
MP/CPI[2]	0.30	0.29	0.27 *	0.32	0.31	0.29 *	0.28 *	0.32	.01
MF/CPI[3]	0.33	0.31	0.30	0.37 **	0.35	0.28 **	0.32	0.34	.01
ME/CPI[4]	6514	6182	5993 *	7058 *	6917	5985 *	6273 *	6756	226

[1] 4% fat corrected milk per kg crude protein intake.
[2] milk protein kg/d " " " " .
[3] milk fat kg/d " " " " .
[4] milk energy kcal/d " " " . (ref. in text).
Differs from control infusion; * p<.10; ** p<.05.

protein, fat and lactose. Responses of efficiency variables to HC were similar within diets S and SG (Table 5), however efficiencies of fat corrected milk, milk protein and milk energy yield for C were only lower than control on diet SG. This mainly reflects the decrease in milk fat percentage obtained with C on diet SG. Higher protein intake by SW cows lowered milk protein efficiency, decreasing the difference between SW and SC to non-significant size ($p > .10$).

Efficiency of protein utilization across diets was not significantly different between ML and controls (Table 4). Within diet effects (Table 5) show that on the soybean meal supplemented diet, S, the efficiency of both fat-corrected milk and milk energy yield per kg crude protein intake were increased by methionine-lysine infusion (SML) over control (SW) ($p < .10$; $p < .05$).

Summary of Production Responses

Infusion of sodium casein (C) per abomasum gave typical increases in milk and milk protein yield, while increasing weight gain and decreasing fat percent but not fat yield. Hydrolyzed casein (HC) did not produce the same response as casein, tending to improve fat yield on the SG diet only. The two sources of casein are apparently not utilized in the same manner or to the same extent. HC may not be as completely absorbed as C because of greater competition between free amino acids for transport sites in the small intestine. Alternatively, the form of casein infused, intact or hydrolyzed, may have affected to proportion of amino acids absorbed in the free versus the peptide form, or the balance among amino acids in the blood at any given time after feeding. Either effect could potentially affect intermediary metabolism of amino acids.

Infusion of 11.3 g L-methionine and 30.1 g L-lysine per day (ML) increased milk protein percentage across both diets and milk fat percentage and yield on diet S. These findings in mid-lactation cows along with other research results suggest that these two amino acids may be limiting for milk component yield in lactating dairy cows, and that benefit may be derived from supplementation. The results also demonstrate that amino acid nutrition of lactating cows is affected by the source

of supplemental protein in the diet. Potential exists for balancing postruminal amino acid supply to promote more efficient milk production.

Plasma Free Amino Acids and Apparent Uptake by the Mammary Gland

Effects of Diet on Plasma Amino Acid Concentrations

Concentrations of plasma free amino acids from the coccygeal vein/artery are shown in Tables 6-8. This blood vessel was sampled because of ease of sampling and because it provides a reasonable estimate of arterial amino acid concentrations (35).

Plasma concentrations of leucine, phenylalanine and tyrosine were higher when cows were fed diet SG, while isoleucine, threonine, lysine, arginine and ornithine were higher for diet S (Table 7). In the cases of leucine, phenylalanine, lysine and arginine, the differences corresponded to the relative amount of the amino acid in the primary protein source (25). Amino acids undergo extensive metabolism, both during and after absorption from the gut (1,11), so that an increase in amino acid intake does not necessarily result in an increase in its plasma concentration. The situation is further complicated by variation in the extent of amino acid breakdown and re-synthesis in the rumen and differences in postruminal digestibility (104,107). Differences in plasma amino acid concentrations may also reflect different rates of utilization by the tissues. The balance of amino acids absorbed can affect the rates of utilization of individual amino acids, thereby affecting plasma concentrations (11). The data here show that amino acid nutrition of the lactating cow can be affected by dietary protein source. The urea cycle amino acids (arginine, ornithine, citrulline) were slightly higher for diet S (Table 6), perhaps due to greater ammonia transfer from the rumen to the portal blood.

Table 6. Effects of basal diet and abomasal infusion on plasma free amino acid concentrations (uM/ml).

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
EAA[1]	98.4	96.6	3.4	84.8	104.0	110.0	91.2	5.4
NEAA[2]	134.6	125.0	4.8	136.4	129.0	132.8	120.9	7.6
					**	**	*	
EAA/NEAA[3]	.74	.77	.03	.64	.80	.83	.76	.04
Total[4]	232.9	221.6	6.6	221.2	233.0	242.8	212.0	10.5
				**	**			
BCAA[5]	55.8	58.3	2.0	49.0	60.2	67.8	51.3	3.2
		*		**	*			
URCY[6]	22.8	21.2	.6	20.2	23.7	23.0	21.1	1.0

Diet * Infusion

Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
		**	**				**		
EAA[1]	83.3	111.6	109.4	89.2	86.2	96.4	110.6	93.1	7.8
NEAA[2]	146.6	137.2	131.8	122.6	126.2	120.9	133.8	119.1	10.9
		**	**						
EAA/NEAA[3]	.58	.82	.83	.73	.69	.78	.83	.80	.06
Total[4]	229.9	248.7	241.2	211.9	212.5	217.3	244.4	212.1	15.1
		**	**				**		
BCAA[5]	45.9	63.6	65.1	48.5	52.1	56.8	70.4	54.1	4.6
						*	**		
URCY[6]	22.0	24.9	22.7	21.7	18.4	22.5	23.3	20.5	1.4

[1]EAA = sum of the essential amino acids.
[2]NEAA = sum of the nonessential amino acids.
[3]EAA/NEAA = ratio of essential to nonessential amino acids.
[4]Total = total amino acids.
[5]BCAA = sum of the branched chain amino acids (Leu, Ile, Val).
[6]URCY = sum of the urea cycle amino acids (Arg, Cit, Orn).
Differs from control infusion; * $p<.10$; ** $p<.05$.

Effect of Infusions on Plasma Amino Acid Concentrations

Infusion of sodium caseinate (C) and enzymatically hydrolyzed casein (HC) into the abomasum elevated plasma concentrations of essential amino acids (Tables 6,7,8) and the ratio of essential to nonessential amino acids. This is in agreement with previous work (14,27). Infusion of C and HC also increased the plasma concentrations of the branched chain amino acids, leucine, isoleucine and valine, over controls. This is also in agreement with previous studies where sodium caseinate was infused into the abomasum (14,27,92). The branched chained amino acids (BCAA) tend to bypass the liver to a greater extent than the other amino acids and have therefore been used as an indication of improved protein status (absorption) (15). However the BCAA were not significantly increased by C infusion on diet SG, suggesting that the rate of their utilization was higher on this treatment. This particular treatment combination was marked by a low milk fat percentage. This in turn may have been related to amino acid balance.

Urea cycle amino acids were elevated in plasma of cows infused with C or HC, which is consistent with catabolism of excess amino acids. Most of the effect was seen on diet SG, due to the relatively low plasma concentration of urea cycle amino acids in controls (SGW).

