# THE EFFECT OF METHIONINE AND METHIONINE HYDROXY ANALOG ON VARIOUS METABOLIC PROCESSES IN THE LACTATING BOVINE

by

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I hereby recommend that this dissertation prepared under my direction by FRANK M. WHITING

entitled THE EFFECT OF METHIONINE AND METHIONINE HYDROXY ANALOG ON VARIOUS METABOLIC PROCESSES IN THE LACTATING BOVINE

be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY

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After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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## ABSTRACT

The effect of supplementing the diet of lactating dairy cows with methionine on methionine hydroxy analog (M-analog) was studied by feeding four experimental concentrates: A. basal; B. basal plus 0.11% DL methionine; C. basal plus 0.11% M-analog and D. basal plus 0.22% M-analog. All experimental concentrates were refused to some extent by the cows during the first feeding period; however, during the second and third periods there was no refusal problem. Free threonine was increased in rumen fluid by supplementation with the higher level of M-analog. No other free amino acids in rumen fluid were influenced by any of the experimental concentrates. The feeding of methionine or M-analog caused no changes in proportions of free amino acids of blood plasma. Methionine and the lower level of M-analog supplementation increased the aspartic acid and valine levels in milk free amino acids.

There appeared to be a close relationship between the levels of free lysine, glutamic acid, arginine, proline, tyrosine and phenylalanine in rumen fluid and in milk. The levels of the other free amino acids in rumen fluid and milk did not appear to be related. There was no apparent relationship between the proportions of free amino acids in blood with those in rumen fluid or in milk.

The experimental concentrates had no effect on the free amino acid content of feces. There did not appear to be any significant relationship between free fecal amino acids and the free amino acid content of rumen fluid, blood plasma or milk.

Supplementation with methionine or M-analog had no significant effect on rumen volatile fatty acids or the fatty acids of blood serum cholesterol esters. The proportion of 18:iso in serum triglycerides was raised by supplementation with methionine and depressed by the lower level of M-analog. Both levels of M-analog supplementation depressed the level of 15:0 in serum free fatty acids while methionine and the higher level of M-analog increased 22:5. A decrease in 18:4 in serum phospho lipids resulted from supplementation with methionine or the higher level of M-analog. The higher level of M-analog depressed the 15:0 and 16:2 fractions of milk fat. All significant effects were found in fatty acids which were present in very small amounts and whose biological significance has never been established. There were no significant changes in the total production or composition of milk, body weights, or the digestibility of feed.

# INTRODUCTION

It is well known that the ruminant animal, because of a symbiotic relationship with rumen microflora, can survive under comparatively marginal feed conditions. The major reason for this is that the ruminant can utilize cellulose whereas monogastric animals cannot. By the same token, any high grade protein fed to a ruminant is essentially wasted, as the microflora of the rumen can completely hydrolyze protein to ammonia and carbon chain compounds. The feeding of compounds which can escape the degradation of rumen microbes could appreciably increase the nutritional state of the animal, thus increasing its productive output.

Methionine is presently thought to be the amino acid most often limiting the metabolic processes of the ruminant. Degradation of methionine in the rumen and sulfur-deficient diets are both possible causes of this condition. Reports in the literature indicate that methionine administered in ways that bypass the rumen or its action cause increased milk or wool production. There are also indications that a metabolic deficiency of methionine in the lactating bovine may depress levels of linoleic acid in blood serum and result in depression of milk production.

Methionine hydroxy analog (M-analog) is presently marketed as a livestock feed supplement as a possible source of methionine for the

animal. It is theorized that the rumen pH prevents the degradation of M-analog by rumen microorganisms. It is then absorbed from the abomasum and subsequently aminated in the liver to form methionine. If, in fact, methionine is the limiting amino acid in ruminant metabolism, and the M-analog can be substituted for methionine, the importance of M-analog to a protein-deficient world would be highly significant.

This experiment was designed to study the effect of supplementing the diet of a lactating bovine with methionine or M-analog. Parameters studied were free amino acid composition of rumen fluid, blood plasma, milk and feces; volatile fatty acid (VFA) proportions in rumen fluid; fatty acid proportions in blood serum cholesterol esters, triglycerides, free fatty acids and phospholipids; fatty acid proportions in milk; milk production and gross composition; body weights; and digestibility of ration.

## REVIEW OF LITERATURE

The following literature was reviewed in an attempt to elucidate previous knowledge of the methionine requirement for mammals in general, but particularly as it applies to the ruminant during lactation. Some attempt was made to separate the nutrition of the rumen microflora from that of the host. It is recognized that this symbiotic relationship cannot, in reality, be separated but that if the care and feeding of both are better understood, a more efficient overall biological unit might result. Select references to non-ruminant methionine metabolism are presented but no attempt was made to exhaust the field due to extensive confirming literature in the area.

As early as 1946, it was thought that methionine might be intimately involved in ruminant metabolism. At that time Shaw (59) unsuccessfully attempted to use methionine to treat bovine ketosis. In contrast McCarthy (35) and McCarthy et al. (36) at a much later date have shown that methionine introduced into the animal by intravenous (I-V) feeding not only helped the ketotic cow, but raised the butter-fat content in the milk of nonketotic cows. Since in lactation there is a considerable requirement for methionine as a methyl donor for the synthesis of milk protein, it is readily seen how stress conditions can be brought about by a slightly marginal nutritional situation. Methionine

was believed to alleviate the stress conditions of ketosis by increasing lipoprotein production.

Russian workers, Kugenev and Razmakhnin (24) attribute the feeding of limited amounts of methionine and methionine plus lysine to minor changes in milk production and ammonia, VFA and nitrogen content of rumen fluid. However, when methionine was infused into lactating dairy cows by others (17, 68) no changes in milk composition or production was detected.

