Lactational and Systemic Responses to the Supplementation of Protected Methionine in Soybean Meal Diets

Daniel J. Illg

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LACTATIONAL AND SYSTEMIC RESPONSES
TO
THE SUPPLEMENTATION OF PROTECTED METHIONINE
IN
SOYBEAN MEAL DIETS

By

DANIEL J. ILLG

A thesis submitted
in partial fulfillment of the requirements for the
degree of Master of Science
Major in Dairy Science
South Dakota State University
1986
LACTATIONAL AND SYSTEMIC RESPONSES TO THE SUPPLEMENTATION OF PROTECTED METHIONINE IN SOYBEAN MEAL DIETS.

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Dr. Joel L. Sommerfeldt
Thesis Advisor

Dr. David J. Schingethe
Head, Dairy Science Dept.
Dedication

To Kim, who was always there for long-distance support; and to my family: Terry, Jerry, Cindy, Tom, Janet, Deann, Marilyn, Kathy, Patty, and Michele and especially Mom and Dad who may not have always understood my motivations but have played a vital role in what I have become.
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Last but not least, thanks to my friends and family for your support without which much of this would not have been possible.

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INTRODUCTION

The high producing dairy cow requires a complex balance of protein and energy. Microbial protein provides a substantial amount of the amino acids absorbed by the small intestine. High production rates cannot be attained with microbial protein as the sole source of amino acids.

The ten classical essential amino acids are required for milk protein synthesis. Increasing the amount of available amino acids to the mammary gland has improved casein production, indicating substrate availability as a controlling factor to milk protein synthesis. A major portion of the nitrogen component of milk is derived from free plasma amino acids. Uptake by the mammary gland is a key to milk protein production. Amino acid absorption is a process of active diffusion and is dependent upon: arterial concentration of amino acids, rate of mammary blood flow, and the extraction process by the alveolus of the mammary gland.

Protein and amino acid research has concentrated on determining the amino acid or amino acids most limiting to milk production and the most appropriate means of presenting these nutrients to the small intestine for absorption. Microbial degradation accounts for a high rate of nitrogen turnover in the rumen. The amino acid profile of feedstuffs
reaching the small intestine are not in the same proportion as those in the diet. To change the status of the amino acid profile reaching the small intestine, protection of dietary proteins and amino acids has become an area of concentrated research. Heat treatment, chemical treatment and encapsulation have been utilized as protection devices.

The amino acid most frequently found limiting to milk production is methionine. Lysine, phenylalanine, and threonine have also been implicated as limiting or co-limiting. Feeding of protected sources of proteins or amino acids has shown variable results. Abomasal infusions of amino acids and casein have exhibited increases in milk production and milk protein production.

The objective of this research was to feed rumen-protected methionine in a diet likely limited in methionine and measure milk production responses to the supplementation.
Rumen Nitrogen Metabolism:

Microbial protein has an important role in the pattern of amino acids entering the small intestine (13, 54). The amino acid composition of microbial protein tends to be constant (7, 85). The digestibility of the microbial protein in the small intestine has been determined to be at least 80% (38, 83) making it a readily available source of amino acids to the animal.

Feed proteins can be segmented into fractions that are degraded by rumen microorganisms at different rates. Pichard and Van Soest (68) categorized proteins the following way: 1) feed protein which is water soluble non-protein nitrogen (NPN) that includes nitrate, ammonia, amines, and free amino acids and is degraded rapidly and completely by the microbial population, 2) feed protein which is insoluble "true" protein and is rapidly degraded, 3) feed protein which is insoluble "true" protein and is more slowly degraded, and 4) feed protein which is unavailable to microbial degradation due to natural protections or induced denaturation of the proteins. The amount of feed protein escaping ruminal degradation is also dependent upon retention time of the feed in the rumen (13). Retention time is dependent on particle size and density of
ingested feed (90) and feed consumption rate (61). Animals at high levels of productivity require the use of feed protein which is unavailable to microbial degradation because the microbial fermentation is not adequate in supplying a proper protein to energy ratio to the animal's system (76). Virtanen (88) fed cows protein-free diets supplemented with urea and other NPN sources and observed lower milk production than would be expected with supplementation of true proteins in the ration.

A continuous supply of fermentable carbohydrates, ammonia, peptides, amino acids, and other nutrients is needed to promote efficient utilization of ATP for microbial protein yield (48). Ammonia is the primary nitrogen source for rumen microorganisms in protein synthesis. Sources of ammonia in the rumen include peptides and amino acids, miscellaneous soluble nitrogen material, ammonia derived from protozoa, and gaseous nitrogen (48). Ammonia not utilized for microbial growth is absorbed through the reticulum-rumen wall and is converted to urea in the liver (14).

An investigation of the nitrogen metabolism in the rumen must be closely related to the energy of the diet. Energy is necessary for the microorganisms to convert the nitrogen of ammonia to microbial protein (13, 61, 93). Protein systems utilized to determine protein available for absorption at the intestinal level need to take into
account: (a) consideration of two sites of protein synthesis, rumen microorganisms and ruminant tissues and (b) linking of protein needs to available energy (14). Halfpenny et al. (36) observed increases in non-essential amino acids and decreases in essential amino acids with increases in the energy intake of cows. Orskov et al. (63) reported increasing amino acid supply stimulated production to the extent that additional energy-yielding nutrients are drawn out of body tissue in early lactation.

Sniffen and Hogue (81) identified the problem of determining amino acid requirements for ruminants as the inability to define requirements of the rumen microorganisms, and therefore the most appropriate combination of amino acids for formulation is not known. The relationship between protein solubility and degradability in the rumen is an important factor for determining the amino acids available to the microorganisms. It is important to balance between degradable and undegradable protein in ruminant diets to insure efficient use of protein or nitrogen. In vitro research (23) has shown that amino acids are not released from feed proteins in the same proportions that exist in the protein. The degradability of lysine, arginine, histidine and leucine was greater than other amino acids in in situ incubations of untreated soybean meal (58).

Ruminant systems of various species function similarly,
however the high producing dairy cow is one of the most specialized (2, 38).

