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PROTECTED METHIONINE AND HEAT-TREATED SOYBEAN

MEAL FOR HIGH-PRODUCING DAIRY COWS

By

CHE-MING JIMMY YANG

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1985

PROTECTED METHIONINE AND HEAT-TREATED SOYBEAN MEAL

FOR HIGH-PRODUCING DAIRY COWS

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
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candidate are necessarily conclusions of the major
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ABSTRACT

The effectiveness of a rumen-protected methionine preparation was studied as an amino acid source for high-producing dairy cows during wk 4 through 16 postpartum. Twenty-four Holstein cows (14 primiparous and 10 multiparous) were fed concentrate containing heat-treated soybean meal without or with 50 g/cow/day of added ruminally protected methionine product which provided 15 g of added DL-methionine daily. Cows were fed 16% crude protein mixed diets containing 30% of dry matter as corn silage, 15% as alfalfa hay, and 55% as concentrate. Milk production and composition were adjusted for pretreatment values (3 wk postpartum) by analysis of covariance. Adjusted milk yields (34.6 and 33.1 kg/day) were higher for cows fed heated soybean meal, but this difference was accounted for by higher pretreatment production (32.6 and 36.9 kg/day) of multiparous cows fed supplemental methionine. Production of 4% fat-corrected milk (28.5 and 27.6 kg/day) and solids-corrected milk (29.0 and 28.5 kg/day) was similar for cows fed both diets. Percentages of fat (2.81 and 2.92) and protein (2.88 and 2.92) were similar, while total solids (11.49 and 12.69) and solids-not-fat (8.68 and 8.77) were higher when cows were fed supplemental methionine. Milk protein percent (2.89 and 2.99) and milk protein production (.97 and 1.00 kg/day)

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were increased for primiparous cows fed supplemental methionine. Fatty acid composition in milk was similar. Dry matter intakes (20.2 and 21.0 kg/day) were higher especially in multiparous cows (21.5 and 23.8 kg/day) when fed supplemental methionine. Body weights (602 and 598 kg) and body weight changes were similar for the two treatments. Ruminal pH, volatile fatty acids, and ammonia, as well as blood serum urea and glucose were generally unaffected by methionine supplementation. Concentrations of methionine in arterial and venous plasma were elevated slightly when fed additional methionine, but the first limiting amino acid for milk production, as calculated by several methods, was not changed by feeding supplemental ruminally protected methionine.

INTRODUCTION

Increased milk yield has been obtained in response to postruminal administration of proteins and amino acids (18, 27, 38, 43, 105, 116, 118) and ingestion of ruminally protected protein sources (22, 69, 86, 88, 109) indicating deficiencies of certain amino acids may limit milk production in lactating cows.

Methionine plays an important role in protein and lipid metabolism. It has been suggested to be the most limiting amino acid for milk protein synthesis (1, 20, 22, 27, 31, 36, 38, 39, 48, 65, 113, 116); however, direct evidence is limited. Feeding methionine hydroxy analog increased yields of milk fat and fat-corrected milk (15, 22, 25, 57, 59, 79, 102, 121); but generally no change in milk production was observed (15, 25, 57, 59, 79, 91, 121). With the exception of a study by Fisher (43), intravenous infusion of methionine (27, 106) or feeding of rumen-protected methionine (18, 23, 96, 97, 128) did not increase production of milk, milk fat, or milk protein. Their diets did not contain protected proteins as concentrate supplement. No responses to protected methionine in the above studies may be due to methionine not being the first limiting amino acid since ruminal fermentation may have altered the sequence of limiting amino acids. Also, other factors may have been colimiting with methionine.

Adequate heat treatment of soybean meal decreased protein solubility and increased protein escaping degradation in the rumen without reducing quality of protein reaching gastro-intestinal absorption sites (1, 28, 69, 88, 109). Amino acid composition of heat-treated soybean meal was similar to composition in the untreated meals (109, 113). Milk production was increased by feeding heat-treated soybean meal to high-producing cows in early lactation (69, 88, 109). Supplementation of ruminally protected methionine with heat-treated soybean meal should provide more balanced amino acid pattern for absorption which may allow additional increased production, since methionine is the first limiting amino acid in heat-treated soybean meal and remained the first limiting amino acid in the insoluble or slowly degradable fraction of heat-treated soybean protein (1, 109, 113).

The objective of this research was to evaluate effects on milk production, milk composition, feed intakes, plasma free amino acid concentrations, and sequence of limiting amino acids when ruminally protected methionine was fed to high-producing cows receiving a ration containing heat-treated soybean meal as protein supplement.

LITERATURE REVIEW

Requirement of Amino Acids for Milk Synthesis

Major Limiting Factors for Milk Synthesis.

The limited milk production when cows are fed nonprotein nitrogen (NPN) diets (9, 125) and the response to postruminal administration of proteins and amino acids (18, 27, 32, 38, 43, 105, 116, 118) indicate amino acids may limit milk production in lactating cows. In vitro studies (34, 114) on amino acid requirements of bovine mammary gland cell cultures suggest that arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine were all essential for milk protein synthesis. Also, it was found (114) that increasing the level of amino acids over the usual concentration or normal physiological level in the medium greatly increased the synthesis of casein without causing a corresponding increase in cell growth, suggesting available substrate rather than the rate of enzymatic reactions in the secretory tissue determines the quantity of milk protein produced. Both of these in vivo and in vitro types of studies indicated a greater production of product from essentially a fixed amount of cellular material as a result of the stimulation provided by increased amounts of available amino acid substrate.

Attempts have been made to maximize milk production, especially during the early stage of lactation. Broster

(21) discussed the many experiments in which there was a correlation between "peak" lactation yield and "total" lactation yield, indicating that a small increase in peak yield can substantially increase the annual yield.

Since availability of energy and amino acids are major nutritional factors affecting milk secretion, metabolism of amino acids in lactating animals is interrelated with glucose availability. Glucose supplies carbon for lactose synthesis and a portion of the energy required for milk synthesis (27). There is also evidence pointing to the use of glucose for synthesis of nonessential amino acids (75). Amino acids supply the carbon and nitrogen for the synthesis of milk protein. Some amino acids have been identified as the main gluconeogenic precursors (27, 40, 54, 55) in ruminants.

Kronfeld et al. (68) reported that glucose uptake by the mammary gland was closely related to milk production. The availability of glucose has been found to influence the rate of milk secretion under a variety of experimental conditions (52, 67, 107). Furthermore, lactose content of milk is constant; therefore lactose synthesis is correlated highly with volume of milk produced (68). Hence, glucose availability to the mammary gland may to a large degree determine milk yield (27).

Little or no glucose is absorbed from the gut into the portal blood of mature ruminants fed their usual diets

(68). During early lactation, especially in high-producing cows, the mammary gland may use 80 to 90% of the total glucose available to the body, and other tissues (eg. liver, kidney, etc.) become competitive with mammary gland for gluconeogenesis (8). Some specific amino acids may become even more limiting for milk synthesis. Owing to a physiological relative insufficiency in energy and protein consumption, and lower protein digestibility in intestine in early lactation (61) as compared to later stage of lactation, highly productive cows draw on body reserves of fat and protein (68). Under such conditions, the development of the metabolic disorder ketosis may spontaneously occur.

Amino acid metabolism may have important influences on ruminant production other than through the process of protein synthesis (90). Methionine supplements given intravenously to cows increased milk fat yield while having no effect on yield of milk protein indicating interaction of methionine with lipid metabolism (24). Slight increases in fat percentages were also found when feeding methionine hydroxy analog (15, 25, 57, 59, 79, 102).

In different circumstances, abomasal infusions of casein into cows in early lactation have had effects on fatty acid metabolism which sometimes resulted in enhanced milk fat yield (66, 94). These effects may involve endocrine responses to changes in amino acid status.

<u>Consistency</u> <u>of</u> <u>Amino</u> <u>Acid</u> <u>Composition</u> <u>in</u> <u>Milk</u> <u>Protein</u> .

Some 20 natural amino acids participate in the structure of milk proteins. Clark et. al. (31) reported that over 92% of the nitrogenous components of cow's milk consists of proteins synthesized de novo in the mammary gland from amino acid precursors extracted from the blood. Featherston et al. (42) reported that under the changed feeding regimen employed the amino acid composition of the milk was not significantly changed. The consistency of amino acid composition in milk protein suggests that high levels of amino acids are required to maximize milk protein synthesis in high-producing dairy cows, especially if the pattern of amino acids available to the mammary gland is not similar to the composition of milk protein.

<u>Factors</u> <u>Affecting</u> <u>Amino</u> <u>Acid</u> <u>Availability</u> <u>for</u> <u>Milk</u> <u>Synthesis</u>

<u>Uptake</u> <u>of</u> <u>Amino</u> <u>Acids</u> <u>by</u> <u>Mammary</u> <u>Gland</u> <u>Tissue</u> .

Amino acid uptake is dependent on three factors: arterial concentrations of amino acid, rate of mammary blood flow (MBF), and extraction process by mammary secretory cell (83). Arterial concentrations of amino acid and MBF together determine quantities of amino acid reaching mammary cells.

The uptake of an amino acid from the plasma by the lactating gland was not highly correlated with the arterial concentrations of that amino acid (76) indicating that

passive difusion does not play a significant role in amino acid transport in this tissue. Data of Mepham and Linzell (84) showed that the hourly uptakes of leucine and tyrosine by the lactating mammary gland were essentially constant over a 12-hr period, even though the arterial concentration of these amino acids fluctuated as much as 40%. These data suggested that amino acid transport into the mammary cell may be via a carrier system (11).

Utilization of Amino Acids by Lacating Mammary Tissue

Once inside the cell, amino acids may 1) undergo RNA-directed polymerization to form milk proteins, subsequently secreted by exocytosis, 2) be retained in the cells in the form of structual proteins or enzymes, 3) enter into metabolic reactions producing CO_2, urea, polyamines, and nonessential amino acids (NEAA), or 4) pass unchanged into milk, blood, or lymph. While for most amino acids the first is the quantitatively major route, this is not the case for all essential amino acids (EAA) or for several EAA in certain conditions of substrate supply (83).

Balance studies (83) demonstrated that the uptakes of amino acid nitrogen (N) and carbon (C) from plasma were adequate to account for the output of these elements in mammary synthesized milk proteins. Although EAA are absorbed in amounts adequate to provide their milk protein residues, uptakes of NEAA vary considerably, and are often deficient relative to uptake.

Within the EAA some (i.e. methionine, phenylalanine, tyrosine, and tryptophan) show apparent stoichiometric transfer to milk protein. Mepham (83) designated them Group 1 to distinguish from other EAA, such as arginine and the branched-chain amino acids (Group 2), which generally show an excessive uptake.

The lack of sufficient NEAA uptakes to supply adequate quantities for milk protein synthesis suggests that a significant portion of these amino acids are synthesized in the gland. The sources of carbon and nitrogen used in their synthesis appear to be met to a large extent from the excess uptakes of arginine, valine, leucine, and isoleucine as well as from ornithine and citrulline (31).

Results presented by Clark et al. (30) show that lactating cow mammary tissue catabolizes ^{14}C-labeled arginine extensively with most of ^{14}C recovered in other amino acids such as proline, ornithine, and glutamate. Studies (30) with ^{14}C-labeled ornithine showed that lactating cow mammary tissue also produced proline and glutamate from the labeled ornithine.