Patton et al. (45) found that lipid synthesis by rumen microorganisms was greatly increased when rumen fluid was incubated with methionine; but, when L-methionine methyl-$C^{14}$ was used no label was transferred to rumen lipids or phospholipids. Methionine apparently performed a specific function in the synthesis of rumen lipids but was not a methyl donor. In another study Patton et al. (46) demonstrated that M-analog fed to cows at 80 g/day in the grain ration resulted in a general stimulation of fatty acid synthesis. Additions of the analog to the ration resulted in an increase in lipid from both protozoal and bacterial sources.

Griel et al. (19) in 1968 fed M-analog to dairy cows in an attempt to improve production. It was shown that 40 g of this compound fed daily increased production while 80 g of the analog was rejected by some of the animals, presumably because of palatability.

The feeding of M-analog to cows from 14 days prepartum to 90 days postpartum enabled Polan et al. (49) to increase milk production. Blood serum of the cows fed the analog showed lowered levels of free fatty acids and triglycerides in the $\alpha$-lipoprotein fraction.

The changes in rumen fluid, blood serum and milk of cows fed M-analog was measured by Patton et al. (47) who found that cows fed 40 or 80 g/day of the analog produced higher amounts of milk containing increased levels of the 18-carbon fatty acids, but there were reduced levels of VFA in the rumen fluid. Feeding the analog also increased total serum lipids, but did not alter the fractions. The analog was apparently responsible for the formation of an unidentified polar serum lipis which was produced at the expense of stearic acid.

The rumen was bypassed by feeding an encapsulated form of methionine to lactating cows by Williams et al. (75). There was no significant benefit shown in 4% fat-corrected milk and no changes were noted in urinary urea, nitrogen, creatinine or amino acids. Blood ammonia and plasma amino acids were also not altered.

Work by Broderick et al. (4) compared the effect of encapsulated methionine and abomasaly introduced casein on milk production. These workers showed that methionine encapsulated with kaolin and tristearin produced no significant change in milk production, however, plasma free methionine and the methionine:valine ratio increased. When these

workers put sodium caseinate and methionine directly into an abomasal fistula, milk protein and total milk production increased, while voluntary grain consumption dropped. Plasma free amino acids were altered as follows: non-essential amino acids decreased, while isoleucine, leucine, valine and phenylalanine increased.

When Hereford steers were fed lysine with or without methionine, Gosset et al. (18) reported no beneficial effects on a protein supplemented diet. The addition of methionine to a basal diet improved weight gain slightly, but feeding 5 g of M-analog did not increase gain and 10 g decreased gain.

In another feeding trial using steers, Oltjen and Putman (44) measured the effect of feeding urea or soy protein purified diets on plasma free amino acid (PFAA) concentration and nitrogen retention. On the urea diet, the PFAA levels of valine, isoleucine and leucine were lowered. They postulated that this was a result of an insufficiency of branched-chain VFA in the rumen.

When lambs were fed urea as the sole source of supplemental nitrogen (urea being 85% of the total dietary nitrogen) McLaren et al. (37) found that methionine or tryptophan increased the retention of absorbed nitrogen. When both amino acids were fed, no synergistic effect resulted.

As early as 1954, considerable interest was shown in attempting to increase animal production by supplemental feeding of sulfur.

Starks et al. (63) experimented with lambs on a low sulfur diet and showed that both elemental sulfur and sodium sulfate gave an increase in weight gain and wool growth.

The increased use of non-protein nitrogen as a source of nitrogen for ruminants has increased the possibility of a sulfur deficiency in these animals. Indications are that it is important to keep the nitrogen-sulfur ratio no wider than 10:1, if the most efficient utilization of nitrogen is to be achieved (38).

Jacobs et al. (22) found that feeding elemental sulfur or sodium sulfate significantly increases ad libitum feed intake and milk production over a basal sulfur deficient diet. Conrad et al. (11) fed sodium or barium sulfide-$S^{35}$ and found that the methionine in the whole organism acted as a single pool which underwent a simple dilution. In a later publication Conrad et al. (10) found that the levels of dry matter and nitrogen ingested by the cow had the most significant effect on rumen synthesis of essential amino acids. These researchers showed that reducted nitrogen absorption in the digestive tract was paralleled by markedly decreased methionine production.

Whanger (74) has shown that the addition of the common chemical sodium sulfate to a purified diet, in which urea was the sole nitrogen source, will alleviate sulfur deficiency symptoms. Rumen microorganisms from sulfur supplemented animals were predominately gram positive, while

those from the sulfur deficient diet were mostly gram negative and were found in appreciably decreased numbers. Evaluation of the rumen fluid from both treatments showed no difference between the sulfur and the non-sulfur diets, other than butyric and higher acids were two to three times higher in the sulfur-supplemented ration. In vivo incubation of the sulfur-adequate organisms with lactate or glucose (or both) yielded more butyrate and valerate than rumen fluid from the sulfur-inadequate diet.

The effect of supplementation on nitrogen utilization and sulfur balance by infusing methionine or sulfur into an abomasal fistula, thereby bypassing the action of the rumen microflora was studied by Steinacker et al. (65). Their efforts resulted in a low response as compared to the same compounds fed in a similar quantity to control animals. This lack of response was credited to one of two facts: either the methionine was not a limiting amino acid in this particular feeding trial or the infused methionine was in too high a concentration causing an amino acid imbalance, which, in turn, abrogated increased nitrogen retention.

Reis and Schinckel (55) found that a continuous daily infusion of cystine or methionine into the abomasum increased wool growth. When casein was fed, wool production nearly doubled. These workers concluded that any protein which can be made available for absorption by the sheep will specifically stimulate wool growth and that availability of sulfur containing amino acids may be especially important for this purpose.

In another study these same men (56) examined the effect of abomasal infusion of casein, gelatin and the sulfur containing amino acids. As before, the sulfur containing amino acids and casein promoted wool growth and increased sulfur deposition, but gelatin gave no response. The increased production caused by casein was credited to the sulfur containing amino acids found in that protein.

Little and Mitchell (30) observed that soybean protein infused into the abomasum did not affect the total digestibility of the diet, but that nitrogen retention was increased over trials by oral feeding. The apparent digestibility of protein, dry matter, and cellulose was not significantly affected by either method of administering casein or gelatin but retention of nitrogen increased when entry was by way of an abomasal fistula.