**Amino Acid Transport:**

Nutrient provision to the lactating mammary gland involves three factors: blood nutrient concentration, blood flow, and cellular uptake (4, 54). The gradient of concentration of substrates across the mammary epithelial cell membrane suggests that a major impediment to substrate supply for milk synthesis is the substrate transport across the membrane (26). Schingoethe et al. (78) reported substrate availability was a major factor in determining the rate of milk protein, mainly casein, synthesis. Net uptake of amino acids, except cysteine, by the lactating mammary gland has been established (19). To accurately assess amino acid concentrations in blood and uptake, Heitmann and Bergman (37), determined that the plasma amino acid concentration needs to be adjusted for packed cell volume. Failure to do so would underestimate the amino acid status of the animal. Baumrucker (3) described the uptake of lysine and arginine as a common pathway in which the concentration of one amino acid had a direct effect on the uptake of the other. In addition to the cationic transport system (3) which supplies lysine and arginine, neutral, anionic, and general transport systems have been discussed
Most systems have been shown to be sodium dependent using transmembrane gradients to transport the amino acids into the cell (4, 43). The cationic pathway is not sodium dependent, however the uptake does not occur as simple diffusion (69). The energy or driving source is not known. The neutral amino acid transport system transports: alanine, glycine, proline, methionine, serine, cysteine, threonine, phenylalanine, tryptophan, and the branched chain amino acids (4, 43). Histidine, glycine, and arginine are believed to employ their own transport system in addition to ones reported (4).

Verbeke and Peeters (86) were able to show a considerable decrease in most amino acids by passage across the mammary gland. Once inside the cell, the amino acids become part of a free amino acid pool. This pool becomes the source for protein synthesis by activation to the aminoacl-\text{-}t\text{-}RNA pool (1). This is where protein synthesis would be inhibited by a limiting amino acid. There is a direct relationship between mammary blood flow and milk production (45), in that the blood carries the amino acids to the tissue where they are to be absorbed. The amino acids from the plasma pool comprise 92\% of the milk protein amino acids (19, 54, 86).
Amino Acid Utilization:

Inside the mammary cell, amino acids may: 1) undergo RNA polymerization to form milk proteins subsequently secreted by exocytosis, 2) be retained in the cell in the form of structural proteins or enzymes, 3) enter into metabolic reactions yielding, *inter alia*, CO₂, urea, polyamines, and non-essential amino acids (NEAA), or 4) pass unchanged into milk, blood, or lymph (54). Contamination of the blood flowing from the udder by other sources may affect amino acid values calculated from arterio-venous difference blood flow data (50). Research has indicated that this is less of a concern than once thought (9). The blood samples from the subcutaneous abdominal vein were similar regardless if the external pudic vein was occluded or not (9).

Amino acids can be categorized into three groups (54). Group 1 amino acids include: methionine, phenylalanine, tyrosine, and tryptophan and are absorbed at a rate nearly equal to their output in milk protein. Group 2 amino acids which are arginine and the branched chain amino acids are absorbed in excess of output. The final group includes the NEAA which have an uptake less than their output in milk protein (25). The uptake to output deficit of the NEAA is an indication of their synthesis in the mammary gland (54, 78). Mepham and Linzell (56) reported excess uptake of arginine and observed the use of arginine carbon in the
synthesis of proline. Other products of the urea cycle, arginine derivatives, have been identified in the mammary gland and their transfer into other NEAA, glutamate, serine, and alanine (56). The branched chain amino acids: valine, leucine, and isoleucine, also group 2 amino acids, are transaminated and the carbon skeletons are modified to enter the Krebs Cycle as acetyl CoA or succinyl CoA (92). The group 2 amino acids are the source of a substantial amount of CO₂ and energy in the mammary gland (56). In the production of NEAA, glucose, and acetate, the group 2 amino acids supply the carbon skeletons and the transamination of the amino acids are the source of nitrogen in the mammary gland. Halfpenny et al. (36) and Gow et al. (34) reported an increase in NEAA plasma concentration with improved energy nutrition. It was proposed that NEAA synthesis in the mammary gland was inadequate (36). However, infusions of NEAA in the goat mammary gland arteries showed no response in milk protein synthesis (55).

Limiting Amino Acids:

The first limiting amino acid of a diet can be defined as the essential amino acid (EAA) in the least amount in relation to tissue requirements for the given amino acid (7). Utilization of all other amino acids is limited to the extent of the available amount of the first limiting amino
acid. Supplementing any other amino acids found in the diet at levels above requirements will not result in an animal response unless the first limiting amino acid has been supplemented (7).

Protein can effect milk production by (a) providing more amino acids, (b) increasing available energy, and (c) altering efficiency or pattern of use of absorbed nutrients (13). Amino acid supply to the mammary gland has been estimated by several means. Arterio-venous difference is used to calculate uptake of amino acids by the mammary gland and uptake to output ratio can be derived for the amino acids. Transfer efficiency (87) measures the amino acid secreted in the milk protein as a percentage of the amino acid in plasma entering the mammary gland.

Broderick et al. (11) postulated that when an amino acid which is limiting is supplied to meet tissue requirements the plasma concentration of the amino acid will increase and the concentrations of the other amino acids will decrease as they are utilized more extensively. Many researchers (11, 21, 22, 27, 31, 66) have attempted to identify the amino acid or amino acids limiting to milk or milk protein production. Schingoethe et al. (78) found that bovine mammary cells require: methionine, lysine, threonine, phenylalanine, leucine, isoleucine, valine, histidine, tryptophan, arginine, and cysteine for synthesis of B-casein and B-lactoglobulin. The amino acids found limiting to milk
and milk protein production are quite varied. Borderick et al. (11) found methionine, valine, and lysine as the amino acids most likely limiting when formaldehyde treated casein was supplemented in diets. Park et al. (66), working with tissue cultures, identified lysine as first limiting and methionine, valine or arginine possibly second limiting. Clark et al. (21) reported that threonine and methionine improved B-lactoglobulin synthesis and cystine increased B-casein production when added to cells in culture. Methionine and threonine were second and third to cysteine in increasing casein synthesis. Clark et al. (22) reported intracellular methionine and tryptophan did not respond to linear increases of amino acids to mammary cells in culture indicating potential for limitation of these amino acids. Derrig et al. (27), infusing sodium caseinate, determined threonine, methionine, and phenylalanine were most limiting. Foldager et al. (31) reported that methionine, phenylalanine, threonine, and lysine was the order of limitation in diets which were protein deficient. The overall ratio of essential to non essential amino acids was depressed on these low protein rations. The role of tryptophan in limiting milk protein synthesis is incomplete because of the complexity of tryptophan transport and difficulties in analyses (26). Fuller and Raush (32) reported a major portion of tryptophan was protein-bound in many warm-blooded animals. The amount of free tryptophan in
the plasma available for milk protein synthesis may be limiting.