Concentrations of individual amino acids (Tables 7,8) showed some differences between C and HC infusions. Plasma leucine, cystine-cystathione, ornithine and histidine were elevated more by HC than by C relative to control infusion (W). Tyrosine, arginine, citrulline and proline were increased more by C than HC. Methyl-histidine was lowered by HC infusion but not by C, versus controls. Both C and HC infusion increased plasma isoleucine, valine, histidine and lysine versus control (W) infusion. Clark et al. (26) reported that abomasal infusion of 425 g/d sodium casein increased plasma arginine, histidine, isoleucine, leucine, valine, lysine, methionine, alanine and ornithine versus water infusion. In the present study sodium casein (C) increased or tended to increase all the above amino acids except alanine and also increased plasma tyrosine, citrulline and proline. Dif-

Table 7. Effects of basal diet and abomasal infusion on plasma free amino acid concentrations (uM/ml).

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
Leu	15.92	21.28***	1.0	16.68	19.50	21.26**	16.96	1.6
Ile	12.83	11.33	0.4	10.63	12.68**	13.94**	11.08	0.7
Val	27.04	25.73	0.9	21.71	28.03	32.56	23.26	1.5
Thr	12.55	11.09*	0.6	11.59	12.62	12.44	10.62	0.9
Phe	4.45	5.05	0.2	4.42	5.17	4.95	4.46	0.4
Tyr	5.78	6.85**	0.3	6.15	7.53	6.14	5.42	0.5
Met	2.61	2.52	0.2	2.21	2.77	2.57	2.72	0.3
Cys[1]	2.16	2.10	0.04	2.06	2.05	2.32**	2.09	0.1
His	4.11	4.43	0.2	3.53	5.04**	5.01**	3.51	0.3
Lys	10.66	8.42**	0.6	7.36	9.88*	9.96*	10.97**	0.9
Arg	8.20	6.72	0.4	6.67	8.30	7.29	7.58	0.6
Orn	6.17	5.49***	0.1	5.35	5.87*	6.41**	5.70	0.2
Cit	8.45	8.99	0.3	8.21	9.51	9.31	7.86	0.5
Asp	1.09	1.08	0.1	1.13	1.06	0.93	1.23	0.2
Glu	5.73	4.93	0.4	5.40	4.90	5.03	5.98	0.6
Gln	28.90	21.60	3.5	34.23	22.41	25.86	18.44*	5.5
Ser	9.38	9.51	0.6	9.01	9.50	9.98	9.29	1.0
Gly	31.96	29.85	1.0	31.55	29.92	30.74	31.41	1.6
Ala	21.75	20.62	0.7	21.53	20.88	21.58	20.76	1.1
Pro	8.35	9.71	0.6	7.97	10.82**	10.23**	7.10	0.9
Taur	4.85	4.29	0.2	3.80	4.56	4.32	5.62**	0.4
Carn	1.41	1.53	0.2	1.32	1.96	1.25	1.35	0.3
3-M-His	0.61	0.65	0.1	0.80	0.82	0.36**	0.54	0.1

[1] includes cysteine plus cystathione.
Differs from control; * p<.10; ** p<.05; *** p<.01.

ferences in the basal diet fed and average milk production, (29 kg/d for Clark et al.) may explain the results of the two studies.

Discrepancies between the effects of C and HC infusion on plasma amino acid concentrations have several possible explanations. Lower plasma 3-methyl-histidine for HC may indicate a lower rate of protein turnover (68). For the urea cycle amino acids, the higher levels of arginine and citrulline for C versus higher ornithine with HC may indicate greater arginase activity for cows receiving infusion C. Plasma urea was slightly higher for C infusion. Other differences in plasma amino acid concentrations between C and HC suggest differential rates of amino acid absorption and/or utilization.

Within diet effects of C and HC on plasma amino acid concentrations are shown in Table 8. In particular, citrulline concentration was increased by C and HC on diet SG. This may reflect poorer amino acid utilization with this diet. Both caseins decreased methyl-histidine levels versus control on diet S. Plasma cystine was elevated by HC on diet S. The cystine may have been derived from methionine. Overall, the values suggest a greater demand for branched chain amino acids on diet SG, as these amino acids were not increased as much by casein infusions as on diet S.

Effects of Methionine-Lysine Infusion on Plasma Amino Acids

Infusion of 11.3 g/d L-methionine and 30.1 g/d L-Lysine (ML) increased the ratio of essential to nonessential amino acids in plasma (IAA/DAA) (Table 6). ML infusion increased plasma levels of lysine and taurine, while decreasing plasma glutamine concentration (Table 7). Therefore part of the effect of ML on the IAA/DAA ratio appears to have been due to decreased levels of nonessential amino acids, presumably due to increased utilization rate. When cows producing 34 kg milk/d were fed 0, 6.9, 12.9, 19.6 or 40.2 g/d rumen protected D-L methionine, plasma methionine was only significantly increased by the two highest levels of supplementation (79). With 12.9 g/d

Table 8. Combined effects of diet and infusion on plasma free amino acid concentrations.

	Diet * Infusion								
Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
Leu	13.97	18.68 **	18.69 **	12.34	19.39	20.31	23.84	21.58	2.2
Ile	10.66	14.29 **	14.57 **	11.81	10.59	11.06	13.31 **	10.34	0.9
Val	21.31	30.63	31.85	24.38	22.10	25.43	33.27	22.13	2.0
Thr	11.98	13.47	12.85	11.89	11.20	11.78	12.02	9.35	1.3
Phe	3.99	5.18 **	4.78	3.86	4.86	5.15	5.13	5.06	0.5
Tyr	5.34	7.68	5.40	4.70	6.97	7.38	6.89	6.14	0.6
Met	2.27	3.04	2.73 *	2.40	2.15	2.50	2.40	3.04	0.4
Cys	1.85	1.95	2.14	1.64 **	1.78	1.69	2.04	1.85	0.1
Cysta	0.24	0.19 **	0.22 **	0.40	0.26	0.28 **	0.24	0.27	0.1
His	3.20	4.76	5.22	3.28	3.86	5.33	4.79	3.74	0.4
Lys	8.32	11.77	10.92	11.65	6.39	7.99	9.00	10.29	1.3
Arg	7.63	9.76	7.77	7.64	5.70	6.84	6.81	7.52	0.9
Orn	5.81	6.39	6.47	6.03	4.90	5.35 **	6.34 **	5.37	0.3
Cit	8.58	8.74	8.43	8.06	7.83	10.29	10.19	7.65	0.7
Asp	1.03	1.08	0.80	1.44	1.22	1.04	1.06	1.02	0.3
Glu	5.53	5.32	5.99	6.07 *	5.27	4.49	4.06	5.89	0.8
Gln	42.94	28.54	25.97	18.09	25.52	16.28	25.75	18.78	7.9
Ser	8.73	9.84	9.97	8.99	9.30	9.17	9.99	9.58	1.4
Gly	32.41	30.47	31.13	33.82	30.69	29.36	30.35	28.99	2.3
Ala	22.28	21.79	21.57	21.36	20.78	19.98	21.59	20.15	1.6
Pro	7.71	10.77	9.42	5.50 **	8.23	10.88	11.04	8.70	1.3
Taur	4.11	4.43	4.33	6.54	3.49	4.68	4.31	4.69	0.5
Carn	1.36	2.26	0.87 **	1.14 **	1.27	1.67	1.63	1.56	0.4
^{3}-M-His	0.96	0.94	0.16	0.36	0.63	0.69	0.56	0.72	0.2

Differs from control; * $p<.10$; ** $p<.05$.