It was reported by Reis (51) that sheep fed methionine or cystine by abomasum had a greatly increased wool production. The feeding of both amino acids at a level of 6 to 8 g daily resulted in reduced wool growth as compared to sheep fed no supplement, yet the sulfur content of the wool was not reduced. It was also determined that M-analog was as effective in promoting wool growth as was DL-methionine when introduced beyond the rumen. D-methionine was less effective than DL-methionine when measured by wool growth.

Ferguson et al. (16) treated casein with a formaldehyde solution in order to shield the casein from the degradative action of the rumen.

Casein thus treated caused no noticeable increase in rumen ammonia concentration in vivo. When wethers were fed 80 g of treated casein/day wool production increased 70%. Reis and Schinckel (55) showed similar results when casein was fed via the abomasum. In another study Reis (52) determined that feeding casein had only a slight effect on wool growth. Casein infused into the abomasum not only increased wool production but also significantly increased the body weight of the sheep. This study further proved that sheep will produce wool at a rate which probably approaches their genetic potential on a relatively low energy ration provided there is ample high quality protein available for digestion and absorption from the abomasum and small intestine.

The young ruminant's digestive system is not functional until it is several weeks of age, therefore, it can be considered in relatively the same light as a monogastric until microbial action begins. Reis (54) undertook the study of the effect of ingested dietary protein and methionine on the sulfur content and growth rate of wool from infant lambs. When results from this trial were compared with adults a direct relationship between the amount of intestinally digested protein and the growth rate of wool was indicated.

Broad et al. (3) have attempted to quantitate the relationship between the sulfur content of wool and high sulfur proteins by comparing the effects of cysteine, methionine and M-analog on these proteins.

The individual variation between sheep showed that M-analog was significantly less responsive in stimulating high sulfur proteins than was cysteine or methionine. It was further observed that a linear relationship between the sulfur content of wool and high-sulfur proteins existed.

The possibility that sulfur containing compounds other than amino acids might be capable of promoting wool growth has also been explored by Reis (53). Supplements of cysteamine and sulfuric acid caused no increase in wool growth, while M-analog almost doubled wool growth rate. This worker also investigated the possibility that several non-sulfur amino acids might enhance wool production. It was found that glycine had little effect on wool growth when added to a casein diet, but that an abomasal supplement of cysteine augmented wool yield. Abomasal supplements of glycine and/or glutamic acid failed to influence wool growth, corrobrating the work by Reis and Schinckel (55) which revealed that a supplement of non-essential amino acids had no effect on wool development.

When M-analog was added to a basal diet, wool growth was not appreciably increased (53), suggesting that M-analog, like methionine, is degraded in the rumen and is not advantageous as a dietary supplement for wool production. It was also shown in this same report that infusion of arginine, lysine or threonine had no effect on wool development, while methionine supplementation alone improved wool growth.

Downes et al. (12) found that both intravenous (I-V) and intraperitoneal introduction of cysteine and methionine increased wool fiber diameter and length. Subcutaneous injection of L-cystine-$S^{35}$ was absorbed by the animal rapidly enough to stimulate wool growth, but was discontinued because of the discomfort of the animals.

Nimrick et al. (41) measured the effect of supplemental amino acids on lambs fed a semi-purified ration in which urea was the only source of nitrogen. Methionine infused into the abomasum increased nitrogen retention, while lysine gave a positive response only after methionine was provided. Threonine caused no response unless introduced in conjunction with methionine and lysine suggesting that, on this dietary regime for growing lambs, the limiting order of these amino acids is first methionine, then lysine and finally threonine. In a later publication these same authors (42) expanded their work to quantitate their previous qualitative efforts. They found when glutamic acid, methionine, lysine-HCl and threonine were abomasaly supplemented at levels of 0.40, 0.10, 0.16 and 0.19% respectively of the basal diet, nitrogen retention was at a maximum level.

Leibholz (26) reported PFAA levels in sheep were much lower than previously published levels for monogastric animals and that PFAA increased two to ten times after feeding while similar free amino acids in the rumen increased in concentration up to one hundred times.

It was demonstrated by Verbeke and Peeters (72) that after passing through a lactating bovine mammary gland most of the PFAA of the blood decrease in concentration. Theurer et al. (69) compared PFAA levels in portal and jugular blood of lambs and reported that any change in jugular plasma was greater than in portal plasma.

Lambs were used by Schelling and Hatfield (57) to determine the effect of dietary protein levels and amino acid supplementation on PFAA. Plasma methionine decreased when dietary protein levels were increased. While most of the other PFAA were increased, the concentrations of lysine and histidine remained relatively constant. Dietary supplentation of lysine or methionine did not increase these compounds in the plasma. The more recent report of Theurer et al. (70) indicated that the source of nitrogen caused differences both in the performance of the animal and in PFAA patterns. The difference in the PFAA picture assumably affects tissue metabolism and consequently animal performance.

The fact that changes in dietary intake can affect PFAA levels was borne out by Hogan et al. (21) who found that the variation of PFAA caused by ration change was greater in the area of the essential amino acids than in the non-essential. A particularly interesting aspect of this study was that the hydrolyzed digesta from a sheep's rumen was almost identical in amino acid composition to hydrolozyed casein. The effect of different sources of nitrogen on PFAA patterns has also been

investigated by Little et al. (29), who found that in steers the amino acids available for absorption vary significantly with the dietary source.

Linton et al. (31) fed encapsulated methionine to bypass rumen action and found a marked increase in plasma free methionine. These authors stated that if methionine had been the first limiting amino acid, the first supplemental increment would have been immediately absorbed and utilized for tissue synthesis and would not have been reflected in an increase in PFAA.

Although there is little evidence indicating that the absorption or tissue requirements and utilization of amino acids is different among most animals, the fashion in which the amino acids are presented does vary greatly. Scott et al. (58) have shown that percent plasma lysine more than doubled when lysine was administered via abomasal fistual rather than orally. Williams (76) reported that in sheep the proportions of amino acids in the portal blood in different subjects was not necessarily the same although the amino acids in the intestine available for absorption were in similar concentrations.