Abomasal Infusions:

Increasing nutrient supply to the mammary gland becomes more of a priority as milk production increases. Cows producing in excess of 30 kg of milk daily fail to produce to their genetic potential because of the lack of some key nutrient (17). A series of abomasal infusion experiments have been conducted to increase the protein, amino acids, glucose, and acetate available to the mammary gland for milk and milk protein production (16, 20, 30, 34, 36, 44, 49, 51, 56, 67, 71, 72, 80, 82, 87).

Casein has been the principle protein utilized in abomasal infusions. Casein is the preferred substrate because it is the primary milk protein and the amino acid composition would most closely duplicate that needed for protein synthesis in the mammary gland. The abomasal infusion of sodium caseinate increased milk production (20, 27, 34, 67, 72, 73, 82) and increased milk protein synthesis (20, 27, 34, 44, 67, 72, 73, 82). The dietary conditions of these trials were variable indicating that the response to casein postruminal infusion was genuine. Casein infusions increased the amount of amino acids presented to the small intestine for absorption and increased milk and milk protein
yield.

The response observed with the casein infusions and knowledge of potentially limiting amino acids for milk production resulted in the abomasal infusions of individual amino acids to further identify which amino acids may be limiting production. The infusion of methionine has received the most attention (16, 30, 72, 73, 30). Rogers et al. (72) reported increased milk and milk protein production in cows fed silage diets when methionine was infused abomasally. The response was similar to casein infusions (72). Schwab et al. (80) observed that methionine and lysine infused in combination accounted for 43% of the increases in production observed with infusions of the 10 EAA. Fisher (30) reported an increase in milk protein production and no change in milk yield with the methionine infusions of 13g per day. This concentration of methionine and response in protein production was similar to (72). No response was indicated by the infusion of 26g per day. Chamberlain and Thomas (16) infused 9g per day of L-methionine and found no effect on milk or milk protein yield however they did report increased fat content and yield.

Infusions of other amino acids have also been applied to lactating mammals. Fisher (30) observed that infusions of histidine decreased milk protein yield. Gow et al. (34) infused arginine into lactating animals with no effect on
milk production.

**Bypassing Dietary Proteins and Amino Acids**

Bypassing dietary proteins and amino acids to the small intestine has been investigated as a practical means of reproducing responses seen with abomasal infusions. Santos et al. (75) feeding protein supplements of varying degradability found that proteins which are less degradable in the rumen were equally available in the small intestine as soybean meal. This indicates that more amino acids were available for absorption and utilization by the animal (75). Bypassing can be accomplished by: heat treatment, chemical treatment, encapsulation, use of amino acid analogs, and esophageal groove closures (12).

Heat treatment of proteins has been researched extensively. Sahlu et al. (74) and Schingoethe and Ahrar (77) reduced the solubility of soybean meal with heat treatment. The heat treated protein supplement increased milk production over unheated soybean meal (74). Janicki et al. (41) and Holter et al. (39) fed diets of varying nitrogen solubility and reported no response in milk production. Kung et al. (46) fed cows diets at three crude protein concentrations and normal soybean meal or heat treated soybean meal. At the two highest concentrations of protein feeding, the cows consuming heat treated soybean
meal produced more milk than cows eating unheated soybean meal (46). Kung et al. (47) reported feeding heat treated soybean meal increased the concentration of plasma essential amino acids and decreased the nonessential amino acids. The amount of amino acids reaching the small intestine were increased with heat treatment.

Formaldehyde treatments have been applied to protein sources to reduce their availability in the rumen (18, 24, 29, 53, 62, 79, 89). Crooker et al. (24) reduced the digestibility of soybean meal with formaldehyde. Schmidt et al. (79) reported protein availability was decreased at the lower concentration of formaldehyde treatment while protein digestibility was not affected. Availability was measured by in vitro rumen fermentation and digestibility was monitored by weight gains in rats. It was assumed rats absorbed and utilized proteins in the same manner as ruminants postruminally (79). Formaldehyde treatment of proteins has shown positive effects on weight gain in growing ruminants (17, 29). Lactating cows have generally shown no response to the feeding of formaldehyde treated proteins (18, 24, 53, 62, 89). Wachira et al. (39) also reported no response in growing lambs. Minson et al. (57) increased milk production in cows consuming ryegrass pasture when formaldehyde treated casein was supplemented in their diets. In the experiments in which no response was seen, the possibility of over-protection of the protein may have
been a factor (18). Rae and Ingalls (71) speculated that a lack of response to feeding formaldehyde treated canola meal may be due to a failure to increase the uptake of tyrosine, a group 1 amino acid (54). Milk production and milk protein production were increased by oral administration of tyrosine.

Work more closely related to the abomasal infusions of individual amino acids is the feeding of encapsulated, rumen-protected, methionine to lactating cows. Generally, no response of milk production or milk protein content has been reported (10, 15, 59, 64, 65, 91, 93). Yang et al. (93) and Mueller et al. (59) fed a heat treated soybean meal with the encapsulated methionine. Yang et al. (93), Mueller et al. (59) and Papas et al. (64) reported increases in dry matter intake with supplementation of rumen-protected methionine. Papas et al. (64, 65) significantly increased plasma methionine concentrations while others (10, 59, 93) reported trends of increased plasma methionine. Oke et al. (60) fed lambs and steers rumen-protected methionine and lysine. Lambs fed protected methionine and lysine in combination had increased nitrogen retention compared to unsupplemented lambs. Plasma concentrations of methionine and lysine were increased indicating the amino acids were protected and available for absorption (60). Growing steers fed diets containing elevated concentrations of the protected amino acids gained better than steers fed
intermediate concentrations of protected methionine and lysine or no supplemental amino acids. Finishing steers, requiring a lower protein concentration in the diet, displayed no response to supplementation (60).