In addition to the role of arginine and ornithine in supplying carbon and nitrogen for NEAA synthesis, they also serve as precursors for the synthesis of spermidine, a proposed modulator of hormonal stimulation upon mammary cells. Spermidine concentration increased in mammary tissue during lactation (108), and was positively

correlated with increased synthesis of RNA and protein. Incubations of mammary tissue with uniformly labeled branched-chain amino acids revealed the ability of this tissue to catablize these amino acids (129).

Catabolism of other EAA by the lactating mammary gland appears to be far less extensive than for arginine and the branched-chain amino acids (31). However, some degradation does occur as shown in studies with lactating goat mammary glands in which label from L-(U-^{14}C) threonine appeared in glycine, glutamate, aspartate and serine of casein.

Cysteine may be derived from methionine in the mammary gland (31), but this apparently occurs to only a limited extent because methionine is not extracted in excess of output in milk protein. Catabolism of tryptophan and histidine have been reported to occur to only limited extent (31).

Factors Affecting Free Amino Acid Concentrations in Blood

Intestinal Absorption .

Amino acids are the principal nitrogenous compounds absorbed from the intestine. In studies using labeled amino acids with sheep, Wolff et al. (131) showed that most of the amino acids, including all the essential ones, were added to portal vein plasma in highly significant amounts. However, uptakes of aspartic and glutamic acids were limited, implying extensive metabolism of these amino acids in the gut wall.

Endogenous Degradation and Utilization of Amino Acids .
The liver removes most of the absorbed amino acids from blood with the exception of some of the branched-chain acids. Large hepatic uptakes of glycine, alanine, and glutamine and to a lesser extent of serine, tyrosine, arginine and phenylalanine occur (131). Following transport to the liver and transport by way of plasma proteins or free amino acids, it was assumed that extensive metabolism occurs in peripheral tissues, particularly muscle.

Some of the early work with ^{14}C-labeled acetate indicated that ruminant tissues apparently cannot synthesize the usual essential amino acids with the exception of arginine (17). Later studies (54) demonstrated quite clearly that amino acids are utilized in substantial amounts for glucogenesis or directly as a source of energy for the animal. Other reports are available giving information on turnover rates and interconversions of amino acids in ruminants (40, 131) or relating to the uptake of ^{35}S and incorporation into tissue proteins.

In the lactating animal, the mammary gland is the major tissue utilizing amino acids for protein formation. A reasonable number of studies have also been done relating milk production (83). The major milk proteins (the caseins, alpha-lactalbumin and beta-lactoglobulin) are

synthesized in the mammary gland from plasma-free amino acids.

Factors Affecting Quality and Quantity of Amino Acids Reaching Absorption Site

Protein and Amino Acid Metabolism in the Rumen .

Potent rumen microbial proteases and deaminases rapidly degrade proteins and amino acids which are soluble in the rumen liquid phase (22). Rumen bacterial proteases are cell bound but are located on the cell surface to provide free access to substrate and are composed of both exo- and endopeptidease.

The enzymatic mechanism necessary for ruminal degradation can be expected to be present under most conditions since bacterial proteases are constitutive enzymes which do not appear to be subject to metabolic regulations (22). For example, additional dietary urea had no sparing effect on ruminal degradation of dietary protein (92).

Hydrolysis of peptide linkages provides minimal quantities of energy (7). Peptides and amino acids produced usually are catabolized to ammonia and carbon sources. Ammonia is the primary but not the only nitrogenous nutrient for rumen bacterial growth (3, 101).

The greatest metabolic importance of protein degradation may be to supply nonammonia nitrogenous nutrients. Some species of rumen bacteria use peptides

directly for microbial protein synthesis. Some amino acids, notably methionine and cysteine, are stimulatory to certain strains of rumen bacteria (22, 25, 46, 111).

Proteolytic enzymes enable rumen protozoa to digest bacterial protein which is the major source of amino acids for growth of these microbes. In general, free amino acids arise as intermediate products in the breakdown of proteins by rumen microorganisms. The low concentration of free amino acids in the rumen suggest rapid utilization, but increased concentrations after feeding imply that the proteolysis occurs faster than does subsequent utilization of free amino acids (72).

Free amino acids in the rumen can be assimilated directly by rumen microbes (3, 101) and can be absorbed from the rumen (35, 58), but most are deaminated to yield ammonia and other intermediate products. Deaminative activity occurs less frequently in rumen bacterial strains than does proteolytic activity (22).

Despite the proteolytic capabilities of rumen microbes, substantial amounts of ingested protein are resistant to degradation and thus bypass degradation in the rumen. In vitro ammonia production (100, 115) and solubility in minerals buffers (37) provide indirect estimates of protein degradation. Researchers concluded (112, 117) that as little as 40% or as much as 80% of the dietary protein normally might be degraded in the rumen and

transferred into microbial protein. There can be wide differences between feed ingredients in the extent of ruminal degradation of the protein fraction. In addition, approximately 30% of the bacterial protein produced in the rumen is also degraded therein (89).

Solubility of protein in rumen liquid and length of time protein is retained in the rumen contribute to differences in apparent ruminal degradation of proteins (82). Lewis and Emery (74) reported Vmax values indicating that arginine and threonine were rapidly degraded; lysine, phenylalanine, leucine, and isoleucine formed an intermediate group; while valine and methionine were amino acids least rapidly degraded in the rumen. Half-lives of 2 h or less for all essential amino acids indicated that for free amino acids to bypass the rumen in significant amounts, supplements greatly in excess of animal requirements would have to be fed (22). Mangan (80) also found rapid in vivo degradation of physiological quantities of amino acids.

Sources of Amino Acids Supplied to Absorption Sites .

Microbial protein synthesized in the rumen is the major source of amino acids reaching the absorption sites in ruminants fed most diets; however, this depends somewhat upon the quantity of dietary protein degraded in the rumen (22, 110). Maximum yield of microbial protein can be obtained only if optimum quantities of energy,

nitrogen, minerals, and other growth factors are supplied
to the microbes (60). The extent of dietary protein
breakdown depends mainly on nature of protein source (82).

Consistency of Amino Acid Composition in Microbial Protein .

Investigations of the amino acid composition of rumen
organisms indicated that the bulk amino acid composition
and the protein quality of the microbial preparation
presented to the host for digestion were not affected by
change of ration (14, 85, 103, 127). Also, digestibility
of the total microbial proteins (ie. bacterial plus
protozoal) was not modified by feeding different rations
(14). However, protozal protein was reported to have a
slightly higher biological value and much greater
digestibility.

Quality of Rumen Undegraded Dietary Protein .

Variations in amino acid composition of rumen
undegraded dietary protein are associated primarily with
dietary regimes. Different sources of protein, even at the
same protein concentration, may affect the amount of
protein entering the abomasum (77). Therefore, the
quantity and quality of amino acids leaving the rumen and
entering the alimentary tract of the ruminant are accounted
for by these fractions.

Means for Bypassing Ruminal Degradation of Proteins and Amino Acids

Postruminal Infusions of Proteins and Amino Acids .

Casein has been a source of protein in most abomasal infusion studies primarily because it is the major milk protein and should offer an ideal pattern of amino acid for synthesis of milk protein (27). Plant and animal proteins, such as soybean meal and fish proteins, have not been infused into dairy cows because of difficulty in getting them into solution or a suitable suspension.

Postruminal administration of casein increased milk production as compared to cows abomasally infused with control solutions (18, 32, 33, 38, 105, 118, 123, 124) even when cows were fed diets to meet their requirements for energy and protein. The greatest increases in milk yield were from high producing cows (18, 32, 38, 118, 124). The lack of response reported by Vik-Mo et al. (124) was attributed to fluctuations in feed intake even though total energy and protein consumption usually exceeded the cow's allowance.

Increased feed intake has been reported (93) when protein was administered to bypass the rumen in animals fed basal diets adequate in protein. The increase in feed intake caused by protein supplied to lower gut may be due to improved protein status of the animal. Nitrogen retention of lactating cows was improved by administering casein into the abomasum (32, 38). The increased nitrogen balance was due largely to decreased urinary nitrogen

excretion. This suggested that casein supplementation in the abomasum improved the pattern of amino acids available for protein synthesis and, thus, improved the efficiency of utilization of the absorbed nitrogen.

Postruminal infusion of essential amino acids either singly or in combination has not given consistent positive responses (27, 43, 116). Intravenous infusions of individual amino acid either methionine, histidine, or lysine did not increase milk and milk protein production significantly (27, 43). Instead, some negative responses were observed when methionine and histidine was infused individually (27, 43, 106). Production increased when more amino acids were administered intravenously. Schwab and Satter (116) infused mixtures of essential amino acids into the abomasum and obtained positive increases in milk protein yield in the cow. However, negative responses in milk production were found when fewer amino acids were given to cows abomasally.

Responses of postruminal infusion trials, either abomasal or intravenous, suggested that bypassing ruminal fermentation with amino acids can increase both milk protein yield and milk protein percent. They are normally increased more when protein or protein hydrolysates are supplemented postruminally as compared to supplying crystalline amino acids. However, it is hardly possible to administer infusates postruminally in practice.

Rumen Protection of Dietary Proteins and Amino Acids

Heat or Chemical Treatment . Heat generated or applied in feed manufacturing procedures not only can destruct enzymatic inhibitors present in some protein sources, such as trypsin inhibitor in soybeans, but can also decrease ruminal degradation of protein (28, 109). The denaturation of protein molecules caused by heat treatment results in decreased protein solubility. However, excess heating can have detrimental effects on nutritive value with reduced digestibility (2, 104). The extent of heat damage is the result of irreversible binding of reducing sugar groups with amino nitrogen (Maillard reaction) or destruction of amino acids.

Attempts have been made to investigate adequate temperature and retention time for preparation of heat-treated soybean meal (HSBM). Netemeyer et al. (88) reported treating soybean meal (SBM) at 75 to 115 C for approximately 30 min reduced protein digestibility index and ruminal degradation without reducing total enzymatic digestion of SBM. Investigations by Kung and Huber (69) indicated heating SBM for 2 h at 149 C reduced N solubility in Burroughs' buffer from 26.5 to 3.9%, decreased in vivo dry matter digestibility from 92.1 to 89.0%, and lowered N disappearance from nylon bags in the rumen from 76.9 to 36.3%. However, treatment for 4 or 6 h only minimally decreased N disappearance but increased acid detergent

insoluble nitrogen to 8.9 and 19.7% of the total N. Results of this study suggested that dry heat treatment of SBM for about 2 h at 149 C appeared optimal for reducing rumen nitrogen degradability with a minimum of heat damage.

Digestion in the small intestine of nonammonia nitrogen (NAN) was equal when feeding HSBM compared to SBM although ruminal degradability of HSBM tended to be lower (1, 71). Ruminal pH, volatile fatty acids (VFA), and ammonia, and serum urea were not affected by feeding HSBM to cows (1, 69, 109).

Amino acid composition of HSBM was similar to composition in the untreated meals (109, 113), and heat treatment tended to reduce solubility of all amino acid in SBM. Kung et al. (71) reported that NAN flow increased to the duodenum for cows receiving HSBM which also related to higher milk yields. Heat treatment of SBM did not alter the profile of amino acid in the arterial or venous blood serum of cows nor affect uptake of amino acids by the mammary gland (1). However, Kung et al. (70) reported EAA and branched-chain amino acids in arterial blood increased and NEAA decreased as amount of HSBM in cow's diet increased.