D-L methionine, there was a non-significant increase in plasma methionine, similar to the present study.

Infusion of ML increased plasma lysine but methionine was not significantly increased (Table 7). However plasma taurine, which is a metabolic product of methionine (45), was increased by ML. This suggests that infused methionine was more actively metabolized than was lysine. Within diet effects (Table 8) show that transformation of infused methionine tended to be greater on diet S. On diet S plasma methionine was increased a small amount by ML infusion, but both cystathione and taurine were increased. On diet SG however, plasma methionine was increased to near significant levels, while no significant increases in its potential metabolites, cystine, cystathione and taurine were found. This is interesting in that metabolism of methionine appears to have been greatest on the diet (S) where ML infusion increased milk fat percentage and yield.

The conversion of methionine to cystine, cystathione and taurine begins with the formation of S-adenosyl-methionine (SAM), which donates methyl groups to a wide array of acceptor molecules (68). Choline is formed by the successive transfer of the methyl groups from three molecules of S-adenosyl-methionine to one molecule of phosphatidylethanolamine (64). Synthesis of choline from L-methionine is the proposed link between methionine and increased milk fat secretion in ruminants (67). Choline is incorporated into phosphatidylcholine (lecithin) which is an important component of lipoproteins, cell membranes and other structures (68). Lecithin-cholesterol acyl transferase, for which lecithin is a primary substrate, is responsible for the formation of cholesterol esters of long chain fatty acids. This enzyme is present in plasma where it forms cholesterol esters by interaction with high density lipoproteins (118). The cholesterol esters formed by this enzyme are especially rich in linoleic and other unsaturated fatty acids, and are a major component of chylomicrons (118). Chylomicrons are a source of lipid for the lactating mammary gland (118) and play a crucial role in lipid absorption from the G.I. tract (68). In addition, the cholesterol esters in the free form may be an important means of cholesterol and fatty acid transport to the liver (118). Therefore methionine can potentially impact lipid metabolism and transport at several key points.

Because choline synthesis in microorganisms is thought to proceed by the same basic pathway as in eukaryotes (34), methionine supply could affect the rate of choline synthesis in the rumen. This may explain some of the beneficial effects of unprotected methionine and methionine hydroxy analog on milk fat secretion in lactating cows (50). In the present study, dietary methionine was lower for diet S, which could conceivably result in lower choline synthesis with this diet, and increase the response to supplemental methionine. In one study, abomasal choline infusion increased fat corrected milk yield in first lactation cows during mid-lactation (39).

Arterio-Venous Differences Across the Mammary Gland

Method

Uptake of plasma free amino acids by the mammary gland were estimated by sampling blood from both the coccygeal vein/artery and the mammary vein. This method has been used by other investigators who have discussed its validity (35,81). The coccygeal vessel was chosen because of ease of sampling and less disturbance to the animal when compared to the internal illiac artery. The latter vessel may be sampled through the rectal wall. In the present study there was concern about possible health risks to the animal from the procedure. However it apparently can be done routinely without risk to the animal (J.H. Clark, personal communication).

The main sources of error in the above technique are due to changes in mammary blood flow due to excitation of the animal and possible backflow of non-mammary blood from the external pudic vein into the mammary vein (13,81). These problems can be overcome by surgical implantation of catheters to measure blood flow directly and of an inflatable balloon to close off the external pudic vein during sampling (81). Neither procedure was possible under the conditions of the present study. Therefore the results are treated as estimates of mammary extraction of amino acids.

Effects of Diet on Mammary Extraction of Amino Acids

Between diets (Tables 9-11) the main differences were that citrulline was extracted to a greater extent when diet S was fed, while removal of taurine was greater for diet SG. There was a tendency for greater extraction of glutamine and glutamate on diet S. Apparent net production of citrulline and urea on diet S may be related to arginase activity in the mammary gland (119). Urea can be produced from arginine by this route, but citrulline is not produced from labeled arginine by perfused goat mammary gland (114). Taurine released by the mammary gland may arise from either methionine or cystine although these pathways have apparently not been investigated in mammary tissue. Uptake of free methionine and cystine are usually barely able to account for their appearance in milk protein (27). However one study found that the lactating mammary gland of cows extracts significant quantities of glutathione (a tri-peptide of glutamic acid, cystine and glycine). It is curious that this has never been pursued since sulphur amino acids may be limiting for milk protein synthesis in ruminants (28,69). It has recently been shown that 5-oxoproline, an intermediate of the gamma glutamyl cycle (responsible for synthesis and breakdown of glutathione), stimulates uptake of most amino acids by rat mammary gland (115).

Extraction of glutamine, glutamate and nonessential amino acids (Table 11) tended to be higher for diet S and may be related to the higher milk protein percent observed with this diet. However milk protein yield was not different between diets.

Effect of Infusion on Mammary Extraction of Amino Acids

The only significant effects of abomasal infusion on mammary extraction of amino acids (Table 10) were that proline extraction was increased over control by casein (C) infusion and ML infusion resulted in mammary production of both citrulline and urea. Proline is usually not extracted in sufficient quantities to account for its appearance in milk (27). However it has been shown that

Table 9. Effect of basal diet and abomasal infusion on mammary arterio-venous differences for plasma free amino acids.

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
EAA[1]	26.0	25.5	8.0	21.7	39.1	23.1	19.0	12.0
NEAA[2]	26.6	15.4	4.5	24.9	32.6	13.6	12.6	7.1
Total[3]	52.5	40.9	11.0	46.6	71.8	36.7	31.7	17.2
SAA[4]	1.4	1.0	0.3	1.0	1.8	0.8	1.2	0.5
URCY[5]	3.7	4.7	1.4	4.3	6.7	3.9	2.0	2.2
BCAA[6]	11.3	14.3	5.0	11.1	19.3	16.3	4.6	8.1

	Diet * Infusion								
Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
EAA[1]	24.9	38.7	34.3	5.9	18.5	39.6	12.0	32.1	17.7
NEA[A2]	43.0	31.4	23.1	8.8 *	6.9	33.9	4.1	16.5	10.3
Total[3]	67.9	70.1	57.4	14.7	25.4	73.5	16.1	48.6	24.6
SAA[4]	1.3	1.7	1.5	1.1	0.8	1.9	0.03	1.3	0.7
URCY[5]	4.9	7.0	4.2	-1.2	3.6	6.3	3.6	5.2	3.1
BCAA[6]	11.7	20.1	19.9	-6.4	10.4	18.5	12.8	15.6	11.6

[1] arterio-venous difference for the sum of essential amino acids.
[2] " " " " " sum of nonessential amino acids.
[3] " " " " " sum of total amino acids.
[4] " " " " " sum of sulpher amino acids.
[5] " " " " " sum of urea cycle amino acids.
[6] " " " " " sum of branched chain amino acids.
Differs from control; * $p<.10$.