By one month of age, most cud-chewing animals are considered to have a functioning rumen. However, Oltjen et al. (43) noticed a marked blood glucose decrease between 42 and 84 days of age with calves, indicating that the calves were still at least partially functioning as monogastrics at the earlier age. During this experiment, when

PFAA from animals on purified diets were compared to those from natural diets there were significant differences at 84 days of age and again at 210 days. The levels of the so-called "essential amino acids" were markedly depressed in calves fed the urea purified diet when compared with calves fed diets containing protein. Hair amino acids and PFAA determinations (at 210 days of age from the animals on the non-protein nitrogen diet) resembled those of African Kwashiorkor subjects.

Leibholz (27) found that within one hour of feeding there was a significant increase in free amino nitrogen and ammonia in the rumen fluid of wethers. There also was a positive correlation between the concentration of these variables in the rumen and in the dietary protein intake. There was no correlation between dietary amino acids and PFAA found in the bloodstream. In another study by this same author (28), the concentration of PFAA and nitrogen metabolism were examined under the conditions of starvation and low nitrogen intake. In plasma of sheep on low nitrogen diets the ratio of essential to non-essential amino acids decreased with a significant increase in glutamic acid, glutamine, glycine, isoleucine, leucine and 3-methylhistidine. The plasma of starved sheep had significantly decreased values in serine, glutamine, glycine, alanine, histidine and arginine and the ratio of essential to non-essential amino acids increased.

Nimrick et al. (41) by infusing urea, methionine or lysine into the abomasum reported that methionine was the first limiting amino acid

when urea was a major source of nitrogen. Concurrent infusions of lysine or methionine with glutamic acid showed that methionine caused a positive response to nitrogen retention over glutamic acid, but lysine caused no response. These results indicated that methionine was again specifically limiting to nitrogen retention. Subsequent work by these same authors (42) showed that there was a relationship between nitrogen retention, free plasma methionine and the level of supplemental methionine.

In the simple-stomached animal amino acid degradation by microbial action is not present or, if present, as in the case of the horse and some rodents, takes place primarily in the caecum. This outpouching of the large intestine is far enough down the intestinal tract so that most amino acids have already been absorbed. Even though there can be no extensive amino acid destruction in the monogastric individual, there is a greater possibility of an amino acid imbalance or deficiency occurring because there is no mechanism present by which amino acid synthesis can take place.

Many researchers have conducted experiments using monogastrics in which factors affecting PFAA levels were studied. Representative of these published reports are Kumta and Harper (25) and Knipfel et al. (23) who used rats, Shao and Hill (61, 62) and Taylor et al. (67) who used chickens, Braude et al. (2) and Grimble and Whitehead (20) who used

swine and finally Nasset and Ju (39) and Swendseid et al. (66) who experimented with man. In general they all reported that diet did not alter PFAA patterns but that PFAA levels influenced appetite. PFAA concentrations reflected the balance between entry of amino acids into the blood both from the digestive system and tissue protein breakdown, and exit of amino acids from the blood to the tissues for synthesis. Amino acid imbalanced diets caused plasma buildup of amino acids present in the diet in adequate amounts because a deficiency of one essential amino acid prevented the utilization of other essential amino acids. The efficiency of utilization of the most limiting amino acid in protein synthesis was increased, while that of the non-limiting indispensable amino acid was decreased as a result of ingesting an imbalanced amino acid diet. These findings were in agreement with those reported from similar experiments with ruminants.

## EXPERIMENTAL PROCEDURES

Twelve Holstein cows were selected from the University of Arizona milking herds for nearness to peak of lactation and high production. The cows were randomly assigned to treatment sequence using Lucas' (32) experimental periods of four weeks each with a one week changeover between intervals.

Four experimental concentrates (Table 1) were formulated to provide the cows with either a basal diet, the basal diet plus 40 g of methionine, the basal diet plus 40 g of M-analog or the basal diet plus 80 g of M-analog per day.

Methionine hydroxy analog is the calcium salt of DL-α-hydroxy, γ-methyl-mercaptobutyric acid (1), with the following molecular structure:

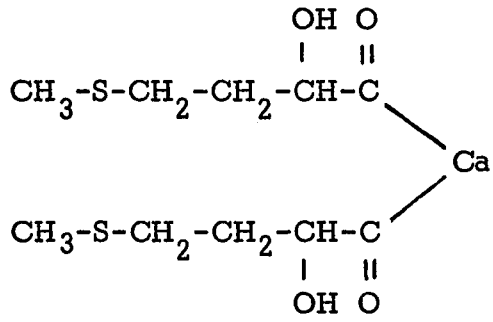

Table 1. Composition of experimental concentrates.

| Ingredient | Type of concentrate: Basal | Basal + 0.11% methionine[1] | Basal + 0.11% M-analog[2] | Basal + 0.22% M-analog[2] |
|---|---|---|---|---|
| | (%) | | | |
| Milo | 35.0 | 35.0 | 35.0 | 35.0 |
| Barley | 18.0 | 18.0 | 18.0 | 18.0 |
| Beet pulp | 20.0 | 20.0 | 20.0 | 20.0 |
| Cottonseed meal | 15.0 | 15.0 | 15.0 | 15.0 |
| Molasses | 7.0 | 7.0 | 7.0 | 7.0 |
| Tallow | 2.0 | 2.0 | 2.0 | 2.0 |
| Mono-sodium phosphate | 1.0 | 1.0 | 1.0 | 1.0 |
| Trace minerals | 1.0 | 1.0 | 1.0 | 1.0 |
| Salt | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin A palmitate[3] (5,500 International Units/kg) | | | | |

[1] Fisher Scientific Co., Chicago, Illinois

[2] E. I. duPont de Nemours & Co.