HSBM supplementation did not change dry matter intake and gains of body weight of lactating cows (1, 50, 69, 88). However, Sahlu et al. (109) reported that cows fed HSBM diet consumed less feed dry matter. Milk production was

increased by feeding HSBM to high-producing cows in early lactation (69, 88, 109), but was less effective with lower producers (50, 109). Heat treatment via extrusion or during desolventizing of SBM seemed to be equally effective for increasing milk production (109). In contrast, Grummer and Clark (50) reported that varying protein solubility in the diet by exposing defatted soybean flakes to various amounts of heat treatment did not affect milk production of early lactation cows, but they only used six cows per treatment in their experiment. The study by Ahrar and Schingoethe (1) noted that milk production was not improved significantly by feeding HSBM to cows past peak lactation. The feeding of HSBM had no effects on milk composition (1, 50, 69, 88, 109).

Certain chemical agents (eg. aldehydes, tannins, etc.) form reversible cross linkages with amino and amide groups which decrease solubility of proteins at the pH of the rumen (22). Chemically-treated proteins subsquently are made available to the host by destruction of these linkages in the more acidic abomasum.

More research has been conducted with formaldehyde (HCHO) treatment of proteins than with other chemcal agents. Minson (86) reported a significant increase in protein and casein content of milk from cows fed HCHO-treated casein. However, most studies (19, 29, 36, 45, 61, 126) showed no beneficial effect on milk production

or milk composition for cows fed HCHO-treated proteins.

Feeding HCHO-treated proteins did not decrease feed consumption (29, 36, 45, 126). However, lower digestibility was found (29, 36, 61) accompanied by little or no responses in production measurements in lactating cows. Treatment of proteins with HCHO reduced protein degradation in the rumen (19, 126). Decreased digestibility indicated that proteins may have been overtreated with HCHO as suggested by lower recovery of applied HCHO in acid-labile HCHO analysis (19).

The study by Broderick and Lane (19) showed that HCHO treatment resulted in losses of available lysine, and most of the treated HCHO was bound to amino acid residues other than lysine. Free amino acid concentrations in arterial or venous plasma or amino acid uptake by mammary gland were not increased by feeding a diet containing HCHO-treated soybean meal (29).

Amino Acid Analogs . Structural manipulation of amino acids creates resistance to ruminal degradation. Methionine hydroxy analog (MHA) has been investigated in numerous studies (12, 15, 25, 41, 47, 49, 57, 59, 79, 81, 91, 95, 102, 110, 121).

In regards to metabolism by tissues, MHA had methionine activity in ruminant animals similar to that in nonruminants. Belasco (12) concluded that MHA had longer stability in rumen fluid than methionine. Salsbury et al.

(110) found that MHA was metabolized in vitro and that while, methionine and MHA had similar affects upon rumen microbial metabolism, MHA was less effective, possibly due to its lower solubility in rumen fluid. However, other investigators (41, 47) concluded that MHA was degraded in the rumen.

Feeding MHA during early lactation increased yields of milk fat and fat-corrected milk (15, 25, 49, 57, 59, 79, 102, 121). Generally no change in milk yield was observed (15, 25, 57, 59, 79, 91, 121) except increased production in the study by Griel et al. (53). No or little responses may due to low producers (59, 91, 121), shorter term studies (121) or experiments conducted beyond the peak of lactation (15). Feeding MHA did not decrease dry matter intake (15, 57, 79, 91, 121) indicating no reduced ration palatability. However, Polan et al. (102) reported reduced silage and concentrate consumption when MHA was fed to cows at .8% of concentrate.

Higher ratios of ruminal acetate to propionate were reported (79) accorded with increased milk fat yield from cows fed MHA. Huber et al. (59) suggested that increased milk fat could have resulted from greater uptake of preformed blood fatty acids by the udder for cows fed MHA. Olson and Grubaugh (121) indicated that fatty acid composition of the milk fat was not different when feeding MHA.

Supplementation of MHA increased methionine, isoleucine, and leucine in serum of coccygeal vein with significantly higher methionine A-V difference across the mammary gland (59).

Encapsulation of Amino Acids . Encapsulation of amino acids has been thought to be the most efficient and economical way of meeting the amino acid requirement for the host tissue. However, the effect is not readily apparent in view of the complexity of ruminant nitrogen metabolism, stability of encapsulated products, and variety of dietary conditions (18, 23, 96, 97, 128).

More work has been done on methionine to protect it from ruminal degradation than on other amino acids. This could be because methionine has been identified as one amino acid that may limit or colimit growth (13, 22), milk, or milk protein production (1, 20, 22, 27, 38, 39, 44, 65, 113, 116) in ruminants. However, feeding MHA or postruminal infusions of methionine for lactating cows has not obtained conclusive results.

Under practical feeding conditions, an increase or no change in feed intake (96, 97, 128) was observed when feeding encapsulted or rumen-protected methionine to lactating dairy cattle. However, previous studies (18, 53, 95) showed a depression in feed consumption when large quantities of methionine or encapsulated methionine were added to the diets of animals.

Broderick et al. (18) found that feeding 5, 15, or 45 g/day of encapsulated methionine to dairy cows during peak lactation had no effect on milk production or composition. Similar results were reported by Williams et al. (128) feeding 12 g per day of encapsulated methionine. However, availability of methionine increased as indicated by elevated methonine to valine concentration ratios in the blood plasma (18), suggesting encapsulation of methionine achieved ruminal protection, intestinal release, and absorption.

Using pH-sensitive polymeric coatings to prepare rumen-protected methionine was described in (96, 97). Papas et al. (96) reported that rumen-protected methionine was 94% stable in pH 5.4 buffer, which simulated ruminal pH, and it released 94% of the methionine in pH 2.9 buffer, which simulated abomasal pH, indicating this preparation of methionine was effective in protecting methionine from ruminal degradation and in releasing it postruminally. However, milk production and milk composition were not significantly affected for cows fed this product during early lactation (96, 97).

A study of a European rumen-protected methionine product by Chalupa and Williams (23) indicated that pellets of methionine coated with fatty acids (C14-18) and $CaCO_3$ was also effective in elevating plasma methionine concentration confirming rumen bypass and absorption in

vivo. However, milk production was not significantly increased for cows fed this product.

Most studies feeding rumen-protected methionine to cows increase methionine plasma concentration, except in (128). The failure to increase plasma methionine concentration in that trial may have been attributed to incomplete ruminal protection or incomplete release for absorption. Concentrations of other plasma amino acids were unaffected significantly by supplemental rumen-protected methionine (18, 23, 96, 97).

Methods in Identifying Limiting Amino Acids for milk synthesis

Comparisons between the amount of amino acids consumed with the amounts excreted in milk has been used to identify amino acids limiting production (63). However, the tempering effect by microbes on the dietary proteins in the rumen may have tremendous alteration on the sequences of limiting amino acids.

Variations in nitrogen intake, preferential catabolism by the liver, interconversion and extensive use of amino acids for glucose also make interpretation of amino acids profiles extremely difficult for identifying limiting amino acids in ruminants (27).

Attempts have been made to determine whether in fact there is a first-limiting amino acid in ruminants by dietary addition of amino acids as in monogastric animals

(63). The results have been largely negative, probably owing to ruminal degradation. However, other approaches have been used to establish the sequences of limiting amino acids in lactating animals. Most of these approaches have involved measurements of changes in the concentrations of plasma free amino acids (20, 33, 38, 39, 118). Based on the premise that a limiting EAA will not accumulate in the plasma until its requirement is met (87, 120), Broderick et al. (20) indicated that methionine, valine, and lysine were the most limiting amino acids for milk production when feeding graded amounts of ruminally protected protein. Differences in arteriovenous concentrations of amino acids can be used to calculate percent extraction of plasma free amino acids by the lactating mammary gland. Clark et al. (33) reported that EAA having the highest percent extraction with lowest concentration in arterial plasma tended to be the most limiting EAA. Drackley and Schingoethe (39) calculated minimum transfer efficiencies (transfer from blood to milk protein) for serum EAA, and suggested EAA showing highest transfer efficiency was the most limiting EAA. More meaningful information can be obtained by expressing uptake of amino acids relative to their output in milk protein. The closer the ratio is to 1 the more limiting the amino acid (33, 38, 39).

Essentiality and Metabolism of Methionine

Methionine is an important amino acid in lactating

ruminant nutrition because of its involvement in protein and lipid metabolism. It has been suggested to be the most limiting amino acid for milk protein synthesis (1, 20, 22, 27, 37, 38, 39, 44, 62, 65, 113, 116).

Methionine may exert its effect on milk production and composition by stimulating rumen microbial growth and metabolic processes in the animal. Although free dietary methionine was degraded readily by rumen organism (73), the addition of methionine accelerated synthesis of microbial protein when ruminal bacteria were maintained on urea as sole source of nitrogen (46). Methionine also improved rumen protozoa growth (25). Preformed methionine was reported to be essential to maximize bacterial growth in steers fed protein and urea-containing diets (111). Methionine also stimulated lipid synthesis by ruminal microorganisms (98), most likely by providing methyl groups for phosphatidylcholine synthesis.

Methionine is also a major precursor in the synthesis of serum lipoproteins (122) in the liver and is involved intimately in deposition and mobilization of adipose tissue and in transport of lipid in the blood (81). Serum lipoproteins are major precursors of long chain fatty acids in milk of the dairy cows (16). Any interference in lipoprotein metabolism from methionine deficiency could be expected to affect production of milk fat.

Methionine and MHA have been used to treat ketosis in

lactating cows (81). Observations in the study by Kronfeld et al. (68) indicated diminished synthesis of short-chain fatty acids by the mammary gland accompanied increased blood acetoacetate concentrations during spontaneous ketosis. This suggested methionine is important for normal de novo fatty acid formation in the lactating mammary gland.

The degradation of methionine (78) is commonly assumed to proceed via a trans-sulphuration pathway in which S-adenosyl-L-methionine donates methyl groups to other molecules. The nature of this degradative pathway can be considered to be anabolic in which diverse substances such as phosphatidylcholine, melatonin, epinephrin etc. are synthesized. Within these, phosphatidylcholine synthesized in the liver is involved in the formation of lipoprotein in animals.

An alternative metabolic pathway which oxidizes the methionine occurs via a transamination pathway. This pathway, which is independent of S-adenosyl-L-methionine formation may be the major degradative route except when its supply is limited. The sequence of the transamination pathway has been established, in which 2-oxoacid (eg. pyruvate, 2-oxoglutarate) accepts the amino group from L-methionine producing alanine or glutamate and 4-methylthio-2-oxobutyrate. Subsequent oxidation of 4-methylthio-2-oxobutyrate to CO_2 occurs in mitochondria.

The transamination pathway is important in the conversion to L-methionine of the dietary substitute, D-methionine with 4-methylthio-2-oxobutyrate as intermediate.

Komarek and Jandzinske (65) reported that different responses were found in cows intraperitoneally infused with graded levels of D, L, and DL-methionine. Their data suggest that there was a need for additional postruminal methionine above the levels the cows were receiving postruminally from rumen and dietary sources. The marked difference between the responses to D versus L-methionine suggested different metabolic pathways for each and indicated that isomerization of D to the L form probably does not occur. The responses to DL-methionine feeding further suggested the combined result of the different metabolism of D and L-methionine. Although L-methionine can be used for both protein synthesis and methyl donor functions, it is postulated that D-methionine is used solely as a methyl donor.