Table 10. Effect of basal diet and abomasal infusion on mammary arterio-venous differences for plasma free amino acids.

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
Leu	3.22	6.62	2.4	4.48	8.60	7.00	-0.44	3.6
Ile	3.85	2.87	0.8	2.88	4.40	2.68	3.48	1.6
Val	4.24	4.84	2.0	3.68	6.32	6.64	1.52	3.2
Thr	3.34	2.37	0.8	2.72	5.28	0.05	3.36	1.6
Phe	1.56	1.59	0.4	1.44	2.64	1.51	0.71	0.6
Tyr	1.13	1.96	0.4	1.32	3.44	1.32	0.12	0.8
Met	1.28	0.92	0.2	0.92	1.56	0.68	1.24	0.4
Cys	0.06	0.09	0.1	0.07	0.15	0.18	-0.10	0.1
Cysta	0.06	-0.01	0.04	0.03	0.06	-0.08	0.08	.04
His	0.96	1.24	0.4	0.76	2.00	1.32	0.30	0.8
Lys	5.16	3.24	0.8	3.08	5.44	2.08	6.16	1.4
Arg	2.36	1.84	0.8	1.72	2.88	1.16	2.68	1.0
Orn	1.99	1.56 ***	0.4	1.60	1.98	1.66	1.86 **	0.8
Cit	-0.64	1.32	0.4	0.92	1.80	1.12	-2.48	0.8
Asp	0.32	0.23	0.2	0.28	0.32	0.06	0.44	0.3
Glu	4.00	2.46	0.8	3.08	3.20	2.52	4.12	1.2
Gln	11.02	1.65	4.0	12.92	5.84	5.80	0.76	6.4
Ser	2.34	1.02	0.8	1.20	3.28	0.08	2.16	1.2
Gly	2.92	0.26	1.6	1.20	3.72	-2.96	4.36	2.8
Ala	2.48	1.80	0.8	2.28	2.88 **	2.48	0.96	1.2
Pro	0.92	3.00 **	1.2	0.03	5.96	1.44	0.40	1.7
Taur	0.44	-0.21	0.16	.003	-0.10	-0.06	0.64	0.2
Carn	-0.49	0.16	0.32	-0.60	0.25	-0.17	-0.16	0.5
3M-His	-0.04	-0.11	0.12	0.0001	0.12	-0.26	-0.16	0.2

Differs from control; * p<.10; ** p<.05; *** p<.01.

arginine, ornithine and citrulline are precursors of proline in mammary tissue (114). In the present study, mammary proline extraction was associated with an increase in milk and milk protein yield. Extraction of essential and total amino acids tended to be greatest for C infusion. HC infusion did not produce the same pattern of extraction of these amino acid groups. Mammary production of citrulline and urea during ML infusion suggests increased arginase activity. This may reflect greater conversion of arginine to nonessential amino acids. Finally, leucine uptake was very low for the ML infusion. Usually, leucine is taken up in excess of its appearance in milk protein (13,69).

Within diet effects of infusion on mammary amino acid extraction (Table 11) show that for ML infusion, most of the citrulline production by the mammary gland occured on diet S, but that urea production occured on both diets. Extraction of total nonessential amino acids was lower than control for ML on diet S (Table 11). Net production of leucine, valine, tyrosine, histidine and proline also apparantly occured during treatment SML. Tyrosine can be produced from phenylalanine by tyrosine hydroxylase (68) but the uptake of phenylalanine was low for SML. Milk production tended to be slightly less than control for SML (Table 3). Apparent net production of amino acids may be due to efflux from red blood cells or hydrolysis of plasma peptides.

Mammary extraction of threonine, tyrosine and proline were greater than controls for C infusion on diet SG. Infusion of C resulted in a more consistent pattern of amino acid extraction than did HC. The latter infusion resulted in several negative values. Thus the milk and milk protein responses obtained with C seem to be associated with a greater overall extraction of plasma free amino acids by the mammary gland. It appears that some type of amino acid imbalance may have existed with the HC and ML infusions as suggested by the apparent release of several essential amino acids by the mammary gland. An imbalance between essential amino acids in plasma could potentially impair mammary utilization of amino acids, leading to inconsistent mammary amino acid uptake. Infusion of hydrolyzed casein did not increase yields of milk or milk protein and was associated with an inconsistent pattern of amino acid extraction by the mammary gland.

Table 11. **Combined effects of diet and infusion on mammary arterio-venous differences for plasma free amino acids.**

	Diet * Infusion								
Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
Leu	4.68	7.35	8.07	-7.23	4.30	9.86	5.94	6.39	5.2
Ile	2.98	4.74	4.66	3.03	2.79	4.07	0.68	3.96	2.1
Val	4.05	8.00	7.13	-2.22	3.32	4.59	6.15	5.29	4.6
Thr	4.64	3.46	2.34	2.89	0.80	7.12*	-2.24	3.80	2.1
Phe	1.83	2.30	2.21	-0.09	1.07	2.97	0.82	1.50	0.9
Tyr	1.77	2.18	2.13	-1.56	0.86	4.66*	0.50	1.80	1.2
Met	1.14	1.42	1.40	1.17	0.68	1.73	-0.02	1.30	0.6
Cys	0.08	0.22	0.16	-0.26	0.05	0.07	0.18	0.06	0.2
Cysta	0.04	0.01	-0.03	0.20	0.02	0.12	-0.13	-0.02	0.1
His	0.58	1.98	1.89	-0.70	0.94	2.02	0.75	1.30	1.1
Lys	3.39	5.64	4.70	6.90	2.76	5.27	-0.52	5.42	2.0
Arg	1.60	3.81	1.88	2.19	1.85	1.95	0.43	3.13	1.4
Orn	1.90	2.29	2.34	1.44	1.31	1.67	0.97	2.28	1.2
Cit	1.36	0.89	0.02	-4.80**	0.48	2.72	2.22	-0.19	0.9
Asp	0.24	0.32	0.07	0.63	0.33	0.30	0.04	0.24	0.4
Glu	3.59	3.77	4.25	4.40	2.57	2.65	0.78	3.84	1.3
Gln	25.90	11.53	5.48	1.14	0.03	0.17	6.12	0.34	9.2
Ser	2.12	2.70	2.32	2.24	0.30	3.82	-2.13	2.10	1.6
Gly	2.68	1.86	-0.10	7.20	-0.30	5.60	-5.80	1.52	9.5
Ala	3.02	3.01	3.18	0.70	1.53	2.76	1.77	1.21	1.6
Pro	0.27	2.58	3.32	-2.56	-0.22	9.36**	-0.44	3.35	2.5
Taur	0.39	0.25	0.21	0.93	-0.38	-0.45	-0.33	0.31	0.3
Carn	-0.90	0.20	-0.42	-0.85	-0.32	0.30	0.08	0.52	0.8
3M-His	0.22	0.43	-0.45	-0.36	-0.22	-0.20	-0.06	0.04	0.3

Differs from control; * $p<.10$; ** $p<.05$.