[3] Pfizer Agricultural Division, New York, NewYork

Methionine hydroxy analog calcium is produced synthetically and marketed under the trade names of Hydan[1] and MHA.[2] Amination of methionine hydroxy analog is thought to take place in the presence of an enzyme(s) containing vitamin $B_6$ (9). The method of converting DL-methionine hydroxy analog to L-methionine as postulated by Fasella and reported by Bishop (1), is shown in Figure 1.

According to Burroughs and Trenkle (9) there are several steps in the utilization of M-analog by the ruminant:

1. Rumen pH conditions are such that microbial degradation of M-analog is either minimal, or non-existent.
2. The lower pH of the abomasum allows M-analog to be absorbed easily into the portal blood system as an altered acid for transport to the liver.
3. Hepatic action transforms altered M-analog acid into methionine. Methionine so gained serves as an additional amino acid source above that supplied to the animal under more common feeding practices.

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1. Hydan is the trade name of methionine hydroxy analog calcium produced as a 90% purity product by the E. I, duPont de Nemours Co., Wilmington, Delaware.

2. MHA is the trade name of methionine hydroxy analog calcium produced as a 90% purity product by the Monsanto Chemical Co., St. Louis, Missouri.

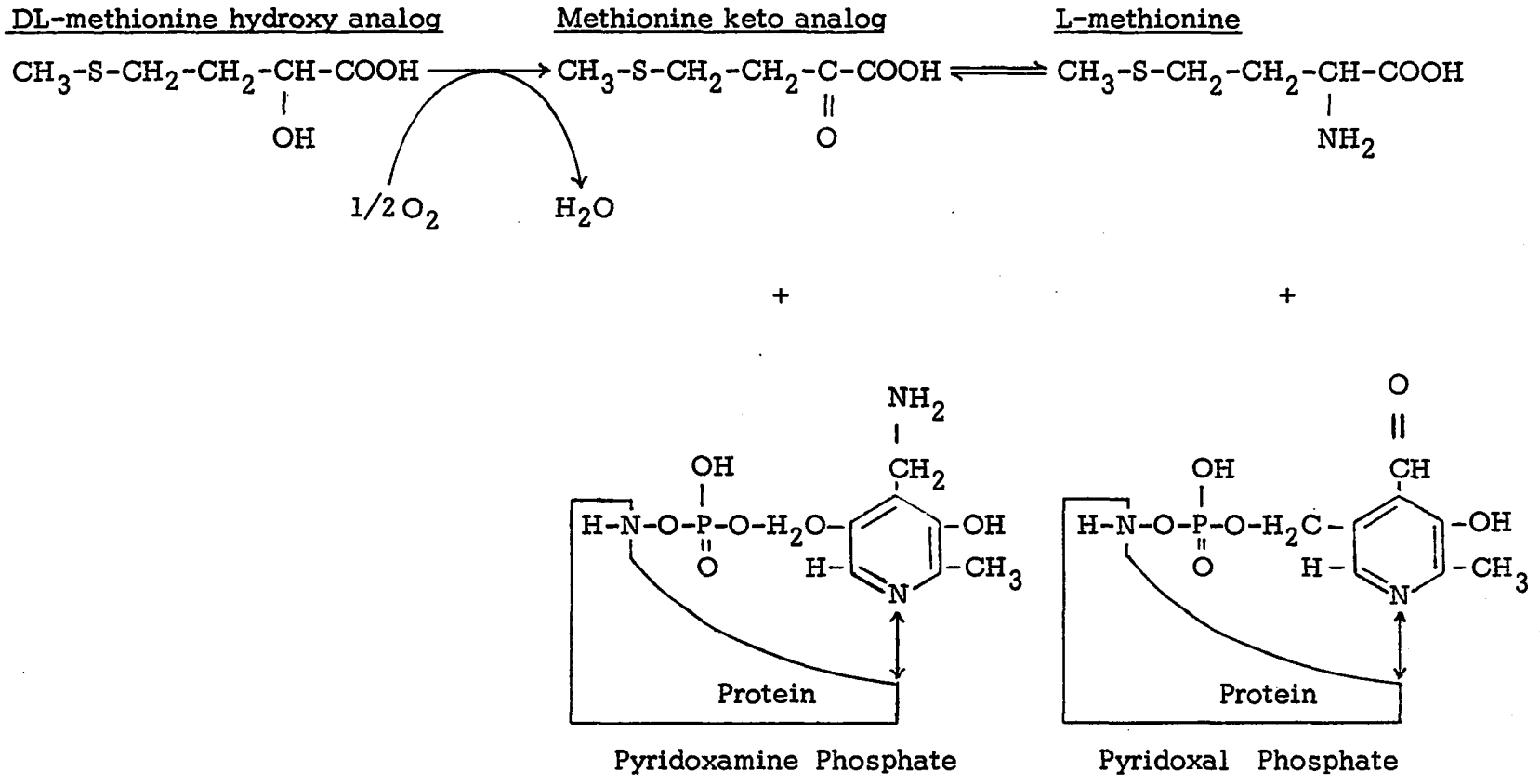


Figure 1. Metabolic pathway of conversion of DL-methionine hydroxy analog to L-methionine as postulated by Fasella and reported by Bishop (1).

The quantity of added methionine can be controlled by feeding the correct amount of M-analog in the ration. This is relatively important as excess methionine in the liver is probably as detrimental to protein production as is insufficient methionine (9).

Each cow was fed individually according to the National Research Council's standards for maintenance and production (40). In addition to high-quality, cubed alfalfa, each cow received one of the four experimental concentrates presented in Table 1. The experimental concentrate made up 45% of the diet by weight, with the remainder being alfalfa. "Hydan" was the M-analog fed in this study.

The experimental concentrates were fed on an individual basis in the milking parlor. Unconsumed feed was weighed and recorded prior to being discarded (Table 2). After each milking (twice daily) the animals were locked into a stanchion and individually fed alfalfa cubes. In contrast to the concentrate feeding there was no refusal problem involved with the roughage, consequently, no weighback records were kept.