MATERIALS AND METHODS

Twenty-four high producing (more than 28 kg milk/day during the third week postpartum) Holstein cows (10 multiparous and 14 primiparous) were randomly assigned to heat-treated soybean meal concentrate without (HSBM) or with added ruminally protected methionine (HSBM+Met) from wk 4 through 16 postpartum. Cows were fed total mixed diets (Table 1) containing 30% of dry matter as corn silage; 15% as alfalfa hay; and 55% as a concentrate mix containing corn, heat-treated soybean meal, and vitamins. Heat-treated soybean meal was subjected to additional heat during desolventizing as outlined by Schingoethe and Ahrar (113).

Diets were formulated to contain 16% crude protein and 18% acid detergent fiber. Methionine supplemented cows received 50 g/cow/day of Ketionin[1], a ruminally protected methionine, blended with the remainder of their diet at time of feeding. This provided 15 g DL-methionine daily.

Cows were housed in a free stall barn, individually fed once daily using a Calan gate feeding system (American

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[1]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, Oslo, Norway.

Table 1. Ingredient content of total mixed diet.

Ingredient	Diet[1]
	(% of dry matter)
Corn	40.25
Heated soybean meal·	13.75
Dicalcium phosphate	.70
Trace mineral salt	.30
Alfalfa hay	15.00
Corn silage	30.00

[1] Diet contains 4,840 IU of added vitamin A, 968 IU of added vitamin D, and .48 IU of added vitamin E/kg dry matter.

Calan, Northwood, NH.) and milked in a double herringbone-type parlor. Milk production and feed intake were recorded daily. Amounts of feed fed were adjusted weekly to the previous week's milk production. Twenty-four hour (p.m. plus a.m.) milk samples were obtained two times during the third week postpartum (pretreatment samples), and once each week throughout the experimental period.

Milk was analyzed for protein by Kjeldahl, fat by Babcock, and total solids by Mojonnier methods(5). Remaining amounts of two milk samples from approximately wk 8 and 13 postpartum were frozen until analyzed for fatty acid distritution. Milk fat was obtained by the Roese-Gottleib extraction procedure (6) and butyl esters of fatty acids were prepared by methods of Jones and Davidson (64) and separated by gas-liquid chromatography using 10% EGSS-X on 100/120 Gas Chrom P5 in a .32 x 305 cm stainless steel column. Temperature of column was programmed at 6 C/min from 75 to 200 C.

Body weights of cows were measured on 3 consecutive days at the beginning and end of experiment, and once every two weeks throughout the experiment.

Concentrates, hay, and corn silage were sampled weekly and composited monthly, and composites dried at 57 C for 72 hrs in a forced air oven to determine dry matter. After drying, feeds were ground in a Willey mill to pass through

a 2-mm screen and analyzed for crude protein, ether extract, and ash by Association of Official Analytical Chemists' methods (5); and neutral detergent fiber, acid detergent fiber, and permanganate lignin by procedures of Goering and Van Soest (48). Feed samples were hydrolyzed in 6N HCl in sealed tubes containing N_2 gas for 4 h at 145 C. Hydrolysates were evaporated to dryness, diluted with sodium citrate buffer (pH 2.2), filtered, and analyzed for amino acid content on an amino acid analyzer (Spinco 120 Automated Amino Acid Analyzer, Beckman Instruments, Inc., Palo Alto, CA.). Separation of amino acids was on ion-exchange columns with sodium citrate buffers ranging in pH from 3.49 to 6.40.

Samples of rumen contents were obtained three times during the trial via esophageal tube and suction strainer approximately 2 to 4 hrs after morning feeding. Samples were collected into bottles containing .5 ml saturated mercuric chloride, measured for pH, and sample was filtered through four layers of cheesecloth. Filtered fluid (10 ml) was centrifuged at 1500 rpm for 10 minutes and the supernatant acidified with .5 ml of .1 N HCl and frozen until analyzed for ammonia by procedure of Chaney and Marbach (26). An additional 10 ml was acidified with 2 ml 25% metaphosphoric acid, centrifuged, and the supernatant frozen for analysis of volatile fatty acids (VFA) using gas-liquid chromatography with a SP-1200/1%

phosphoric acid on 80/100 Chromosorb W AW in a 183 x 2 mm ID glass column (Supelco Inc., Bellefonte, PA.) as described by Baumgardt (10).

Samples of jugular vein blood were withdrawn into heparinized tubes at the time of rumen sampling. Samples were centrifuged for 20 minutes at 2000 rpm. The supernatant fraction was decanted and frozen until analyzed for urea nitrogen (26), and glucose (Sigma chemical Company, St. Louis, MO.). Samples of tail vessel and mammary vein blood were obtained from approximately 6 to 8 weeks postpartum. Serum amino acid composition was determined in samples from 6 cows per treatment group by methods described by Drackley and Schingoethe (39). Amino acid uptake from blood transversing the mammary gland was estimated using differences in amino acid concentration of arterial and venous blood (AV) with mammary blood flow (MBF) estimated by the regression equation reported by Kronfeld et al. (75). Packed cells content (99) in the blood was used as correction factor to calculate mammary serum flow. Averaged daily milk production and Kjeldahl nitrogen content of the milk produced during the arterio-venous blood sampling week and amino acid composition of milk (63) were used to estimate the amino acid output in milk.

Percent extraction, transfer efficiency of amino acid by mammary gland and ratios of amino acid uptake to output

as described in (39) were used to identify amino acids limiting milk secretion.

Data were analyzed statistically by analysis of variance as described by Steel and Torrie (119) using the Statistical Analysis System (SAS) general linear model program (56). Milk production and composition were adjusted by covariance analysis using pretreatment (wk 3 postpartum) milk production and composition as covariates. Differences due to treatment, time, lactation number (first lactation versus second and later lactations), and all possible interactions were considered. Significant level was P<.05.

RESULTS AND DISCUSSION

Chemical composition of feeds and total ration is in Table 2. The results were similar to as formulated except lower neutral detergent fiber percent in the ration. This was because this portion was not analyzed in grain mix. Based on similar concentrate formulation reported by Sahlu et al. (109), 5.5% of acid detergent fiber (ADF) and 19.3% of neutral detergent fiber (NDF) were obtained by averaging ADF and NDF fractions in heat-treated soybean meal and extruded soybean meal concentrates. Including these values would give a recalculated ADF of 17.4% and NDF of 32.3% in the total ration.

Production of milk and milk compositions are listed in Table 3. Actual milk yield was not increased (P>.05) by protected methionine supplementation. However, after covariant adjustment for pretreatment productions, cows receiving the HSBM diet produced more milk (P<.01) than cows fed HSBM+Met. This difference was accounted for by multiparous cows assigned to HSBM+Met having considerably higher pretreatment milk production (Table 4). It is not known why cows fed supplemental methionine had less covariant adjusted production, but this response was not likely caused by the dietary treatment. It may have been a artifact of more of the cows assigned to the HSBM+Met diet having fewer complications after calving, and thus attaining peak production earlier postpartum.

Table 2. Chemical composition of concentrate, forages, and total ration.

Chemical composition	Ration component			
	Concentrate mix[1]	Corn silage	Alfalfa hay	Ration[2]
Dry matter (DM), %	90.3	38.7	86.2	74.5
	------------ (% of DM) ----------			
Crude protein	18.3	8.1	17.2	15.1
Ether extract	3.3	2.7	1.6	2.9
Acid detergent fiber	...	27.8	40.4	(14.4)
Neutral detergent fiber	...	46.6	51.6	(21.7)
Lignin	...	4.8	9.4	(2.8)
Ash	4.8	5.0	8.5	5.2

[1] Acid detergent fiber, neutral detergent fiber, and lignin analyses were not included in grain mix.

[2] Calculated.

Table 3. Milk production and composition from cows fed diets containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met).

Measurement	Primiparous		Multiparous		All cows		
	HSBM	HSBM +Met	HSBM	HSBM +Met	HSBM	HSBM +Met	SE
Milk, kg/day	34.4 [1] (30.8)	33.7 (31.7)	34.9 (37.3)	32.6** (38.8)	34.6 (34.5)	33.1** (35.2)	.30 .36
4% Fat-corrected milk, kg/day	28.8 (26.2)	27.8* (27.2)	28.1 (29.9)	27.5 (31.4)	28.5 (28.0)	27.6 * (29.3)	.28 .39
Solids-corrected mild, kg/day	29.2 (27.0)	28.7 (27.8)	28.8 (30.4)	28.4 * (32.2)	29.0 (28.8)	28.5 * (30.0)	.26 .21
Fat, %	2.90 (2.85)	2.90 (3.07)*	2.71 (2.66)	2.93* (2.77)	2.81 (2.75)	2.92 * (2.92)	.03 .05
Fat, kg/day	1.00 (.90)	.95 * (.97)	.94 (1.00)	.97 (1.06)	.97 (.95)	.96 * (1.01)	.01 .02
Protein, %	2.89 (2.93)	2.99** (2.95)	2.87 (2.83)	2.84 * (2.91)	2.88 (2.88)	2.92 * (2.93)	.01 .02
Protein, kg/day	.97 (.93)	1.00* (.94)	1.02 (1.05)	.94** (1.12)	1.00 (.99)	.97 * (1.03)	.01 .02
Total solids, %	11.64 (11.70)	11.74 (11.90)	11.34 (11.23)	11.64** (11.50)	11.49 (11.46)	11.69 ** * (11.67)	.04 .06
Total solids, kg/day	3.96 (3.70)	3.92 (3.75)	3.97 (4.19)	3.85 * (4.42)	3.97 (3.94)	3.89 * (4.09)	.03 .04
Solids-non-fat, %	8.75 (8.83)	8.83 (8.79)	8.62 (8.57)	8.71 (8.71)	8.68 (8.70)	8.77* (8.75)	.02 .03
Solids-non-fat, kg/day	2.95 (2.80)	2.95 (2.78)	3.04 (3.20)	2.92 * (3.36)	3.00 (3.00)	2.94 (3.07)	.05 .03

[1]Unadjusted data are in parentheses.

*Different from HSBM, P<.05.

**Different from HSBM, P<.01.

Table 4. Pretreatment period (3 wk postpartum) milk yield and composition.

Measurement	Primiparous		Multiparous		All cows	
	HSBM	HSBM +Met	HSBM	HSBM +Met	HSBM	HSBM +Met
Milk, kg/day	26.8	27.5	32.6	36.9	29.5	30.3
FCM[1], kg/day	25.4	28.1	30.8	33.6	28.0	30.0
SCM[2], kg/day	26.1	27.8	30.9	34.2	28.4	29.9
Fat, %	3.66	4.15	3.64	3.40	3.65	3.93
Fat, kg/day	.98	1.14	1.19	1.25	1.08	1.19
Protein, %	3.08	2.85	2.86	3.17	2.98	2.95
Protein, kg/day	.83	.78	.93	1.17	.88	.90
TS[3], %	12.77	12.93	12.42	12.28	12.60	12.74
TS, kg/day	3.42	3.56	4.05	4.54	3.71	3.86
SNF[4], %	9.11	8.78	8.78	8.88	8.95	8.81
SNF, kg/day	2.44	2.41	2.86	3.28	2.64	2.67

[1]FCM = 4% fat-corrected milk.