**Methionine Hydroxy Analog (MHA)**

Chalupa (12) categorized the feeding of analogs of amino acids as a means of bypassing the rumen. In vitro (5) and in vivo (6) research indicated that MHA was more resistant to ruminal degradation than L-methionine. Emery (28) reported MHA was degraded in the rumen. The feeding of MHA has shown increases in milk fat production and fat-corrected milk production (6, 8, 35, 40, 52, 70). Griel et al. (35) reported increased milk production. Stokes et al. (84) observed no production responses from MHA feeding. Polan et al. (70) observed decreased dry matter intake in animals fed MHA.


56. Mepham, T. B., and J. L. Linzell. 1974. Effects of


86. Verbeke, R., and G. Peeters. 1965. Uptake of free plasma amino acids by the lactating cow's udder and


Lactational and Systemic Responses to the Supplementation of Protected Methionine in Soybean Meal Diets

D. J. ILLG

Dairy Science Department
South Dakota State University
Brookings 57007-0647

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2 Animal Science Department, University of Minnesota, 130 Haecker Hall, St. Paul, MN 55108.
ABSTRACT

Ruminally protected methionine was supplemented to soybean meal diets to evaluate the response of lactational and systemic parameters. Twenty-seven Holstein cows (14 primiparous and 13 multiparous) were randomly assigned to diets containing soybean meal without or with 15 g of added DL-methionine daily, provided as 50 g of ruminally protected methionine product, during wk 4 through wk 16 postpartum. Cows were fed mixed diets of (dry matter basis) 30% corn silage, 15% alfalfa hay, and 55% concentrate mix. Diets were formulated to contain 16.0% crude protein and 18.0% acid detergent fiber. Yields of milk (32.9 and 35.2 kg/day), 4% fat-corrected milk (27.8 and 29.5 kg/day) and solids corrected milk (28.5 and 30.1 kg/day) were higher for cows fed supplemental methionine. Milk protein percentage (2.99 and 3.06) was increased with supplemental methionine while, the percentage of fat (2.96 and 3.00), solids-not-fat (8.69 and 8.73), and total solids (11.67 and 11.71) were similar among diets. Dry matter intake (19.3 and 21.3 kg/day) was higher for the SBM+ cows while production efficiency (1.74 and 1.69 kg milk/kg dry matter) was not different. Serum urea, ruminal ammonia, and molar concentrations of acetate, propionate, and butyrate were similar between diets. Serum amino acid concentrations were similar between diets. Milk production and milk protein
percentage were increased with the addition of 15 g of protected DL-methionine.
Microbial protein has an important role in the pattern of amino acids entering the small intestine (21). The inadequacy of microbial protein in supplying sufficient amounts of amino acids to support high levels of milk production (34) has resulted in an interest in feeding bypass proteins and amino acids. Several means of treating proteins to decrease ruminal degradation have been employed. These include: heat treatment (30), formaldehyde treatment (4), and encapsulation (3, 5, 22, 24, 25, 26, 35, 36).

Abomasal infusions of casein have been used to identify amino acids limiting to milk and milk protein production (6, 8, 29, 31). Methionine was consistently one of the amino acids found most limiting to milk production based on relative amino acid concentrations in the plasma. Other amino acids frequently cited as limiting or co-limiting include phenylalanine, lysine, and threonine (21).

Research with forms of methionine which were to have rumen bypass potential has shown variable results. Methionine hydroxy analog (MHA), once thought to bypass the rumen, is degraded extensively by the rumen microorganisms (10) and the responses seen with MHA are believed to be a result of changes in the rumen. Methionine hydroxy analog increased milk fat percent and 4% fat-corrected milk production (14, 15, 19, 20) in several experiments, but
increased milk production in only one of the studies (14). Feeding of encapsulated methionine did not affect milk production (5, 22, 25, 26, 35, 36).

Individual amino acids and groups of amino acids were injected abomasally into lactating cows and goats (6, 8, 11, 29, 31). Schwab et al. (31) observed increased milk and milk protein production with the injection of the essential amino acids and concluded that methionine and lysine accounted for a major portion of the increase. Rogers et al. (29) reported a response from the abomasal infusion of methionine comparable to that with casein infusions. Fisher (11) reported increased milk protein production with intravenous infusions of methionine. Other researchers (6, 8) have had less consistent results with the infusions of methionine.

This research was designed to evaluate the lactational and systemic responses of early lactation cows to the supplementation of ruminally protected methionine in soybean meal diets.
MATERIALS AND METHODS

Diets were composed of 55% (dry basis) concentrate mix (Table 1), 15% alfalfa hay, and 30% corn silage. Diets were formulated to contain 16% crude protein and 18% acid detergent fiber (ADF). Treatments were complete mixed diets without (SBM) or with (SBM+) 15g/cow/day of DL-methionine provided as 50 g/cow/day of ruminally protected methionine product\(^3\) fed wk 4 through wk 16 postpartum.

Twenty-seven cows (14 primiparous and 13 multiparous) were randomly assigned to treatment. Treatments were balanced for primiparous cows. Multiparous cows were producing at least 27 kg of milk and primiparous 23 kg of milk per day by wk 3 postpartum.

Cows were fed at ad libitum intake once daily in individual feeding gates. Amounts fed and refusals were recorded daily. Cows were acclimated to experimental diets during wk 3 postpartum. Body weights were recorded three consecutive days at the beginning and end of experiment and biweekly during the trial.