Composite milk samples for individual cows were taken from four consecutive milkings at the end of each week. Milk weights were recorded and averaged weekly. Milk for free amino acid and fatty acid analysis was taken at the end of each experimental period and stored by freezing at -20$^{o}$C. Percent fat was determined by the standard Babock method, and solids not fat by the method of Watson (73). The last

Table 2. Refusal of experimental concentrates during each four week feeding period by dairy cows fed different types of concentrates.

| Type of concentrate | Total kg of concentrate refusal (period) | | | Percent of concentrate refusal during all three periods |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Basal | 147.6 | 2.1 | 1.4 | 6.29 |
| Basal + 0.11% methionine | 91.5 | 0.5 | --- | 3.74 |
| Basal + 0.11% M-analog | 107.1 | 12.3 | 0.6 | 4.78 |
| Basal + 0.22% M-analog | 146.8 | 12.5 | --- | 6.37 |

two analyses were performed weekly on fresh, unpreserved samples.

Prior to gas liquid chromatographic analysis, approximately 0.5 g of fat was separated from the milk by the TeSa Fat Test[3] and converted to methyl esters by the method of Luddy et al. (33). Methyl esters were extracted in hexane and separated on a diethylene glycol succinate column (2 m by 6.3 mm) using a Perkin-Elmer Model 800 instrument.[4] The esters were identified by comparison with retention times of standard compounds; their relative amounts were determined by comparison of areas under peaks drawn by the recorder and calculated by a Perkin-Elmer Model 186 printing integrator.[4]

Rumen fluid samples were taken by rumen tube three to four hours after the morning feeding at the end of each experimental period. The rumen liquor was filtered through two layers of cheesecloth and preserved with one part saturated mercuric chloride solution to ten parts rumen fluid then stored at -20°C until analyzed by the method of Erwin et al. (15).

Body weights were determined by averaging weights after milking on the last three evenings during each experimental period. Fecal samples were obtained by rectal removal twice daily (morning and evening) on three consecutive days at the end of each feeding period.

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3. Technical Industries, Fort Lauderdale, Florida.

4. Perkin-Elmer Instrument Division. Monrovia, California.

Samples were composited for each animal, air dried, ground and stored at room temperature in sealed containers until analysis.

Composite fecal samples, alfalfa cubes and concentrate rations were analyzed for percent ether extract, fiber (71), and lignin (p3). Apparent digestibility was estimated by the lignin ratio technique (34). Percent protein was determined by standard Kjeldahl method. Combustible energy was determined by an adiabatic oxygen bomb calorimeter.[5]

Two blood samples were collected on the last day of each period. One sample was heparinized and centrifuged; plasma was drawn off for free amino acid analysis. The other sample was allowed to coagulate at room temperature and then centrifuged; serum was collected for fatty acid analysis (6).

Free amino acids from all classes of samples were extracted by deproteinization with a 10% solution of sulfosalicylic acid (50), filtered, centrifuged at 3,000 RPM for thirty minutes and refrigerated until analysis.

Amino acids were separated by the method of Stein and Moore (64) on a Beckman Model 121 Automatic Amino Acid Analyzer.[6] Amino acids were identified by comparison of retention times with known

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5. Parr Instrument Co., Moline, Illinois.

6. Beckman Instruments Inc., Spinco Division, Palo Alto, California.

standards. Quantitation was determined by using an Infotronics Digital Integrator, Model CRS-220A.[7] In a few instances of integrator malfunction, quantitative values were measured manually by calculating the area of the peak.

Deproteinized fecal samples were injected automatically onto the column in 250 µl quantities, using 6 mm cuvettes in the colorimeter. Milk free amino acid concentrations were so minimal that determinations were made by employing 2,500 µl for the short, and 5,000 µl for the long columns, respectively, and 6 mm cuvettes were used.

Two of the plasma samples were analyzed in a manner similar to that of milk and the remainder determined in 1,000 µl and 2,500 µl quantities.

Due to the low concentration of free amino acids in the milk and blood samples, injection was made manually. For this purpose the instrument was modified by the addition of two manual sample injection valves. Sample metering coils were calibrated by weighing with water.

Twelve of the deproteinized rumen fluid pellets were injected automatically from 250 µl metering loops. The last 24 rumen fluid samples and feed samples were run automatically with 750 µl metering

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7. Infotronics, Houston, Texas.

loops and all read through 12 mm cuvettes as were the concentrate rations. Free amino acid concentrations of the alfalfa were high enough to be analyzed with the 250 µl loop.

## RESULTS AND DISCUSSION

No digestive or physiological disturbances to the animals were noted during the course of this experiment. While some rejection of the experimental concentrates occurred early in the first feeding period (Table 3), it is obvious that the cows soon adjusted to the feed as insignificant amounts were refused during subsequent feeding periods. There was no refusal of the alfalfa cubes. There were no significant differences in feed consumption caused by the addition of methionine or M-analog to the diet. As initial concentrate refusal was somewhat similar for all rations and was no different from that reported by Brown et al. (5), weighback was considered as not having any effect on the final outcome of the experiment.

The feed consumption of the ration containing the higher level of M-analog was in direct contrast to the work of Griel et al. (19) who reported a relatively high level of refusal when the cows were fed 80 g of M-analog/day. It is conceivable that the manner of presentation of the supplement could influence ration acceptance or refusal. In the present study M-analog was mixed directly into the concentrate blend prior to pelleting, while Griel et al. (19) merely mixed the supplement loosely with grain.