[2]SCM = solids-corrected milk.

[3]TS = total solids.

[4]SNF = solids-non-fat.

Figures 1 and 2 illustrate actual and covariate-adjusted milk yield of cows during the entire experiment. Methionine supplementation did not increase persistency of milk production as observed in (128) when encapsulated methionine was fed to cows. As shown in Figure 1, cows receiving HSBM+Met had earlier (6 wk postpartum) and higher (37.6 vs. 35.8 dg/day) peak yield, whereas cows fed HSBM maintained peak production for a longer period of time (wk 6 through 10). Methionine-supplemented cows tended to be more variable in milk production from wk 6 to 12 postpartum. Differences in pretreatment milk production caused covariant adjusted milk yield to be greater for cows fed HSBM after wk 7 postpartum (Figure 2). HSBM supplementation to high-producing cows increased milk production in early lactation relative to production with regular soybean meal (69, 88, 109). However, a recent study by Kung et al. (69) indicated maximum responses to HSBM feeding were obtained when NPN was also incorporated in the diets. The lack of responses for cows fed HSBM in some previous studies (1, 50) may imply that insufficient soluble nitrogen was available for maximum microbial protein biosynthesis when soybean protein was protected to bypass the rumen. Other trials in which different rumen-protected methionine products were supplemented in the diets showed that milk yields were not increased (18, 23, 96, 97, 128).

Figure 1. Actual milk production of cows fed diets containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met).
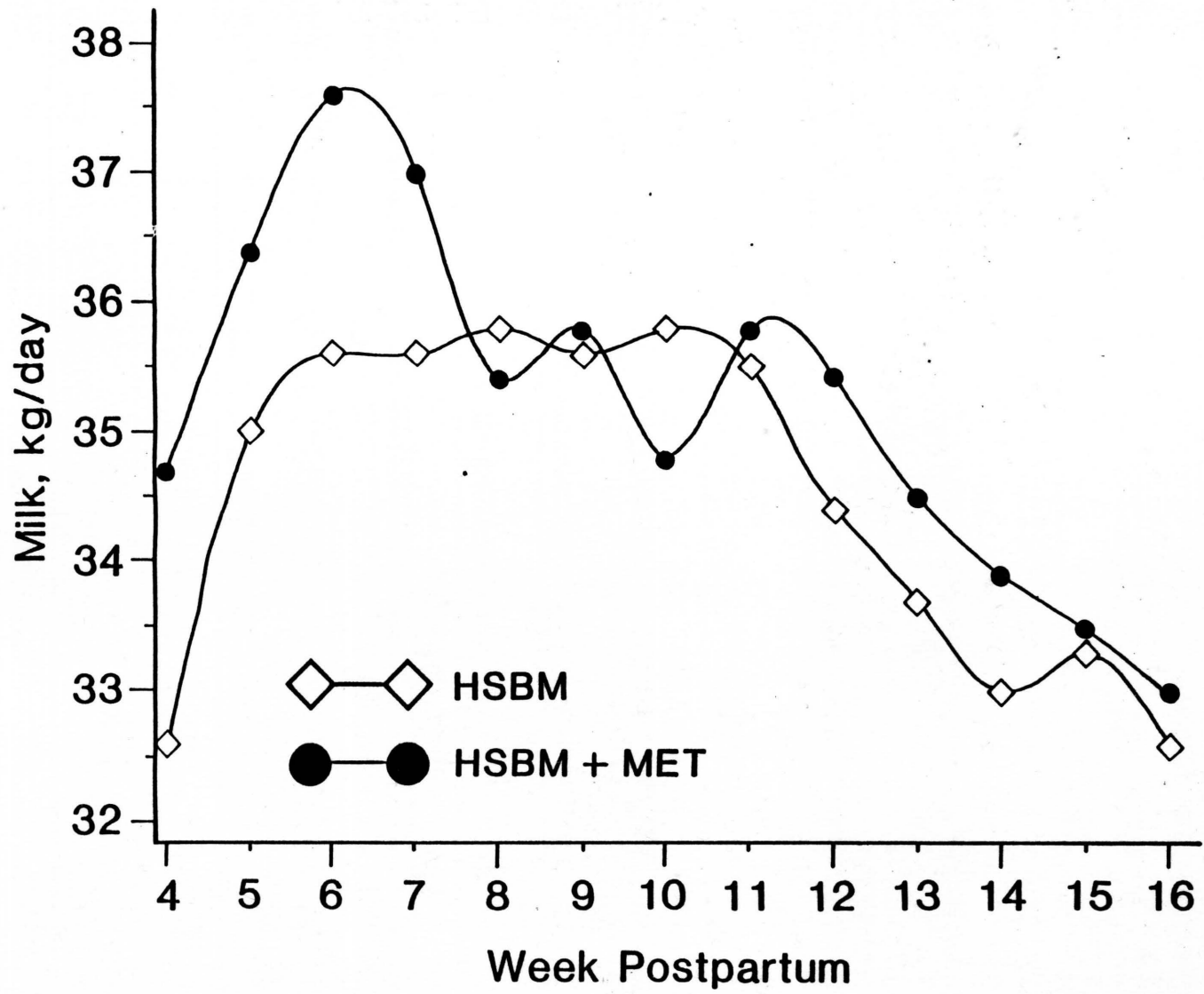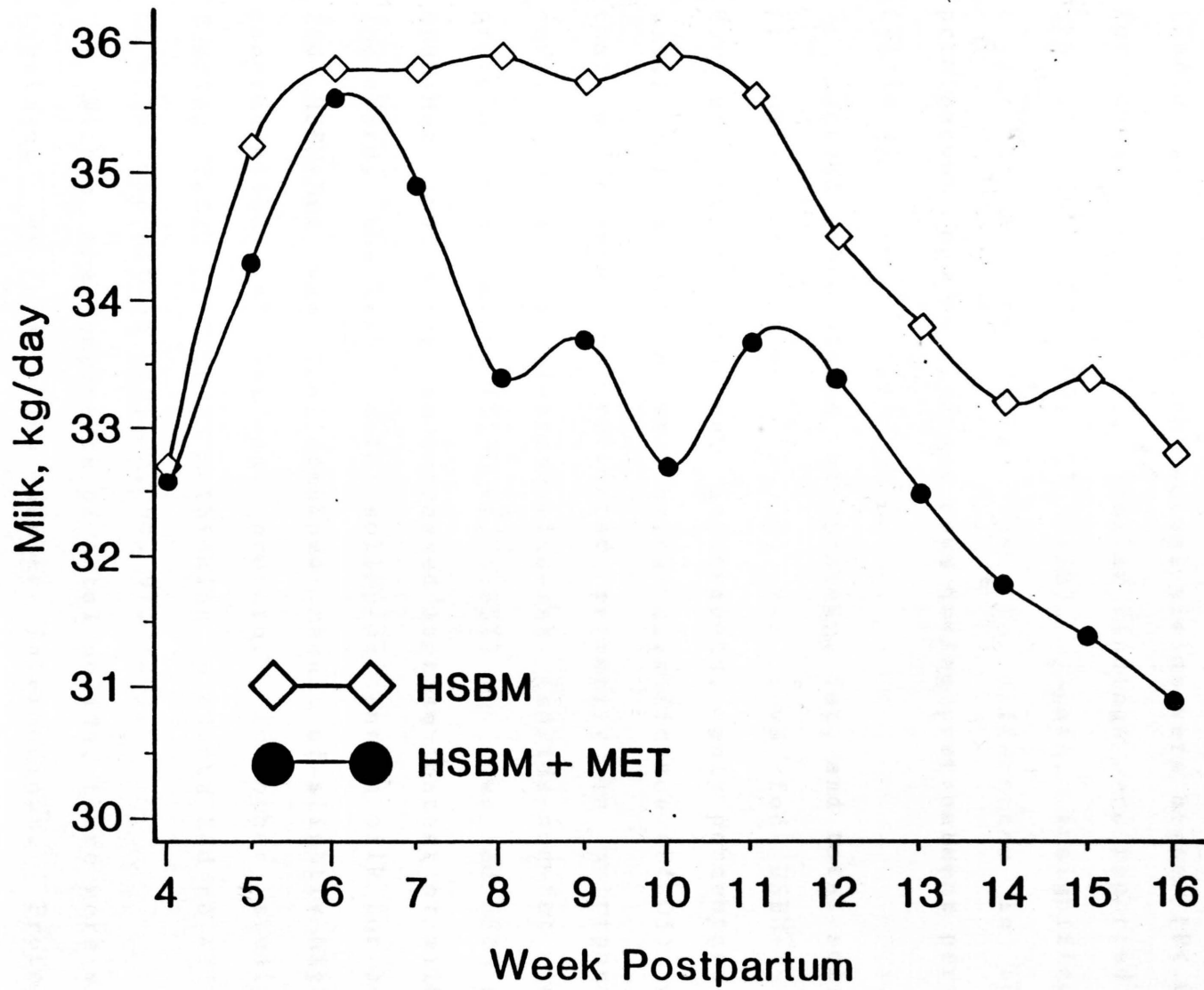
Figure 2. Covariate adjusted milk production of cows fed diets containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met).

Yield of covariant adjusted 4% fat-corrected milk and solids-corrected milk were similar between treatments (Table 3), even though actual yields were higher (P<.05) for cows fed HSBM+Met. Similar findings were reported by others (18, 23, 96, 97, 128). Again, insignificant treatment effects were due to differences in both primiparous and multiparous cows during pretreatment period (Table 4).

Actual percentages of protein, fat, and total solids in milk were higher (P<.05) for cows fed HSBM+Met. However, after covariant adjustments, only percentage of total solids revealed statistical significance (P<.05), and that difference was reflected primarily in multiparous cows. Percent of lactose-plus-ash (solids-non-fat minus protein) was similar (5.80 vs. 5.85%) in cows fed HSBM and HSBM+Met indicating no increased lactose content of milk. Therefore, the higher total solids percent in milk for cows fed HSBM+Met was the combined result of slightly higher concentrations of fat and protein. In other studies, feeding rumen-protected methionine products had no effect on milk composition (18, 23, 96, 97, 128).

Within the components of total solids, there were some treatment by lactation number interactions. Protein percentage increased in primiparous cows when fed added methionine, while fat percentage increased in multiparous cows. The route by which supplemental methionine affects

lactating cows may be different in older than in younger cows.

The higher (P<.05) actual milk fat, protein and total solids production by cows fed added methionine were due to more (P>.05) actual milk production and higher (P>.05) percentages of these components in the milk. Although, grouped data adjusted for pretreatment performance were similar, methionine supplementation increased (P<.01) milk protein production in primiparous cows but did not increase milk yield. Similar results were reported by Fisher (43) and Schwab et al. (116) in which crystalline amino acids were supplemented postruminally.

Fatty acids in milk fat (Table 5) were similar for cows fed both diets with no significant treatment by time or treatment by lactation number interactions. Relative proportions of short chain fatty acids were unaltered indicating no effect of protected methionine on de novo synthesis of short chain fatty acids in mammary gland or on preformed fatty acid transportation in blood stream (16, 68, 81). Similar amounts of unsaturated fatty acids (31.5 vs 32.5 g/100g total fatty acids) may imply unchanged degree of hydrogenation in the rumen (39).