Limiting Amino Acids for Milk Production

Limiting amino acids for milk production were estimated by the method of Clark et al. (26). The concentration of each IAA in blood plasma and milk is divided by the concentration of total IAA (excluding tryptophan which was not measured). The relative concentration of each amino acid in plasma is divided by its relative concentration in milk. The amino acid with the smallest ratio is declared most limiting for milk protein synthesis (26).

By this method the order of limiting amino acids were:

1. W - Phe Met Lys His Leu Ile Arg Cys Thr Val

2. C - Phe Met Lys His Leu Ile Arg Cys Thr Val

3. HC- Phe Met Lys His Arg Leu Ile Thr Cys Val

4. ML- Phe Met His Lys Leu Ile Arg Thr Cys Val

Using the same method Clark et al. (26) reported that the first three limiting amino acids during water infusion (different basal diet) were; lysine, methionine and phenylalanine. For casein the order of limiting amino acids was; methionine, phenylalanine and lysine. These amino acids have been suggested as limiting for milk protein synthesis by other methods of calculation (13). Threonine, histidine and arginine were associated with increases in milk yield in the study of Schwab et al. (101). Therefore a combination of these six amino acids may account for most of the production responses to casein infusion. In the present study, the order of limiting amino acids was not markedly changed by abomasal infusions except ML resulted in lysine becoming less limiting.

Rumen Metabolites and pH

Rumen Ammonia, Plasma Urea, Rumen and Duodenal pH.

Rumen pH was not significantly different between diets, infusions or diet*infusion combinations (Table 12). Both diets were adequate in fiber content (Table 1) so that normal rumen pH (6 to 7) would be expected. Values are similar to those reported by Chapin (21) and Zerbini (121).

Duodenal pH was within values reported for ruminants (111). Abomasal infusion of casein, especially HC, tended to increase pH slightly, especially on the SG diet. This is presumably due to the buffering capacity of functional groups of amino acids. Greater buffering would be expected from HC which contains about 35% of the amino acids in free form.

Rumen ammonia tended to be higher ($p < .15$) for diet S. This is consistent with the more extensive rumen hydrolysis of soybean meal versus corn gluten meal. Although not significant, rumen ammonia was slightly higher than controls for HC and lower for ML. These trends were primarily on diet S and may reflect the net exchange of rumen ammonia and blood urea across the rumen wall. Plasma urea nitrogen values are also shown (Table 12). Generally, plasma urea nitrogen (PUN) is positively correlated with rumen ammonia concentration in that ammonia diffuses down its concentration gradient across the rumen wall and into the portal blood (58). The rate increases with pH (58). However blood urea also diffuses into the rumen, assisted by the activity of ureolytic bacteria attached to the rumen wall (34). During the course of a 24 hour day, both processes probably occur, depending on the time since the last meal, nitrogen intake and rumen degradability (54). The data presented here are composites of six separate samplings of rumen fluid and blood, representing several time points relative to feeding.

Plasma urea paralleled rumen ammonia concentration across diets and infusions (simple regression, $r = .9097$). However, within each diet the relationship between rumen ammonia and plasma urea

Table 12. Effects of basal diet and abomasal infusion on rumen parameters.

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
Rumen pH	6.07	6.07	.03	6.13	6.03	6.03	6.09	.05
Duodenal pH	2.83	2.83	.03	2.81	2.83	2.96**	2.72	.05
Rumen NH3	8.78	7.85	.40	8.13	8.36	9.01	7.75	.70
PUN mg/dl[1]	11.12	10.97	.70	10.87	11.90	12.40	9.01	1.20

Diet * Infusion

Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
Rumen pH	6.21	6.09	5.98	6.02	6.05	5.98	6.08	6.17	.07
Duod. pH	2.84	2.81	2.92	2.77	2.78	2.85	3.00*	2.67	.07
Rumen NH3	8.52	8.83	10.02	7.74	7.74	7.89	8.00	7.76	1.00
PUN mg/dl[1]	9.48	13.78	11.11	10.10	12.26	10.03	13.69	7.92	1.70

[1] Plasma urea nitrogen
Differs from control infusion; * $p<.10$; ** $p<.05$.

was lost ($r = .2050$, diet S, $r = .2983$; diet SG, $r = .5449$). Because each value is a composite of six samplings, certain diurnal fluxuations in the two parameters which may better explain their relationship may have been masked. Plasma urea concentration is also affected by the rate of amino acid catabolism in the body. Generally, plasma urea increases when excess protein is ingested or when protein is not being efficiently utilized, as in the case of amino acid imbalance (112). The treatments in which plasma urea appears to be high relative to rumen ammonia levels; SC, SML, SGW, SGC and SGHC; may have resulted in greater amino acid catabolism due to excess amino acid supply (SC, SGC and SGHC) or lack of certain limiting nutrients (SGW and SML).

Rumen Volatile Fatty Acids

Molar percentages of rumen volatile fatty acids (VFA) and total VFA concentrations are shown in Table 13. Molar percentages of acetate and propionate were similar between diets. This would be expected when the diets contain the same proportion of fiber (ADF) from the same main source (corn silage). Molar percentage of butyrate was lower in cows fed diet SG ($p < .05$) as was valerate ($p < .01$). These differences are probably due to the protein supplementation of the diets. Amino acids are fermented in significant quantities by rumen bacteria to form volatile fatty acids (6,34). Therefore the observed differences in VFA proportions may be due to amino acid availability from the protein sources. However corn gluten meal, which was present only in diet SG, was previously found to increase rumen butyrate concentration (47). The authors attributed the effect to the high leucine content of corn gluten meal. However in their study the percentage of corn gluten meal in the diet was higher than in the present study. Although other studies have found differences in rumen VFA due to dietary protein source the biochemical reasons for these differences have not been investigated. The effects of protein source on rumen VFA could be of significance in animal production.

Molar percentage butyrate and total VFA were lower for C infusion versus W, while molar percentage valerate was lower for SC and SML versus SW and for SGC and SGHC versus SGW. These results are probably not biologically significant.