Two 24 h (am plus pm) milk samples were collected from

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\(^3\) Ketionin, prills of a ruminally protected methionine composed of 30% DL-methionine; 58% saturated and unsaturated fatty acids with 12 to 22 carbon atoms; 6% calcium carbonate; 1 to 2% glucose; and 4% flavoring, antioxidant, and stabilizer. Supplied by Rumen Kjemi a/s, division of Peter Moller a/s, Oslo, Norway.
each cow during wk 3 postpartum and one 24 h sample was taken each week throughout the balance of the trial. Milk was analyzed for protein by Kjeldahl (1), fat by Babcock (1), and total solids by Mojonnier (2). Milk yield and composition during wk 3 postpartum were used for covariance analysis (32) of the trial period.

Feed samples were collected weekly and composited monthly for analyses. Dry matter (DM) was determined by drying samples for 72 h at 57°C in a forced air oven. Dried samples were ground through a 2mm screen and analyzed for crude protein (1), ether extract (1), ash (1), neutral detergent fiber (NDF) (28), ADF (13), and acid detergent permanganate lignin (13).

Samples of ruminal contents were collected monthly by esophageal tube 2 to 4 h after feeding into sample bottles containing .5 ml saturated mercuric chloride. Samples were tested for pH. Samples were prepared and analyzed for rumen ammonia and volatile fatty acids (VFA) as described in (30).

Jugular blood was obtained at the time of rumen sampling into heparinized vacuum tubes and analyzed for serum urea (7). At peak production (wk 6-wk 8 postpartum), blood samples were taken from the coccygeal artery and subcutaneous abdominal vein. Samples from the six highest producing cows (3 multiparous and 3 primiparous) from each treatment were prepared and analyzed for amino acid composition as described in (9). Mammary blood flow was
estimated using the formula of Kronfeld et al. (17) and was adjusted for 30% packed cell volume (27). Mammary blood flow was calculated to be 450 l/kg milk and was used to determine amino acid concentrations presented to the mammary gland. Milk protein amino acid composition reported by (16) was used to compute amino acid outflow from the mammary gland. Transfer efficiencies (33) were also calculated.

Data were subjected to analysis of variance (32) using the Statistical Analysis System (SAS) General Linear Model program (SAS Institute, Cary, NC). Effects due to treatment, time, age (primiparous vs. multiparous) and interactions between factors were tested. Significance level was $P<.05$. 
RESULTS AND DISCUSSION

The chemical composition of the feeds and total diet is in Table 2.

Milk yield (Table 3) was increased (P<.01) when cows were fed supplemental protected methionine. Figure 1 shows the SBM+ cows peaked at a higher level of milk production and maintained the increased level throughout the trial. The increased milk yield (Table 3) is comparable to increases observed with abomasal infusions of casein (6, 8, 29, 31) and methionine (29). Oke et al. (24) observed improved growth with steers fed protected methionine and lysine in combination.

Other researchers (3, 5, 22, 25, 26, 35, 36) did not observed a response from encapsulated methionine supplementation. Papas et al. (26) fed diets supplemented for protein at 125% of National Research Council (NRC) requirements (23). This plane of nutrition may have provided ample protein to the animals and the additional supplementation of methionine was not beneficial. Yang et al. (36) and Mueller et al. (22) fed a heat-treated protein supplement which had bypass potential (30), thus a lack of response may have been due to sufficient amino acids bypassing the rumen from the soybean meal so that the added methionine was not utilized. Another potential problem of heat-treatment is that the process tends to be more
detrimental to lysine than the other amino acids in soybean meal (30). Schwab et al. (31) found lysine and methionine close to co-limiting for milk production. If the lysine was bound by heating, lysine may have replaced methionine as most limiting to milk production when heated proteins were fed (22, 36).

Percent protein (Table 3) in milk was increased (P<.01) in cows fed supplemental protected methionine. Increased protein percentage of milk was also observed with the abomasal infusions of casein (6, 8, 29, 31) and methionine (11, 29). Figure 2 illustrates that the SBM+ cows maintained a higher percentage of protein in their milk, especially during peak lactation when the animal would most likely be in a protein deficit as dry matter intake was not sufficient to account for production. Broderick et al. (4) increased nitrogen in the milk of cows fed formaldehyde treated casein and reported that 82% of the increase in nitrogen was due to true protein and not merely an increase in the nonprotein nitrogen of the milk. They concluded that the increased amino acids absorbed from the small intestine due to the protection stimulated protein synthesis in the mammary gland.

Percent fat in milk (Table 3) was not different between the two treatment groups. Supplementation with MHA (14, 15, 20, 21) has increased the fat percentage in milk. Chamberlain and Thomas (6) increased milk fat percentage
with intravenous infusions of methionine. However research (3, 5, 22, 25, 26, 35, 36) with protected methionine products showed no effect on the fat content of the milk.

The percentage of solids-not-fat and total solids of the milk (Table 3) were not different and agree with previous research (3, 22, 25, 26, 35, 36).

The increased milk and milk protein production of the SBM+ cows resulted in increases (P<.01) in output of 4% fat-corrected milk, solids-corrected milk, fat per day, and protein per day (Table 3).

Dry matter intake was higher (P<.01) for the SBM+ cows (Table 4). Figure 3 shows that the SBM+ animals increased their dry matter intake at a higher rate than the control (SBM) cows and continued to consume more dry matter throughout the trial. Yang et al. (36), Mueller et al. (22), and Papas et al. (25) reported increased dry matter intakes with supplementation of protected methionine, while Papas et al. (26) and Williams et al. (35) reported no difference in dry matter intake. Broderick et al. (3) observed decreased intakes in ruminants fed protected methionine.

Milk production efficiencies (Table 3), kg of milk, fat-corrected milk or solids-corrected milk per kg dry matter intake, were not different between treatments. Although the SBM+ animals consumed more dry matter they converted the increased dry matter to milk production and
not to body weight gain.

Ruminal volatile fatty acids (VFA), pH, and ammonia, as well as serum urea concentration are listed in Table 5. Molar percentages of acetate, propionate, and butyrate were similar. The molar percent of valerate was reduced in the SBM+ cows (P<.01). Ruminal ammonia and pH as well as blood serum urea concentration were similar. Rumen function was not altered with the supplementation of the protected methionine indicating that methionine bypassed the rumen.