Table 3. Free amino acids composition of experimental concentrates.

| Amino acid | Type of concentrate | | | |
|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog |
| | (%) | | | |
| Lysine | 1.06 | 0.60 | 1.05 | 0.93 |
| Histidine | 0.46 | 0.20 | 0.61 | 0.42 |
| Arginine | 11.97 | 5.94 | 11.33 | 9.89 |
| Aspartic acid | 19.94 | 14.88 | 19.86 | 16.48 |
| Threonine | 1.50 | 1.09 | 1.57 | 1.66 |
| Serine | 7.38 | 4.31 | 7.60 | 7.22 |
| Glutamic acid | 19.16 | 10.39 | 20.40 | 20.45 |
| Proline | 5.56 | 3.54 | 5.41 | 6.46 |
| Glycine | 3.45 | 1.95 | 3.52 | 3.96 |
| Alanine | 14.72 | 8.42 | 15.40 | 17.03 |
| Cysteine | 0.00 | 0.00 | 0.00 | 0.19 |
| Valine | 3.91 | 2.18 | 4.13 | 4.50 |
| Methionine | 2.97 | 42.01 | 0.46 | 0.75 |
| Isoleucine | 3.34 | 1.87 | 3.55 | 4.17 |
| Leucine | 2.46 | 1.23 | 2.61 | 3.04 |
| Tyrosine | 1.61 | 0.87 | 1.62 | 1.95 |
| Phenylalnine | 1.39 | 0.36 | 0.88 | 0.90 |

The free amino acid composition of the four experimental concentrates are shown in Table 3. Free amino acid analysis revealed several compounds which were not identified. Table 4 lists ninhydrin positive compounds found in deproteinized feed, rumen fluid, blood plasma, milk and feces. These data are presented in one sample of each type analyzed to illustrate the relationship of the unknown substances and ammonia to the identified amino acids. All metabolic materials were obtained from a single cow on the basal concentrate. Although integrator measurements were obtained for the unknowns and ammonia in all samples, these compounds were quantitated against the "knowns" only in this table. Values for the unknowns and for ammonia were obtained by the use of the integrated value under the chromatographic peak for each compound. It was beyond the scope of this study to determine molecular weight or structure, or determine whether or not these compounds were in any way affected by the feeding of the experimental rations.

The effects of the diet upon free amino acid patterns in rumen fluid are presented in Table 5. Free threonine levels were significantly ($P < 0.05$) higher in the rumen fluid of the cows receiving 80 g of M-analog/day than in cows on the basal ration. The percentage values of free threonine in the rumen fluid of the cows receiving 40 g of methionine/day or 40 g of M-analog/day were between the basal and the high M-analog intake values, however, they were not significantly different

Table 4. Ninhydrin positive compounds found in deproteinized samples of alfalfa, basal concentrate, rumen fluid, blood plasma, milk and feces.

| Retention time (min.) | Compound | Alfalfa | Basal concentrate | Rumen fluid | Blood plasma | Milk | Feces |
|---|---|---|---|---|---|---|---|
| | | | | (%) | | | |
| Short Column | | | | | | | |
| 15 | Unknown | 0.05 | 0.15 | 0.09 | 0.26 | .01 | .95 |
| 19 | Lysine | 2.11 | 0.47 | 4.85 | 3.09 | 4.56 | 8.22 |
| 25 | Histidine | 0.63 | 0.16 | 0.89 | 1.15 | 0.17 | 0.33 |
| 31 | Ammonia | 30.88 | 44.45 | 72.98 | 16.15 | 47.68 | 26.57 |
| 37 | Unknown | ----- | ----- | ----- | ----- | .03 | .22 |
| 51 | Arginine | 1.42 | 3.86 | 1.29 | 2.17 | 1.06 | .40 |
| Long Column | | | | | | | |
| 75 | Unknown | 1.32 | 2.77 | ----- | ----- | ---- | ---- |
| 96 | Unknown | 1.62 | ----- | ----- | ----- | ---- | ---- |
| 103 | Aspartic acid | 13.52 | 8.40 | 5.30 | 1.38 | 2.49 | 6.29 |
| 107 | Unknown | ----- | ----- | ----- | 0.41 | ---- | 0.37 |
| 111 | Unknonw | ----- | ----- | ----- | 0.68 | ---- | ---- |
| 117 | Threonine | 21.12[a] | 10.89[a] | 0.59 | 3.74 | 1.48 | 3.85 |
| 120 | Serine | 21.12[a] | 10.89[a] | 2.33 | 6.91 | 2.08 | 3.82 |
| 129 | Glutamic acid | 4.34 | 7.61 | 5.89 | 3.73 | 23.05 | 8.64 |
| 136 | Proline | 2.66 | 0.50 | 0.07 | 2.92 | 0.51 | 2.05 |
| 146 | Unknown | ----- | ----- | ----- | 0.17 | ---- | 0.02 |
| 153 | Glycine | 1.79 | 2.70 | 2.11 | 11.43 | 6.06 | 4.91 |
| 160 | Alanine | 7.77 | 9.98 | 1.63 | 8.10 | 2.85 | 9.48 |
| 168 | Unknown | ----- | ----- | ----- | 0.27 | ---- | 0.02 |
| 173 | Cysteine | ----- | ----- | ----- | 0.36 | 0.09 | 0.11 |
| 188 | Valine | 3.98 | 1.83 | 0.28 | 16.50 | 3.60 | 5.22 |
| 202 | Methionine | ----- | 1.17 | 0.53 | 0.66 | 0.66 | 1.83 |
| 208 | Isoleucine | 2.19 | 1.48 | 0.08 | 5.90 | 0.90 | 4.35 |

Table 4--Continued.

| Retention time (min.) | Compound | Alfalfa | Basal concentrate | Rumen fluid | Blood plasma | Milk | Feces |
|---|---|---|---|---|---|---|---|
| 212 | Leucine | 2.22 | 1.13 | 0.42 | 9.70 | 1.43 | 6.55 |
| 218 | Unknown | ----- | ----- | ----- | 0.06 | ---- | 0.37 |
| 223 | Unknown | ----- | ----- | ----- | 0.01 | ---- | ---- |
| 233 | Tyrosine | 0.58 | 1.98[b] | 0.31 | 1.90 | 0.45 | 2.68 |
| 238 | Phenylalanine | 1.80 | 1.98[b] | 0.24 | 2.34 | 0.25 | 2.59 |
| 244 | Unknown | ----- | ----- | ----- | ----- | ---- | 0.05 |
| 260 | Unknown | ----- | 0.51 | 0.12 | 0.01 | 0.34 | 0.01 |
| 277 | Unknown | ----- | ----- | ----- | ----- | 0.25 | 0.10 |
| Total Unknown Compounds | | 2.99 | 3.44 | 0.21 | 1.87 | 0.63 | 2.11 |

[a]Threonine and Serine are reported together in alfalfa and ration no. 1 due to improper separation.