Apparent Total Tract Digestibilities

Apparent digestibilities of nitrogen (N), organic matter (OGM) and acid detergent fiber (ADF) are shown in Table 14. Values were similar between diets. Apparent N digestibility was increased over control by infusion of sodium casein (C) or hydrolyzed casein (HC). This reflects the fact that the nitrogen infused (&eqv.55 g/d, 2% increase in crude protein) was highly digestible relative to dietary N. Values were slightly higher for C versus HC. Within diet effects show that most of the overall difference in apparent N digestion was on diet SG. Apparent N digestibility of SGW tended to be lowest of the eight treatments, suggesting that the digestibility of diet SG tended to be lower than diet S. Apparent digestibility values can also be affected by endogenous nitrogen secretion such as enzymes and sloughed cells. Endogenous N losses may have been greater with diet SG. Diet SG contained a greater proportion of rumen escape protein. This could have increased nitrogen supply to the large intestine, resulting in greater microbial growth. This would increase the fecal flow of microbial organic matter and nitrogen, which would decrease decrease apparent digestibilities. Absolute values for apparent digestibility of N, OGM and ADF tended to be somewhat lower than those reported by some other investigators (21,89,121). This suggests that forage quality may have been lower than typically found with corn silage.

Duodenal Flow of Dry Matter and its Constituents

Duodenal flows of dry matter and constituents are shown in Tables 15 and 16. Duodenal flows and rumen digestion of dry matter (DM), organic matter (OGM) and acid detergent fiber (ADF)

Table 13. Effects of basal diet and abomasal infusion on rumen volatile fatty acid concentrations.

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
MP Ac[1]	70.60	70.90	.50	70.50	70.00	71.10	71.40	.80
MP Pro[2]	16.20	16.50	.60	16.50	17.70	16.30	15.00	1.00
MP IB[3]	.72	.68 **	.02	.70	.67 **	.70	.74	.03
MP B[4]	10.20	9.70	.20	10.20	9.30	9.80	10.40	.30
MP IV[5]	1.40	1.30 ***	.04	1.30	1.25	1.38	1.42	.07
MP V[6]	1.10	1.00	.02	1.04	.98 *	1.00	1.02	.03
Total (mMol/ml)	122.60	122.70	1.70	126.10	117.90	123.40	123.10	2.70

Diet * Infusion

Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
MP Ac[1]	69.50	71.10	70.00	71.60	71.40	68.80	72.20	71.20	1.10
MP Pro[2]	17.40	16.30	17.10	14.10	15.60	19.20	15.40	15.80	1.40
MP IB[3]	.71	.71	.68	.76	.68	.63	.72	.71	.04
MP B[4]	10.20	9.40	10.00	10.90	10.10	9.10	9.50	9.90	.35
MP IV[5]	1.30	1.30 **	1.30	1.50 **	1.30	1.20 **	1.50 **	1.30	1.00
MP V[6]	1.10	1.00	1.10	1.00	1.00	.90	.90	1.0	.04
Total (mMol/ml)	125.60	114.90	125.20	124.70	126.70	120.90	121.50	121.60	3.90

[1] Molar percentage of acetate
[2] " " of propionate
[3] " " of isobutyrate
[4] " " of butyrate
[5] " " of isovalerate
[6] " " of valerate

Differs from control infusion; * p<.10; ** p<.05; *** p<.01.

Table 14. Effects of basal diet and abomasal infusion on apparent total tract digestibilities of nitrogen, organic matter and acid de

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
					**	**		
N[1]	63.9	62.2	.01	60.1	66.7	65.6	59.8	.02
OGM[2]	64.5	63.7	.01	63.6	64.1	64.9	63.8	.02
ADF	43.3	42.6	.01	42.2	42.9	42.2	44.3	.02

Diet * Infusion

Item	SW	SC	SHC	SML	SGW	SGC	SGHC	SGML	se
						**	**		
N[1]	63.6	67.1	66.8	58.2	56.6	66.2	64.5	61.4	.02
OGM[2]	63.3	64.7	67.2	62.8	63.9	63.6	62.6	64.9	.02
ADF	39.9	43.6	44.7	44.9	44.5	42.2	39.8	43.7	.03

[1] Nitrogen
[2] Organic matter
Differs from control infusion; ** $p<.05$

were not significantly different between diets. Duodenal flows of OGM and ADF tended to lower and apparent rumen digestibilities lower than reported values of 30 to 43% for OGM and 35 to 43% for ADF in lactating dairy cows (89,99,105). Robinson et al. (88) using lactating cows fed at four levels of intake and fed grass-legume hay, corn and soybean meal, reported apparent rumen digestibilities of 42 to 45% for OGM and 46 to 51% for ADF. Santos et al. (99) reported values of 30 to 33% for OGM in cows fed diets of corn silage, alfalfa hay, corn and soybean meal or corn gluten meal.

Of primary interest in the present study was the effect of basal diet on the total supply of nitrogen and protein reaching the duodenum. Total duodenal N flow tended to be higher for diet SG versus diet S, while duodenal recovery of dietary N was 91.2 and 105.5% for diets S and SG respectively. These results are consistent with the presence of rumen escape protein in diet SG, in the form of corn gluten meal. Stern et al. (105) reported that duodenal N recovery was 109% in cows fed mixed diets containing urea and a comparable amount of corn gluten meal to the present study. Precipitable protein comprised 45 and 52.6% of duodenal N for diets S and SG, although the amount of liquid phase protein was lower for SG. These results are consistent with greater duodenal flow of intact dietary protein with diet SG. Average rumen degradabilities of soybean meal and corn gluten meal are 70 and 45% respectively (76). Therefore diet SG (60:40 soybean meal/corn gluten meal) should supply more intact feed protein postruminally than diet S (100% soybean meal).

Total duodenal N flow was significantly higher for infusions C and HC versus control (W) as would be expected. Liquid phase flow of total N and precipitable protein was higher for C and HC versus W. Hydrolyzed casein (HC) tended to increase liquid N and protein flow more than C relative to control (W). This is consistent with the large proportion of free amino acids and short peptides present in HC, most of which would not be precipitated by trichloroacetic acid. Apparent rumen N digestion is decreased by postruminal infusion of protein. The fact that the effect of infusion was not as significant as might be expected in increasing duodenal N flow is related to the very large variation encountered in duodenal solids flow. This suggests that acid detergent insoluble ash may

Table 15. Effect of basal diet and abomasal infusion on duodenal flow of dry matter and its constituents.