Feed consumption, body weights, and body weight changes are shown in Table 6. Dry matter intakes were greater (P<.05) when methionine was supplemented. Depressed dry matter intakes were observed in previous

Table 5. Fatty acid composition of milk fat from cows fed diet containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met)[1].

| | Diet | | |
| | HSBM | HSBM +Met | SE |
Fatty acid[2]			
	-- (g/100g) --		
4:0	3.2	3.4	.13
6:0	2.3	2.3	.08
8:0	1.4	1.5	.05
10:0	3.8	4.0	.12
12:0	4.2	4.5	.13
14:0	12.2	12.3	.24
16:0	29.9	28.1	.57
16:1	4.4	4.1	.14
18:0	11.2	11.3	.32
18:1	22.5	23.2	.52
18:2	4.6	5.2	.35
18:3	trace	trace	...
Short chain (4:0-14:0)	27.3	28.0	.64
Long chain (16:0-18:2)	72.3	72.0	.58

[1] Means of samples taken averaged week 8 and week 13 postpartum.

[2] Expressed as number of carbons : number of double bonds.

Table 6. Dry matter intake (DMI) and body weight (BW), and body weight changes (BWC) for cows fed heat-treated soybean meal concentrate without (HSBM) or with added protected methionine (HSBM+Met) during weeks 4 to 16 postpartum.

Measurement	Primiparous		Multiparous		All cows		SE
	HSBM	HSBM +Met	HSBM	HSBM +Met	HSBM	HSBM +Met	
DMI, kg/day	18.8	18.3	21.5	23.8**	20.2	21.0*	.21
DMI, kg/100 kg BW	3.24	3.28	3.45	3.74**	3.35	3.52**	.03
Beginning BW, kg	573	562	609	631	591	597	13.9
Ending BW, kg	600	571	635	652	618	611	13.9
BWC wk 4-10, kg/day	− .14	− .22	.26	.00	.06	− .12	...
BWC wk 11-16, kg/day	.52	.43	.31	.38	.43	.40	...
BWC wk 4-16, kg/day	.30	.10	.28	.23	.30	.15	...

*Different from HSBM, P<.05.

**Different from HSBM, P<.01.

studies (18, 53, 95) when large concentrations of methionine or MHA were added to the diets of animals. However, feeding rumen-protected type of methionine did not alter DMI in most studies (23, 97, 128). The flavoring agent used in this protected methionine product may have enhanced voluntary feed consumption. Increased DMI was also found in the study by Papas et al. (96) in which rumen-protected methionine was fed to cows. Significant treatment by lactation number interactions were found in which multiparous cows had higher (P<.01) DMI when fed HSBM+Met, while DMI was similar in primiparous cows.

Figure 3 illustrates dry matter intakes as percent of body weight during whole trial. Methionine-supplemented cows showed some fluctuation in dry matter intake after 5 wk postpartum, while cows fed HSBM consistently increasing in dry matter intake toward the end of the experiment. This may have been related to the variability in milk yield from wk 6 to 12 postpartum for cows fed HSBM+Met. Vik-Mo et al. (124) proposed that the lack of response to bypassed protein was attribuated to fluctuations in feed intake even though total energy and protein consumption was greater than requirements.

Body weight and body weight changes were not different (P>.05) between treatment groups (Table 6). Cows in both groups gained some weight over the 12-wk experimental period, 27 and 14 kg, respectively with HSBM and HSBM+Met.

Figure 3. Dry matter intakes (DMI) as a percent of body weight for cows fed diets containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met).
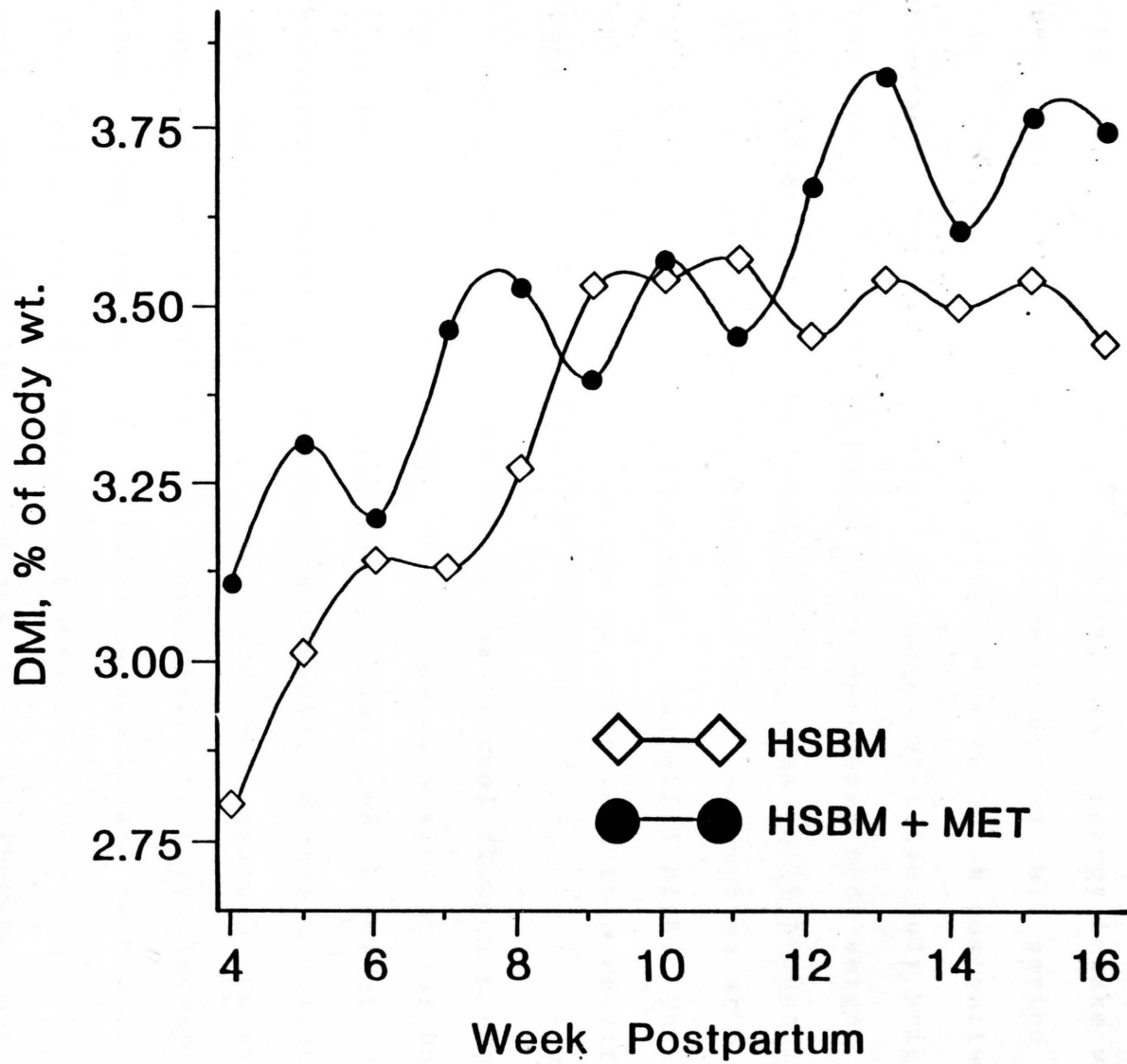
Most of this weight gain was during the last 6 weeks of the experimental period (Figure 4). This was expected since energy requirements in lactating dairy cows decrease with the decrease in milk production, and energy intake was generally greater than requirements during this period of time. Cows fed HSBM+Met lost weight 4 to 10 wk postpartum; however, HSBM cows tended to have positive body weight changes throughout the trial. The decreased body weight in methionine-supplemented cows during peak milk production may have been due to decreased feed comsumption at 6 wk postpartum when milk production was still high. During early lactation, energy intake is often less than required (28).

Proportions of the VFA in the ruminal fluid are shown in Table 7. Total VFA production was similar for both treatments; however, primiparous cows fed HSBM+Met had increased total VFA production (P<.01). Of the individual VFA, molar percent of isobutyrate and isovalerate were lower for cows fed HSBM+Met, especially in first lactation cows. The ratios of acetate to propionate were similar when all cows were pooled for analysis. However, a lower ratio was found in multiparous cows fed HSBM+Met due to increased propionate concentration.

Concentrations of ruminal ammonia (Table 7) 2 to 4 hr after morning feeding were not affected by treatments. These values may reflect no changed rate of proteolysis for

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Figure 4. Bodyweights of cows fed diets containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met) during week 4 to 16 postpartum.

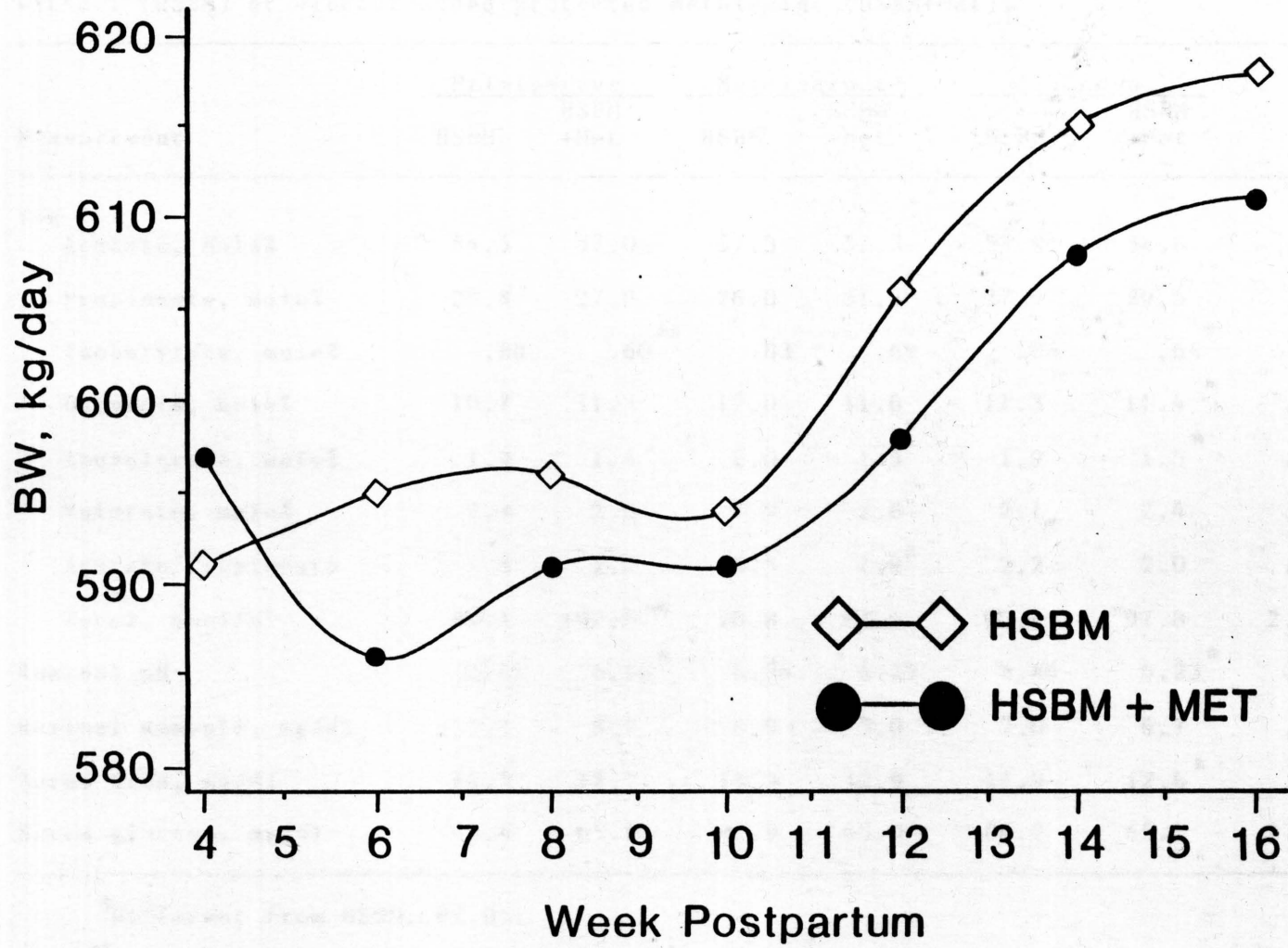Figure showing BW, kg/day versus Week Postpartum for HSBM and HSBM + MET.