Concentrations of amino acids in the arterial and venous serum and arterio-venous (A-V) differences are in Table 6. The addition of protected methionine to the soybean meal diets slightly increased the methionine concentrations of arterial and venous blood. Similar increases were observed in (22, 36). Papas et al. (25, 26) dramatically increased arterial methionine by feeding encapsulated methionine. Arterio-venous differences were similar between treatments. Feeding additional methionine did not alter the concentrations of other amino acids in the serum. Broderick et al. (4) postulated that when a limiting amino acid was supplied in excess of requirement it would accumulate in the serum and be associated with a decrease in the serum levels of the other amino acids. Since this did not occur, perhaps the supplementation of more methionine would have been advantageous, or another amino acid became limiting and prevented plasma changes from occurring.
Uptake and output of amino acids are listed in Table 7. There was no difference in the uptake of the amino acids. Output of all amino acids was greater (P<.01) for the SBM+ cows due to the increase in milk and milk protein production. Using uptake to output ratio as an indicator of amino acid limitation, tryptophan is suggested as the first limiting amino acid for both groups of cows. Fuller and Rausch (12) reported that a large proportion of tryptophan can be bound to proteins in tissues of warm-blooded animals therefore reducing the amount of free plasma tryptophan available for metabolic processes. Tryptophan has an uptake value very close to its output level and could be limiting to milk production (21). Linzell and Mepham (18) cited tryptophan as the potentially limiting amino acid for milk production in goats. Whether the animals in this trial could have produced at the rate in which they did (34.0 kg/day) with the tryptophan deficit the uptake to output ratio indicates is not known.

Another means of evaluating amino acid status to determine limiting amino acids is to calculate transfer efficiencies. Transfer efficiency ranks amino acids based on their output divided by their arterial concentration and serum blood flow. Based on the transfer efficiencies in Table 8, methionine is most limiting in both treatment groups. The reduced transfer efficiency of methionine in the SBM+ cows than in SBM cows would indicate that the
methionine status of these animals was improved by supplementing diets with ruminally protected methionine.

The increased production of milk and milk protein when cows were fed ruminally protected methionine would render support to methionine as the amino acid limiting milk production under these experimental conditions.
LITERATURE CITED


tryptophan to plasma proteins in several species. Comp. Biochem. Physiol. 46B:273.


or glucose. J. Dairy Sci. 57:1024.


Table 1. Ingredient composition of the concentrate mix.1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Corn</td>
<td>73.2</td>
</tr>
<tr>
<td>Soybean Meal, 44% CP</td>
<td>25.0</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>1.3</td>
</tr>
<tr>
<td>Trace Mineral Salt</td>
<td>.5</td>
</tr>
</tbody>
</table>

1 Plus 8,800 IU of added vitamin A, 1,760 IU of added vitamin D, and .9 IU of added vitamin E per kg.
Table 2. Chemical composition of concentrate mix, alfalfa hay, corn silage, and total diet.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentrate mix</th>
<th>Alfalfa hay</th>
<th>Corn silage</th>
<th>Total diet &lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (DM), %</td>
<td>89.0</td>
<td>89.3</td>
<td>49.1</td>
<td>76.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>19.4</td>
<td>16.3</td>
<td>7.4</td>
<td>15.3</td>
</tr>
<tr>
<td>Ether extract</td>
<td>2.2</td>
<td>.9</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>25.4</td>
<td>52.0</td>
<td>45.7</td>
<td>35.5</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>5.6</td>
<td>38.8</td>
<td>26.0</td>
<td>16.7</td>
</tr>
<tr>
<td>Ash</td>
<td>4.9</td>
<td>9.0</td>
<td>4.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Lignin</td>
<td>1.5</td>
<td>12.3</td>
<td>5.8</td>
<td>4.4</td>
</tr>
</tbody>
</table>

<sup>1</sup> Computed.
Table 3. Milk yield and composition for cows fed soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>SBM</th>
<th>SBM+</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, kg/day</td>
<td>32.9</td>
<td>35.2**</td>
<td>.27</td>
</tr>
<tr>
<td>4% Fat-corrected milk, kg/day</td>
<td>27.8</td>
<td>29.5**</td>
<td>.25</td>
</tr>
<tr>
<td>Solids-corrected milk, kg/day</td>
<td>28.2</td>
<td>30.1**</td>
<td>.25</td>
</tr>
<tr>
<td>Fat, %</td>
<td>2.96</td>
<td>3.00</td>
<td>.04</td>
</tr>
<tr>
<td>Protein, %</td>
<td>2.99</td>
<td>3.06**</td>
<td>.01</td>
</tr>
<tr>
<td>SNF, %</td>
<td>8.69</td>
<td>8.37</td>
<td>.03</td>
</tr>
<tr>
<td>Total Solids, %</td>
<td>11.67</td>
<td>11.71</td>
<td>.06</td>
</tr>
<tr>
<td>Fat, kg/day</td>
<td>.96</td>
<td>1.04**</td>
<td>.01</td>
</tr>
<tr>
<td>Protein, kg/day</td>
<td>.98</td>
<td>1.07**</td>
<td>.01</td>
</tr>
<tr>
<td>Milk/DMI&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.74</td>
<td>1.69</td>
<td>.03</td>
</tr>
<tr>
<td>FCM&lt;sup&gt;2&lt;/sup&gt;/DMI</td>
<td>1.45</td>
<td>1.44</td>
<td>.02</td>
</tr>
<tr>
<td>SCM&lt;sup&gt;3&lt;/sup&gt;/DMI</td>
<td>1.48</td>
<td>1.46</td>
<td>.02</td>
</tr>
</tbody>
</table>