Diet				Infusion				
Item	S	SG	se	W	C	HC	ML	se
DM g/d	9738	10171	1294	8808	9557	10704	10750	1952
RDDM %[1]	43.7	42.1	5.1	47.4	44.7	39.9	39.7	7.7
OGM g/d	7586	7496	1029	6472	7419	8036	8235	1537
RDOGM %[2]	51.9	51.0	6.3	57.5	52.6	48.5	47.2	9.4
ADF g/d	1704	1751	317	1595	1538	1801	1976	474
RDADF %[3]	51.6	46.3	8.8	50.3	55.1	48.8	41.5	13.1
N intake (g/d)	434.5	410.2 ***	5.9	390.0	456.3	448.4	394.9	9.4
N flow (g/d)	398.9	431.1	31.1	344.1	437.1	477.1	416.3	46.5
Liquid N flow (g/d)	173.3	156.6	9.2	136.7	178.1 *	194.0 **	151.1	14.6
Solid N flow (g/d)	228.8	290.9	37.0	207.4	259.0	283.1	265.2	55.3
RDN %[4]	2.8	-15.6	8.7	12.2	-10.4	-20.0	-7.4	13.0

[1] Rumen digestibility of dry matter, apparent.
[2] Rumen digestibility of organic matter, apparent.
[3] Rumen digestibility of acid detergent fiber.
[4] Rumen digestibility of nitrogen, apparent.
Differs from control infusion; * $p<.10$; ** $p<.05$; *** $p<.01$.

Table 16. Effect of basal diet and abomasal infusion on flow and composition of duodenal nitrogen.

	Diet			Infusion				
Item	S	SG	se	W	C	HC	ML	se
Protein flow (g/d)[1]	179.9	226.7	29.0	158.9	200.0	229.3	225.1	43.4
Liquid phase (g/d)	7.5	5.3 **	0.3	5.4	7.0 **	8.2 **	4.9	0.5
Solid phase (g/d)	172.6	221.5	29.0	153.2	193.0	221.9	220.2	43.4
Microbial N flow (g/d)	169.3	184.7	26.0	138.8	166.8	189.0	213.3	38.8
Liquid (g/d)	13.7	12.4	1.6	11.7	12.3	16.5	11.7	2.5
% liquid N	6.7	6.5	0.5	7.0	5.8	7.4	6.2	0.9
Solid (g/d)	155.6	172.3	26.2	127.1	154.5	172.5	201.6	39.2
% solid N	65.0	53.9 ***	2.2	58.6	57.4	56.4	65.5	3.4
Efficiency[2]	23.2	27.6	6.2	16.9	22.5	29.8	32.4 *	9.3
Residual N[3]	200.9	220.3	7.0	207.3	211.5	220.7	203.0	10.8
RDN%[4]	50.6	40.8 **	2.1	-	-	-	-	-

[1] nitrogen precipitated by trichloroacetic acid.
[2] g microbial N / kg OGM apparently digested in the rumen.
[3] total N - (microbial N + infused N).
[4] percent of dietary crude protein degraded in the rumen.
Differs from control infusion; * p<.10; ** p<.05; *** p<.01.

not be as desirable a marker as some of the rare earth markers which can be measured more precisely in small quantities. Assay of ADIA is particularily variable in duodenal liquids. The amount of dry matter in duodenal liquids is very low, and as such any variation in the amount of ADIA present is magnified when expressed on a dry matter basis. In some cases the concentration of ADIA was higher in duodenal liquid dry matter than in duodenal solids dry matter. This violates a requirement of the dual marker calculation, that the solid phase marker associate preferentially with the solid phase of digesta (3,42).

Duodenal Flow of Microbial Nitrogen

The nitrogen and cytosine content of isolated rumen microbes are shown in Appendix Table 1. Nitrogen content was comparable to values summarized by Czerkawski (34) for rumen liquid phase bacteria. There is considerable variation in the reported composition of mixed rumen bacteria even when effect of solid and liquid phase organisms is accounted for. Further studies would be useful. Cytosine content, expressed as uMol/g DM, averaged 30.6. Means for the four cows used in the study ranged from 26.4 to 34.2. Armentano (3) reported somewhat higher values for both nitrogen and cytosine for mixed rumen liquid phase bacteria from lactating cows fed corn silage and soybean meal or dried brewers grains.

Microbial N flow to the duodenum averaged about 46% of total nitrogen (corrected for infused N). This value is somewhat lower than typically reported for lactating cows fed mixed diets supplemented with soybean meal, 50 to 70% (88,99,104), but is comparable to that reported by Stern et al. (105) for cows fed varying levels of corn gluten meal, 45 to 51% of the grain mix. Similar contribution of microbial nitrogen to duodenal digesta was reported by Chapin (21) in lactating cows fed corn silage diets supplemented with 2:1 soybean meal:fishmeal or 2:1 fishmeal:soybean meal. Values were similar between diets S and SG. In some cases the inclusion of rumen escape protein in the diet has reduced the flow of microbial N to the duodenum (21,47,121). This did not happen in the present study, probably because diet SG still contained 60% of supplemental protein

as soybean meal. Thus the diet formulated according to NRC guidelines for rumen degraded crude protein supported rumen microbial growth as well as the more soluble protein-soybean meal diet. The percentage of microbial N in duodenal solids was in agreement with that reported by Zerbini (121), while the value obtained for duodenal liquids was lower (Table 16). Part of the effect is due to infused ^{15}N because the solid and liquid phases could not be individually corrected for infused N, and because a larger proportion of the infused N appeared in the liquid phase.

The efficiency of microbial N synthesis (g microbial N per kg OGM apparently digested in the rumen) averaged 25.4, which is in the range of 25 to 40 reported in other studies (104). A review of literature values gave an average figure of 27 (104). Stern et al. (105) found that efficiency of microbial N synthesis decreased linearly from 44.3 to 34.9 g N/kg OGM apparently digested as the percentage of corn gluten meal in the grain mix was increased from 3.5 to 38%. In their study the 15% corn gluten meal treatment was similar to diet SG in the present study, and resulted in an efficiency value of 39. Therefore in the present study, the efficiency of microbial N synthesis appears to be somewhat lower in some other studies. As mentioned earlier, the extent of rumen organic matter digestion was higher than typically reported. This would tend to decrease the calculated efficiency of microbial N synthesis. The similarity in rates of microbial N synthesis for diets S and SG suggest that partial substitution of corn gluten meal for soybean meal in the diet did not impair rumen microbial N synthesis. In some cases, diets supplemented with corn gluten meal have yielded less microbial protein at the duodenum than diets supplemented with more soluble, readily degraded proteins such as soybean meal and linseed meal (47,105). These investigators reported that rumen ammonia concentrations were lower with the corn gluten meal diets versus soybean meal or linseed meal. In one study (47) rumen ammonia concentration was 6.71 mg/dl for a corn gluten meal supplemented diet. This is only slightly higher than the minimum concentration of 5 mg/dl required for maximal rates of microbial protein synthesis in vitro (100). However 5 mg/dl has not always been adequate to maintain rumen microbial N synthesis in vivo (104). In the present study, rumen ammonia concentrations 8.78 and 7.85 mg/dl for diets S and SG, respectively. The concentration was slightly higher for diet S ($p < .15$). However both values were lower than

expected based on diet and intake and may have been inadequate to support maximal rates of rumen microbial protein synthesis.