Table 7. Volatile fatty acids (VFA), ammonia, and pH in ruminal fluid, and urea and glucose in serum in cows fed diet containing heat-treated soybean meal without (HSBM) or without added protected methionine (HSBM+Met).

| Measurement | Primiparous | | Multiparous | | All cows | | |
	HSBM	HSBM +Met	HSBM	HSBM +Met	HSBM	HSBM +Met	SE
VFA							
Acetate, mole%	54.5	57.0	57.3	52.3	55.9	54.6	.70
Propionate, mole%	29.8	27.8	26.0	31.2*	27.9	29.5	.77
Isobutyrate, mole%	.88	.60**	.83	.69	.86	.64*	.04
Butyrate, mole%	10.7	11.3	12.0	11.6	11.3	11.4	.32
Isovalerate, mole%	1.9	1.4	2.0	1.5	1.9	1.5*	.09
Valerate, mole%	2.3	2.0	1.9	2.8	2.1	2.4	.14
Acetate/Propionate	1.9	2.2	2.5	1.8*	2.2	2.0	.10
Total, umol/ml	89.4	107.6**	90.8	87.9	90.1	97.8	2.56
Ruminal pH	6.51	6.16*	6.38	6.29	6.44	6.23*	.04
Ruminal ammonia, mg/dl	7.2	8.4	6.9	5.0	7.0	6.7	.61
Serum urea, mg/dl	14.5	12.3	15.3	12.9	14.9	12.6*	.50
Serum glucose, mg/dl	69.6	65.5	62.9	65.0	66.2	65.2	1.13

*Different from HSBM, P<.05.

**Different from HSBM, P<.01.

animals receiving added methionine. Serum urea concentrations (Table 7) were lower (P<.05) after feeding HSBM+Met than after feeding HSBM ration (14.9 vs. 12.6 mg/100 ml serum). This may suggest that the protein in HSBM diet was degraded at a slower rate by the ruminal microorganisms when methionine was supplemented. The decreased serum urea levels may also represent decreased diffusion of ammonia through the rumen wall or improved microbial and peripheral nitrogen utilization. Increased VFA production for primiparous cows with methionine supplementation and similar ruminal ammonia concentration between treatments may imply increased microbial activities and fermentation but not growth. A possible mechanism in stimulating microbial activity may be explained that methionine was partially degraded in the rumen supplying more methionine for microorganisms compared to control cows.

Rumen pH was lower (P<.05) in methionine-supplemented cows. This was due to lower (P>.05) rumen ammonia concentrations together with higher VFA production in rumens after feeding HSBM+Met. A significant (P<.01) treatment by lactation number interaction on ruminal pH showed in primiparous cows in which lower ruminal pH reflected significantly higher total VFA concentrations. Ruminal pH of multiparous cows was similar between treatments.

Serum glucose concentrations were similar for the two treatment groups. It could be expected that overall energy effect on nitrogen metabolism was similar. Blood glucose must be maintained at 40-60 mg/dl to maintain normal function in many body tissues in the dairy cow (68). Serum glucose concentrations in cows of both treatments appeared adequate for normal body function. Similar distribution of fatty acids in milk fat and adequate concentrations of serum glucose (68, 81) could imply no deficiency in glucose supply nor potential incidence of ketosis between treatments although higher actual milk production and less body weight gains were found for cows fed HSBM+Met in early lactation (Table 4).

Amino acid contents of feeds and total ration are shown in Table 8. Methionine, histidine, threonine, isoleucine, and lysine were the first five limiting amino acids in the concentrate and also in total ration, when compared to the amino acid content of milk (63).

Concentrations of amino acids in arterial and venous serum, and A-V differences are given in Table 9. Concentrations of most amino acids were higher in tail arteries than in mammary veins. This was expected since most amino acids are utilized for milk protein synthesis by mammary glands. Individual amino acid concentrations in the serum were similar for both treatments. Total essential and nonessential amino acid concentrations in

Table 8. Amino acid content of concentrate mix, alfalfa hay, corn silage, and total ration.

Amino acid	Concentrate	Alfalfa hay	Corn silage	Ration[1]
	---------------------- (% of dry matter) ----------------------			
Arginine	1.11	.57	.26	.77
Histidine	.49	.29	.15	.36
Isoleucine	.77	.56	.29	.59
Leucine	1.76	.91	.74	1.34
Lysine	.91	.68	.23	.67
Methionine	.30	.21	.32	.29
Phenylalanine	.94	.70	.36	.73
Threonine	.65	.51	.28	.52
Valine	.95	.88	.47	.80
Total essential amino aicd	7.88	5.37	3.10	6.07
Alanine	.99	.71	.59	.83
Aspatic acied	1.75	2.96	.56	1.57
Half-cystine	.22	.12	.31	.23
Glutamic acid	3.41	1.34	1.13	2.42
Glycine	.73	.68	.33	.60
Proline	1.20	1.41	.52	1.03
Serine	.81	.56	.31	.62
Tyrosine	.70	.46	.40	.57
Total nonessential amino acid	9.81	8.24	4.15	7.88
Total amino acids[2]	17.69	13.61	7.25	13.95
Ammonia	.38	.64	.21	.37

[1]Calculated.

[2]Tryptophan not included.

Table 9. Concentration of amino acids in arterial and venous serum, and arteriovenous (A-V) difference in cow fed diets containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met).

Amino acids	Arterial serum			Venous serum			A-V difference		
	HSBM	HSBM +Met	SE	HSBM	HSBM +Met	SE	HSBM	HSBM +Met	SE
	--------------------------------- (umoles/dl) ----------------------------------								
Arginine	18.0	16.5	.49	11.3	11.8	.88	6.7	4.7	.85
Histidine	5.0	6.0	.30	4.4	4.6	.27	.6	1.4	.36
Isoleucine	12.8	12.9	.71	7.9	7.1	.77	4.9	5.8	.51
Leucine	19.1	20.3	1.26	11.2	11.2	1.26	7.8	9.1	.58
Lysine	7.8	8.2	.36	3.6	3.6	.32	4.2	4.6	.38
Methionine	2.0	2.2	.13	.6	.7	.06	1.4	1.5	.10
Phenylalanine	5.1	5.3	.20	2.4	2.7	.22	2.6	2.6	.16
Threonine	9.1	10.2	.49	5.8	6.5	.44	3.3	3.7	.31
Tryptophan	3.2	3.8	.34	2.3	2.8	.27	.9	1.0	.28
Valine	27.6	27.0	1.64	21.2	20.4	1.59	6.4	6.6	.50
Total essential amino acids	109.6	112.3	3.79	70.8	71.3	4.28	38.8	41.0	3.03
Alanine	23.7	24.4	1.21	19.0	20.7	1.30	4.7	3.7	.44
Asparagine	4.2	5.8[+]	.42	2.7	3.3	.31	1.4	2.5	.34
Aspartic acid	1.4	.9	.18	1.6	1.2	.16	- .2	- .3	.21
Half-cystine	.2	.2	.01	.2	.3	.04	.0	- .1	.04
Glutamic acid	9.4	8.5	.59	4.9	3.7	.26	4.5	4.8	.26
Glutamine	20.5	20.1	.94	15.8	14.4	.59	4.8	5.7	.59
Glycine	44.8	49.4	2.42	43.2	50.4	.90	1.6	- .9	.90
Proline	11.2	11.5	.50	9.4	9.4	.63	1.7	2.1	.32
Serine	11.9	13.0	.65	8.6	9.8	.61	3.2	3.3	.61
Tyrosine	4.6	5.4	.42	2.2	2.8	.16	5.4	2.7	.16
Citrulline[1]	9.4	8.2	.67	8.9	8.5	.68	.5	- .3	.23
Ornithine[1]	4.8	5.3	.44	2.9	2.6	.42	1.9	2.7	.27
Taurine[1]	8.0	6.2[+]	.67	7.4	6.5	.36	.6	- .3	.34
Total nonessential amino acid	131.8	139.5	3.22	107.7	116.0	3.54	24.1	23.6	2.21

[+] Different from HSBM, P<.1.

[1] Not included in total nonessential amino acid.

arterial serum were slightly higher in HSBM+Met cows, however these differences were not significant. Higher ratios of EAA to NEAA in arterial serum (.84 and .81) as compared to the results in (39, 97) indicated a more adequate protein supply from both treatments. Lower ratios of essential (EAA) to nonessential amino acid (NEAA) (.84 vs. .81 in artery and .66 vs. .61 in vein) in the venous serum for both groups indicated that relatively more EAA were taken up by mammary gland cells than NEAA, as expected. Similar observations were also reported by others(1, 39).

Concentrations of methionine in arterial and venous plasma were elevated slightly and mammary gland uptake of methionine was greater $(P>.1)$ when fed additional methionine. This slight but consistent increase in serum methionine concentrations indicated that some additional methionine was absorbed from the digestive tract. The increases in blood methionine were slightly less than the increases observed by Papas et al. (96, 97) when feeding methionine protected by a different mechanism. However, their plasma methionine concentrations were obtained from jugular blood. Based on the slight increase of methionine concentration in arterial serum for cows fed additional methionine compared to control cows and the blood flows assumed to be occurring, an estimated 5.2 g/day methionine may have been absorbed into blood. Thus, approximately 35%

of the supplemental DL-methionine was present in the form of plasma free methionine presented to the mammary gland. Losses of methionine due to mastication (96), partial degradation in the rumen, lower rate of release and absorption in gastrointestinal tract, and tissue uptakes may also account for other uses of the added methionine. The ratio of methionine to valine in blood has been considered as an indicator of increased blood methionine concentrations (18, 128), since valine undergoes negligible catabolism by the ruminal microbes and the liver. A higher ratio (7.32 vs. 8.23) in arterial serum for HSBM+Met cows may further indicate the effectiveness of protected methionine in elevating methionine level in the blood. However, this difference was not significant (P>.1).

The activity of serine-threonine dehydratase has been reported to increase with an increase in protein intake (4). Hepatic degradation was not increased to a significant extent since arterial threonine concentration tended to be higher with HSBM+Met feeding, although protein intake was higher (3.04 vs. 3.18 kg/day) (P<.05) for cows on HSBM+Met.