** Means with unlike superscripts differ (P<.01)
<sup>1</sup> Dry Matter Intake
<sup>2</sup> 4% Fat-corrected Milk
<sup>3</sup> Solids-corrected Milk
Table 4. Nutrient intakes and body weight (BW) changes for cows fed soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBM</td>
<td>SBM+</td>
<td>SE</td>
</tr>
<tr>
<td>Dry matter intake, kg/day</td>
<td>19.3</td>
<td>21.1**</td>
<td>.22</td>
</tr>
<tr>
<td>Dry matter intake, kg/100 kg BW</td>
<td>3.23</td>
<td>3.49**</td>
<td>.04</td>
</tr>
<tr>
<td>BW, kg</td>
<td>599.2</td>
<td>606.8</td>
<td>4.30</td>
</tr>
<tr>
<td>BW change weeks 4 to 16, kg</td>
<td>57.3</td>
<td>68.8</td>
<td>14.29</td>
</tr>
</tbody>
</table>

** Means with unlike superscripts differ (P<.01)
Table 5. Volatile fatty acids (VFA), ammonia, and pH in ruminal fluid, and urea in serum in cows fed soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diet</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBM</td>
<td>SBM+</td>
</tr>
<tr>
<td>VFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate, moles/100 moles</td>
<td>55.2</td>
<td>56.7</td>
</tr>
<tr>
<td>Propionate, moles/100 moles</td>
<td>31.3</td>
<td>29.9</td>
</tr>
<tr>
<td>Butyrate, moles/100 moles</td>
<td>9.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Isobutyrate, moles/100 moles</td>
<td>.7</td>
<td>.7</td>
</tr>
<tr>
<td>Isovalerate, moles/100 moles</td>
<td>1.1</td>
<td>1.2**</td>
</tr>
<tr>
<td>Valerate, moles/100 moles</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Acetate/Propionate</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Total, umoles/ml</td>
<td>109.4</td>
<td>104.7</td>
</tr>
<tr>
<td>Ruminal pH</td>
<td>6.36</td>
<td>6.32</td>
</tr>
<tr>
<td>Ruminal Ammonia, mg/dl</td>
<td>8.4</td>
<td>8.1</td>
</tr>
<tr>
<td>Serum Urea, mg/dl</td>
<td>15.3</td>
<td>15.5</td>
</tr>
</tbody>
</table>

** Means with unlike superscripts differ (P<.01)
Table 6. Concentration of amino acids in arterial and venous serum, and<br>arterio-venous (A-V) difference in cows fed soybean meal (SBM) and soybean<br>meal plus methionine (SBM+) diets.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Arterial serum</th>
<th>Venous serum</th>
<th>A-V Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBM</td>
<td>SBM+</td>
<td>SE</td>
</tr>
<tr>
<td>Arginine</td>
<td>14.05</td>
<td>14.35</td>
<td>1.26</td>
</tr>
<tr>
<td>Histidine</td>
<td>6.19</td>
<td>5.56</td>
<td>.47</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>8.68</td>
<td>9.55</td>
<td>.88</td>
</tr>
<tr>
<td>Leucine</td>
<td>13.76</td>
<td>14.83</td>
<td>1.37</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.77</td>
<td>7.30</td>
<td>.91</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.88</td>
<td>2.03</td>
<td>.13</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.80</td>
<td>4.06</td>
<td>.22</td>
</tr>
<tr>
<td>Threonine</td>
<td>10.29</td>
<td>9.24</td>
<td>.81</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.53</td>
<td>2.99</td>
<td>.38</td>
</tr>
<tr>
<td>Valine</td>
<td>20.60</td>
<td>21.60</td>
<td>2.29</td>
</tr>
<tr>
<td>Total</td>
<td>90.57</td>
<td>91.51</td>
<td>8.72</td>
</tr>
<tr>
<td>Essential</td>
<td>90.57</td>
<td>91.51</td>
<td>8.72</td>
</tr>
<tr>
<td>Alanine</td>
<td>28.28</td>
<td>23.68</td>
<td>2.37</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.51</td>
<td>1.45</td>
<td>.09</td>
</tr>
<tr>
<td>Cystine</td>
<td>.27</td>
<td>.23</td>
<td>.03</td>
</tr>
<tr>
<td>Glutamate</td>
<td>6.86</td>
<td>7.67</td>
<td>.60</td>
</tr>
<tr>
<td>Glycine</td>
<td>50.35</td>
<td>43.05</td>
<td>4.69</td>
</tr>
<tr>
<td>Proline</td>
<td>11.43</td>
<td>9.75</td>
<td>1.09</td>
</tr>
<tr>
<td>Serine</td>
<td>11.95</td>
<td>11.44</td>
<td>1.01</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.94</td>
<td>3.67</td>
<td>.33</td>
</tr>
<tr>
<td>Non-essential</td>
<td>141.45</td>
<td>127.13</td>
<td>11.52</td>
</tr>
</tbody>
</table>
Table 7. Uptake and output of amino acids by the mammary gland, and ratio of uptake to output in cows fed soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Category</th>
<th>SBM</th>
<th>SBM+</th>
<th>SE</th>
<th>SBM</th>
<th>SBM+</th>
<th>SE</th>
<th>Uptake/Output</th>
<th>SBM</th>
<th>SBM+</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td></td>
<td>79.74</td>
<td>137.72</td>
<td>46.75</td>
<td>38.33</td>
<td>41.67</td>
<td>.89</td>
<td>2.12(10)^1</td>
<td>3.14(10)</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td></td>
<td>39.32</td>
<td>15.56</td>
<td>8.56</td>
<td>29.57</td>
<td>32.15</td>
<td>.69</td>
<td>1.33(9)</td>
<td>.47(2)</td>
<td>.27</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td></td>
<td>85.91</td>
<td>106.54</td>
<td>8.30</td>
<td>64.62</td>
<td>70.25</td>
<td>1.50</td>
<td>1.33(8)</td>
<td>1.53(9)</td>
<td>.14</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td></td>
<td>140.47</td>
<td>156.27</td>
<td>14.44</td>
<td>106.24</td>
<td>115.49</td>
<td>2.46</td>
<td>1.32(7)</td>
<td>1.37(7)</td>
<td>.15</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td>106.34</td>
<td>87.13</td>
<td>20.01</td>
<td>88.71</td>
<td>96.44</td>
<td>2.06</td>
<td>1.22(5)</td>
<td>.88(3)</td>
<td>.21</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
<td>27.24</td>
<td>31.17</td>
<td>3.07</td>
<td>28.47</td>
<td>30.96</td>
<td>.66</td>
<td>.96(3)</td>
<td>1.03(4)</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
<td>58.87</td>
<td>64.56</td>
<td>5.97</td>
<td>53.67</td>
<td>58.34</td>
<td>1.24</td>
<td>1.11(4)</td>
<td>1.12(5)</td>
<td>.12</td>
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</tr>
<tr>
<td>Threonine</td>
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<td>44.12</td>
<td>67.38</td>
<td>8.94</td>
<td>50.38</td>
<td>54.77</td>
<td>1.17</td>
<td>.87(2)</td>
<td>1.24(6)</td>
<td>.20</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
<td>7.59</td>
<td>3.48</td>
<td>6.53</td>
<td>15.33</td>
<td>16.67</td>
<td>.36</td>
<td>.53(1)</td>
<td>.23(1)</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td></td>
<td>93.76</td>
<td>117.68</td>
<td>10.34</td>
<td>72.29</td>
<td>78.58</td>
<td>1.68</td>
<td>1.30(6)</td>
<td>1.49(8)</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>Total Essential</td>
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<td>683.36</td>
<td>787.49</td>
<td>93.11</td>
<td>547.62</td>
<td>595.32</td>
<td>12.70</td>
<td>1.26</td>
<td>1.32</td>
<td>.18</td>
<td></td>
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<tr>
<td>Alanine</td>
<td></td>
<td>73.99</td>
<td>76.33</td>
<td>12.38</td>
<td>37.24</td>
<td>40.48</td>
<td>.86</td>
<td>1.98</td>
<td>1.89</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td></td>
<td>4.98</td>
<td>7.08</td>
<td>1.27</td>
<td>86.52</td>
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<td>.08</td>
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<tr>
<td>Cystine</td>
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<td>-.94</td>
<td>.46</td>
<td>8.76</td>
<td>9.52</td>
<td>.20</td>
<td>-.19</td>
<td>-.12</td>
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<tr>
<td>Glutamate</td>
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<td>105.50</td>
<td>11.95</td>
<td>239.86</td>
<td>260.75</td>
<td>5.56</td>
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<td>.41</td>
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<td>21.90</td>
<td>23.81</td>
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<td>-.53</td>
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<tr>
<td>Proline</td>
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<td>34.44</td>
<td>18.82</td>
<td>108.43</td>
<td>117.87</td>
<td>2.51</td>
<td>.28</td>
<td>.26</td>
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<tr>
<td>Serine</td>
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<td>51.31</td>
<td>43.02</td>
<td>11.04</td>
<td>61.33</td>
<td>66.68</td>
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<td>.86</td>
<td>.63</td>
<td>.19</td>
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<tr>
<td>Tyrosine</td>
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<td>60.06</td>
<td>73.28</td>
<td>7.90</td>
<td>55.86</td>
<td>60.72</td>
<td>1.30</td>
<td>1.08</td>
<td>1.23</td>
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<tr>
<td>Total Non-essential</td>
<td></td>
<td>469.85</td>
<td>570.01</td>
<td>91.96</td>
<td>619.91</td>
<td>673.91</td>
<td>14.37</td>
<td>.76</td>
<td>.85</td>
<td>.15</td>
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</table>