The flow of residual nitrogen to the duodenum, which consists of undegraded feed N, endogenous nitrogen and infused N, tended to be greater for diet SG. Residual nitrogen, corrected for infused nitrogen, was not significantly different between infusions, but tended to be greater ($p < .15$) for diet SG (Table 16). Percent rumen degradation of dietary crude protein was greater ($p < .05$) for the soybean meal supplemented diet (50.6%) than for the soybean meal-corn gluten meal diet (40.7%). These values are lower than most previously reported for corn silage based diets supplemented with soybean meal or corn gluten meal (76). However, Stern et al. (105) found that a diets similar to diet SG in the present study were approximately 42 to 47% degraded in the rumen. The lack of agreement between results from diet S and some previous studies with soybean meal may be due to low rumen degradability of crude protein in the corn silage, high moisture corn or soybean meal used in this study. Rumen ammonia concentrations were lower than typically obtained with mixed corn silage-corn-soybean meal diets containing about 14% crude protein (85). Therefore the extent of ruminal degradation of protein in diet S appears to have been lower than typical reported. Variation in protein quality of soybean meal and other protein concentrates occurs due to processing (25,100).

Conclusions From Digesta Flow Data

In general, duodenal flow of nitrogen and protein was enhanced by partial substitution of corn gluten meal for soybean meal in corn silage based diets fed to lactating dairy cows. Most of the increase was in the form of non-microbial nitrogen. Microbial N yield was not significantly different between diets. Rumen degradabilities of dietary crude protein were lower than predicted for both diets. However, rumen degraded N for diet SG was similar to that reported by Stern et al. (105) for a similar diet fed to lactating cows. The rumen degradability of diet S was lower than typically reported for corn/soy diets in lactating cows.

Abomasal infusion of casein and hydrolyzed casein were reflected in total and non-microbial duodenal nitrogen flow, especially in duodenal liquids, which lends validity to the methods used in the study.

Recommendations for Future Studies

If the present experiment were to be repeated, the digestion trial would be conducted separately from the abomasal infusions; first because infusions may interfere with measurements of duodenal digesta, and second because little new information is gained unless the cows are equipped with ileal cannulae. A longer sampling period would be employed, perhaps with more sampling times (n = 12) included. A rare earth element might be probably be preferable as the solid phase marker. Acid insoluble ash has been used successfully but this was in studies where duodenal digesta was not separated into solid and liquid phases for analysis (47). In the present study, the concentration of ADIA (acid detergent insoluble ash) was sometimes higher in duodenal liquid phase dry matter than in solid phase dry matter. The dual marker technique requires that the solid and liquid phase markers associated preferentially with the solid and liquid phases, respectively (3,42). It appears that ADIA tends to solubilize in the abomasum or doudenum. This is in contrast to results reported for acid insoluble ash (47), although these workers centrifuged duodenal digesta samples at ten times the force (30,000 * g) used in the present study (3000 * g) to separate solid and liquid phases. If insoluble ash is used, either ADIA or acid insoluble ash, it is highly recommended that only one source of silage or hay be fed, as considerable variation in ADIA content was found between corn silage from different silos. In general, it would be best if one source of silage were fed throughout the study. The liquid phase marker could be continuously infused, although values for duodenal liquid flow were rather consistent even though CoEDTA was administered as a bolus three times per day. Cobalt concentration in duodenal liquid phase dry matter was at least ten times higher than in solids. This method would appear to be as effective as mixing the marker with the feed. The bolus method allows equilibration of the marker with the larger mass of rumen digesta,

whereas a highly soluble marker in the feed could easily be washed directly from the reticulum into the omasum. There is also the advantage of knowing the exact dosage of marker administered. Ideally, both feeding and marker administration would be done four times daily or more.

Summary and Conclusions

1. Infusion of 11.3 g/d L-methionine and 30.1 g/d L-lysine increased milk protein percentage across both diets and increased milk fat percentage and yield on diet S (soybean meal supplemented) in cows during mid-lactation. Therefore there is potential benefit from supplementing these amino acids in rumen protected form in the diets of lactating cows.

2. Abomasal infusion of 400 g/d sodium casein increased milk and milk protein production while decreasing milk fat percentage but not milk fat yield. The decrease in milk fat percent was pronounced on diet SG (60/40 soybean meal/corn gluten meal). Bodyweight gain was increased by sodium casein infusion.

3. Infusion of 400 g/d enzymatically hydrolyzed casein did not produce significant increases in milk or milk protein production or bodyweight gain, but tended to improve milk fat production on diet SG. The difference in production responses between intact and hydrolyzed casein suggests amino acids from the two sources were not utilized with the same efficiency, probably because of differences in patterns of amino acid absorption.

4. Source of supplemental protein in the basal diet affected the response to abomasal infusion of protein or amino acids. This indicates that amino acid nutrition of a ruminant animal, in this case the dairy cow, can be affected by manipulation of dietary protein degradability (source).

5. Infusion of sodium casein or hydrolyzed casein elevated plasma concentrations of the essential amino acids. However there were differences between the two casein sources for certain amino acids suggesting that there were differences in amino acid absorption.

6. Methionine and lysine infusion increased plasma lysine and taurine concentrations and gave a non-significant increase in plasma methionine. These results suggest that infused methionine was metabolized to a greater extent than was lysine. This is consistent with methionine's role as a methyl donor and was associated with the milk fat response observed with methionine-lysine infusion on diet S. Methionine appears to exert significant effects on ruminant lipid metabolism.

7. Total duodenal flow of nitrogen, precipitable protein and non-microbial nitrogen tended to be greater for diet SG, in which corn gluten meal was substituted for part of the supplemental soybean meal. Percent rumen degradation of dietary crude protein was lower for the diet supplemented with 2:1 soybean meal:corn gluten meal. Microbial nitrogen flow was similar between diets, although microbial nitrogen as a percent of total duodenal nitrogen tended to be greater for diet S. Apparent efficiency of rumen microbial protein synthesis was similar to both diets and averaged 25.4%.

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Table 17. Nitrogen and cytosine content of mixed rumen bacteria.

	Nitrogen		Cytosine		Nitrogen/Cytosine	
Cow	g N/g DM	SD	µMol C/g DM	SD	mg N/µmol C	SD
1592	.0543	.003	26.36	.20	2.058	.11
1691	.0607	.003	30.28	.40	2.004	.07
1764	.0641	.002	31.37	3.50	2.062	.16
1797	.0684	.002	34.17	.01	2.002	.05
Diet						
S	.0628	.007	31.48	3.50	1.996	.07
SG	.0609	.004	29.61	2.90	2.067	.13
mean	.0619	.006	30.55	3.20	2.031	.11

[1] fluid phase bacteria isolated as described in text.

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