Arterio-venous differences were similar between treatments for all amino acids. Negative values for half-cystine and taurine (Table 9) concentrations may be related to higher uptake and metabolic rate (31) of methionine in mammary tissue with methionine

supplementation. Higher A-V differences of branched-chain amino acids and ornithine (Table 9), precursors of other NEAA synthesized in mammary glands (27, 30, 31, 83), were found in HSBM+Met cows. However, total A-V difference of NEAA in both treatments was similar (P>.1).

Higher (P>.05) milk (38.0 vs. 38.7 kg/day) and milk protein (1.05 vs. 1.10 kg/day) production in HSBM+Met cows during the week of arterial-venous blood sampling concided with higher (P>.1) A-V difference of total EAA. Higher A-V difference of ornithine (Table 9), a precursor of spermidine, and DNA (27, 31, 83), may be related to higher milk protein yield in this group of cows. The negative value of ammonia A-V difference (6.7 vs. -.7 umole/dl, data not shown) may be due to higher metabolic activities of amino acids, such as deamination, in the mammary gland (90).

The lack of a significant increase in arterial serum concentration of methionine when 50 g protected methionine product/day (ie. 15 g DL-methionine/day) was fed to cows in this study may be due partially to increased utilization of methionine for milk and milk protein production, since actual milk protein percent and milk protein yield were increased, and total milk yield was slightly higher for cows fed HSBM+Met (Table 3). However, different extent of responses were observed between primiparous and multiparous cows fed additional methionine as data adjusted for

pretreatment performance (Table 3). The maturity of animals may have played a part in partitioning utilization of additional methionine when supplemented with HSBM. Although responses to rumen-protected methionine in multiparous cows masked overall results, supplementation of this product tended to be effective in primiparous cows.

Although previous studies showed significant increases in plasma methionine concentration when feeding rumen-protected methionine at different levels (18, 23, 96, 97), their basal diets were different from the diets used in this study in which protected protein was used as protein supplement. Methionine may have been more limiting in their diets, even though methionine was still the most limiting amino acid in the insoluble or slowly degradable portion of soybean protein (1, 109, 113) or in the total ration (Table 8).

Feeding protected methionine did not alter ($P > .1$) the plasma concentrations of any other amino acids. This also indicates that supplying this quantity of methionine did not decrease absorption of other amino acids, which agreed with observations of others (18, 23, 96, 97) when similar types of rumen-protected methionine were supplemented.

Data of calculated uptake (A-V difference times daily blood flow and output of amino acids in milk protein by the mammary gland are listed in Table 10. Calculated estimate of blood flow as described in (39, 68) was 645 liters/kg

Table 10. Uptake and output of amino acids by the mammary gland, and ratio of uptake to output in cows fed diets containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met).

Amino acids	Uptake[1]			Output			Uptake/Output		
	HSBM	HSBM +Met	SE	HSBM	HSBM +Met	SE	HSBM	HSBM +Met	SE
	------------------ (g/day) ----------------								
Arginine	188.9	140.5	25.2	33.6	36.2	2.5	5.63(10)[2]	4.03(10)	.68
Histidine	24.3	37.2	5.9	25.9	27.9	1.2	.92(1)	1.39(1)	.22
Isoleucine	102.2	135.2	13.0	54.6	58.8	2.4	1.89(7)	2.30(7)	.18
Leucine	166.0	211.1	13.0	93.2	100.4	4.2	1.79(6)	2.11(7)	.11
Lysine	98.7	118.0	9.4	77.8	83.8	3.5	1.27(2)	1.41(2)	.09
Methionine	33.1	39.0	2.7	25.0	26.9	1.1	1.31(3)	1.46(3)	.08
Phenylalalanine	69.7	76.0	3.8	47.1	50.8	2.1	1.51(5)	1.50(4)	.08
Threonine	63.4	77.4	5.6	44.2	47.7	2.0	1.46(4)	1.63(5)	.12
Tryptophan	29.5	36.3	9.4	13.4	14.4	.6	2.24(9)	2.64(9)	.71
Valine	120.7	134.2	9.2	63.4	68.3	2.8	1.92(8)	1.99(6)	.13
Total essential amino acids	897.6	1004.9	65.9	478.4	515.3	21.3	1.89	1.97	.13
Alanine	67.5	57.6	6.6	32.7	35.2	1.4	2.08	1.64	.18
Aspartic acid	− 6.5	− 5.1	4.7	75.9	81.8	3.4	− .06	− .08	.06
Half-cystine	.1	− 1.5	.8	8.2	8.8	.4	.01	− 2.0	.10
Glutamic acid	108.7	123.8	5.5	210.4	226.6	9.4	.52	.56	.03
Glycine	17.4	− 15.6	12.6	19.2	20.7	.8	1.05	− .58	.59
Proline	32.1	40.8	5.7	95.2	102.5	4.2	.35	.41	.06
Serine	52.5	62.4	11.7	53.8	57.9	2.4	1.01	1.05	.20
Tyrosine	69.7	86.1	5.1	49.0	52.7	2.2	1.43	1.64	.08
Total nonessential amino acids	341.5	348.6	28.3	544.3	586.3	24.2	.64	.60	.05
Ornithine	39.6	63.1	6.5						

[1] Calculated as A-V difference mutiplied by estimated blood flow per day.

[2] Numbers in parentheses indicate apparent sequence of limiting amino acids.

milk produced, corrected for an average packed cell volume of 30.5% (99). This generated a serum flow to milk yield ratio 450:1.

Ratios of uptake to output for most EAA were greater than unity (1.0) indicating EAA were taken up in adequate or excessive amounts relative to outputs in milk protein. Uptakes of histidine, lysine, and methionine were very close to output in milk protein, and uptakes of phenylalanine and threonine only slightly in excess of output in milk protein, similar to other studies (38, 118). The mean ratio for histidine for cows fed HSBM was less than 1.0 because of a lower A-V difference for this amino acid but still within one standard deviation of 1.0. Negative A-V differences (data not shown) of histidine were observed from 2 cows fed HSBM. Results of study by Spires et al. (118) also showed a similar value.

Ratios of uptake to output of branched-chain amino acids by the mammary gland were subtantially greater than unity as also observed in other studies (33, 38, 118). Isoleucine and valine may be oxidized or converted to NEAA (27, 31, 83). Uptake of arginine was greatly in excess of secreted in milk protein as in previous studies (31, 34, 39, 40, 137). Arginine was reported to serve as precursor for the synthesis of NEAA (27, 30, 31, 83).

The uptake of NEAA showed a great deal of variation as reported in (33, 38, 118). Uptakes of glutamic acid and

proline were not in adequate supply as required for milk protein synthesis. Halfpenny et al. (51) suggested that glutamic acid and proline were limiting for milk protein synthesis. Although these amino acids can be synthesized in the mammary gland (30, 75), they may become limiting if mammary gland extraction and de novo synthesis fail to meet requirements for these amino acids (27).

Data on the ratio of uptake of amino acids by the mammary gland to their output in the milk protein indicated that histidine, methionine, lysine, phenylalanine, and threonine were the five EAA used most completely by the mammary gland for milk protein synthesis. Similar conclusions were stated in (33, 118). Data were similar for cows fed HSBM and HSBM+Met.

Extraction percentages of amino acids from arterial plasma by mammary gland were not different between treatments as shown in Table 11. Results were similar to those reported by others (33, 38). Methionine was extracted at the highest percentage in both treatments with lysine and phenylalanine ranked second and third, respectively. It has been suggested that EAA which show lowest concentration in the arterial plasma coupled with a high percent extraction are the ones which are most likely to be limiting for milk synthesis (38). On this basis, methionine was the first limiting amino acid for milk production in both groups, even when fed supplemental

Table 11. Extraction percentages and transfer efficiencies of serum essential amino acids in cows fed diet containing heat-treated soybean meal without (HSBM) or with added protected methionine (HSBM+Met).

Amino acid	Extraction[1]				Transfer efficiency[2]			
	HSBM	HSBM +Met		SE	HSBM	HSBM +Met		SE
				(%)				
Arginine	36.9 (7)[3]	29.3	(7)	4.6	6.6 (10)	7.1	(10)	.2
Histidine	10.1 (10)	21.8	(10)	7.0	20.8	(8)	17.4 (7)	1.0
Isoleucine	39.4 (5)	45.2	(5)	4.1	20.8	(6)	19.7 (6)	1.1
Leucine	42.9 (4)	45.2	(4)	3.3	24.2	(5)	21.4 (5)	1.5
Lysine	53.4 (2)	56.0	(2)	3.6	42.4	(2)	40.2 (2)	1.3
Methionine	68.8 (1)	66.9	(1)	2.4	54.1	(1)	46.7 (1)	2.6
Phenylalanine	52.5 (3)	49.2	(3)	2.9	35.2	(3)	32.7 (3)	1.4
Threonine	37.1 (6)	36.4	(6)	3.0	25.0	(4)	22.9 (4)	1.0
Tryptophan	13.4 (9)	25.3	(8)	12.4	18.9	(7)	10.6 (9)	4.5
Valine	24.0 (8)	24.2	(9)	2.1	12.5	(9)	12.3 (8)	.7
Alanine	20.7	15.1		2.2	9.8		9.1	.4
Aspartic acid	− 32.9	− 72.5		21.3	280.7		522.1	62.8
Half-cystine	.8	− 35.4		18.4	172.9		187.0	4.5
Glutamic acid	49.6	56.3		2.7	97.0		102.1	4.9
Glycine	3.3	− 1.6		1.9	3.6		3.2	.2
Proline	16.3	18.2		2.7	46.2		43.6	1.7
Serine	26.0	25.2		5.2	27.3		23.9	1.4
Tyrosine	56.1	49.7		4.3	39.5		30.4	2.9

[1] A-V difference/arterial concentration *100.

[2]
$$\frac{\text{Amino acid output in milk (g/day)} * 100}{\text{Arterial serum amino acid (g/day)} * \text{serum flow (liter/day)}}$$

[3] Numbers in parenthesis indicate apparent limiting order.

methionine.

Transfer efficiencies are also shown in Table 11. If it is assumed that amino acids utilized most efficiently are those limiting production (39), then methionine, lysine, phenylalanine, threonine, and leucine were the first five limiting amino acids in both treatments. Tyrosine would be third-limiting in HSBM and fourth-limiting in HSBM+Met, if considered an EAA. A lower (P>.1) transfer efficiency of methionine when fed HSBM than when fed HSBM+Met may be due to higher arterial concentration, which resulted from the methionine supplementation, and were more adequate after its supplementation.

Although methionine, lysine, and threonine were the first three limiting amino acids for milk protein synthesis as determined by these two methods (ie. extraction percentages and transfer efficiencies), intercellular utilization of amino acids in mammary gland could not be determined in this study. Amino acid may be utilized for purposes other than direct incorporation into milk protein (31, 83). Under such conditions, an amino acid may become limiting for milk synthesis if a sufficient quantity is not extracted by the mammary cells.

Ratios of uptake of amino acids by mammary glands to their output in milk protein may give more meaningful data in determining the sequence of limiting amino acid. Both

blood flow to mammary glands and A-V difference are considered in this technique. However, hourly blood flow to mammary glands, extraction process of individual amino acid, and fate of amino acid in mammary cells may be considerably variable in individual animal (31, 83).

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