** Output of all amino acids for SBM+ was increased (P<.01).

1 () indicate the order of limiting essential amino acids.
Table 8. Transfer efficiencies\(^1\) of essential and non-essential amino acids of cows fed soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Diet</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>SBM</td>
<td>SBM+</td>
<td>SE</td>
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<tr>
<td>Essential</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>9.84(10)(^2)</td>
<td>9.24(10)</td>
<td>.97</td>
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<tr>
<td>Histidine</td>
<td>19.00(8)</td>
<td>20.84(7)</td>
<td>1.93</td>
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<tr>
<td>Isoleucine</td>
<td>37.62(5)</td>
<td>30.68(5)</td>
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</tr>
<tr>
<td>Leucine</td>
<td>38.88(4)</td>
<td>32.34(4)</td>
<td>4.76</td>
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<tr>
<td>Lysine</td>
<td>52.36(3)</td>
<td>50.30(2)</td>
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<tr>
<td>Methionine</td>
<td>62.29(1)</td>
<td>57.98(1)</td>
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<tr>
<td>Phenylalanine</td>
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<td>47.56(3)</td>
<td>3.35</td>
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<tr>
<td>Threonine</td>
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<td>27.06(6)</td>
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<tr>
<td>Tryptophan</td>
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<td>15.69(9)</td>
<td>1.68</td>
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<tr>
<td>Valine</td>
<td>20.70(7)</td>
<td>16.96(8)</td>
<td>2.95</td>
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</tr>
<tr>
<td>Nonessential</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Alanine</td>
<td>9.85</td>
<td>9.75</td>
<td>2.15</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>264.12</td>
<td>273.70</td>
<td>18.76</td>
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</tr>
<tr>
<td>Cystine</td>
<td>173.34</td>
<td>197.25</td>
<td>20.40</td>
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<tr>
<td>Glutamate</td>
<td>150.70</td>
<td>127.33</td>
<td>11.50</td>
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</tr>
<tr>
<td>Glycine</td>
<td>3.56</td>
<td>4.35</td>
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<tr>
<td>Proline</td>
<td>52.83</td>
<td>58.70</td>
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<tr>
<td>Serine</td>
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<td>32.06</td>
<td>3.80</td>
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<tr>
<td>Tyrosine</td>
<td>49.24</td>
<td>51.31</td>
<td>5.18</td>
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</tbody>
</table>

\(^1\) Transfer efficiency = \(\frac{\text{Amino acid output in milk (g/day) x 100}}{\text{Arterial serum x serum flow amino acids (g/liter x liter/day)}}\)

\(^2\) () indicate order of limiting essential amino acids
Figure 1. Milk yield of cows fed diets containing soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.
Figure 2. Percent protein in milk of cows fed soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.
Figure 3. Dry matter intake of cows fed soybean meal (SBM) and soybean meal plus methionine (SBM+) diets.