[b]Tyrosine and phenylalanine are reported together in ration no.1 due to improper separation.

Table 5. Mean free amino acids in rumen fluid from dairy cows fed different types of experimental concentrates.

| Amino acid | Type of concentrate: Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | F value |
|---|---|---|---|---|---|
| | (%) | | | | |
| Lysine | 19.23 | 16.42 | 12.71 | 12.69 | 2.17 |
| Histidine | 4.08 | 3.76 | 3.63 | 5.05 | 2.06 |
| Arginine | 6.55 | 6.14 | 5.35 | 6.01 | 0.48 |
| Aspartic acid | 18.20 | 18.06 | 19.52 | 18.88 | 0.19 |
| Threonine | 2.88 | 3.43[a,b] | 3.41[a,b] | 4.08[b,c] | 4.78 |
| Serine | 8.40 | 8.61 | 8.61 | 8.42 | 0.05 |
| Glutamaic acid | 24.85 | 26.43 | 28.06 | 27.84 | 1.38 |
| Proline | 1.93 | 4.10 | 1.89 | 2.65 | 0.36 |
| Glycine | 4.12 | 3.73 | 4.27 | 3.88 | 0.67 |
| Alanine | 3.82 | 3.32 | 5.16 | 4.39 | 2.40 |
| Cysteine | ---- | ---- | ---- | ---- | --- |
| Valine | 0.81 | 1.07 | 1.58 | 1.12 | 2.51 |
| Methionine | 1.77 | 2.00 | 1.13 | 1.25 | 1.08 |
| Isoleucine | 0.36 | 0.39 | 0.56 | 0.49 | 2.76 |
| Leucine | 0.90 | 0.72 | 1.10 | 0.88 | 1.50 |
| Tyroeine | 1.10 | 1.11 | 1.64 | 1.43 | 1.16 |
| Phenylalanine | 0.96 | 0.74 | 1.20 | 0.87 | 1.41 |

[a, b, c] Values within a group of means with different superscripts indicate a significant difference ($P < 0.05$). Those with no superscripts indicate no significant differences ($P > 0.05$).

from either. It is noteworthy that the free methionine levels in rumen fluid were higher for the cows receiving methionine in the diet than for the other cows even though this difference is not significant ($P > 0.05$). It was assumed that the rumen microorganisms rapidly deactivated the added methionine. No other explanation of this phenomenon seems evident at this time, except that McCarthy (35) indicated that in vitro synthesis of total lipid synthesis was depressed by the addition of M-analog. This could mean that the microbial activity was reduced, but how this would affect methionine metabolism remains unclear.

The experiments by Leibholz (26, 27) are the only known studies where free amino acid determinations were made on rumen fluid. There were fairly large variances within the different amino acids and, generally, there was little agreement between the figures given by Leibholz and the results of this study. For example, Leibholz (27) reported lysine values ranging from 11.6 to 29.3% of the rumen fluid free amino acids. The present study showed a range of 12.7 to 19.2% for the same acid in rumen fluid. The fact that no significant differences ($P > 0.05$) were apparent in these figures indicates that a large variation between animals existed. There was an appreciable difference between the glutamic acid values reported by Leibholz (27) (7.01-22.29%) and those reported in this study (24.85-28.06%). The present study found only trace amounts of free cysteine in rumen fluid, while Leibholz (26)

reported values from 9 to 7.65%. Another large difference between these studies was in alanine levels with Leibholz (26) reporting a range of 10.00 to 19.45% while this study reported only 3.32 to 5.16%.

Table 6 indicates the effect of diet upon free amino acid patterns in blood plasma. There were no significant differences ($P > 0.05$) in any of the PFAA which could be attributed to supplementing the diet with methionine or M-analog. This agrees with Schelling and Hatfield (57) who reported that the addition of methionine to the diet resulted in no increase in plasma free methionine. As there were no increases in methionine levels in the rumen fluid of cows fed supplementary methionine in the experiment reported here, it follows that there would be no increases in the blood. Results comparing the plasma of control animals to to plasma of animals receiving methionine, were as expected. However, in the case of the cows receiving the M-analog supplementation results were not as predicted. As previously reported, Burroughs and Trenkle (9) postulated that M-analog would not be altered by rumen microorganisms because of unfavorable pH conditions and would pass unchanged into the abomasum. At this point a lower pH would allow the analog to be absorbed into the protal blood system where it would be transported to the liver. Here, hepatic enzymes (Figure 1) theoretically convert M-analog into methionine.

Several possible explanations exist as to why there were no increases in plasma free methionine values following the M-analog

Table 6. Mean free amino acids in the blood plasma from dairy cows fed different types of experimental concentrates.

| Amino acid | Type of concentrate | | | | F value |
|---|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | |
| | (%) | | | | |
| Lysine | 7.83 | 9.44 | 7.69 | 8.71 | 1.08 |
| Histidine | 3.63 | 4.09 | 3.85 | 4.17 | 0.60 |
| Arginine | 9.85 | 8.78 | 9.59 | 9.28 | 0.14 |
| Aspartic acid | 1.41 | 1.52 | 1.45 | 1.42 | 0.26 |
| Threonine | 3.08 | 3.46 | 3.28 | 3.13 | 1.40 |
| Serine | 4.43 | 4.65 | 4.43 | 4.57 | 0.24 |
| Glutamic acid | 3.79 | 4.26 | 4.20 | 3.93 | 1.44 |
| Proline | 17.54 | 15.42 | 16.85 | 15.97 | 1.16 |
| Glycine | 6.04 | 7.06 | 7.98 | 7.34 | 0.98 |
| Alanine | 6.01 | 5.93 | 5.36 | 5.36 | 0.72 |
| Cysteine | 0.86 | 0.60 | 0.66 | 0.88 | 0.42 |
| Valine | 13.43 | 12.57 | 13.87 | 13.05 | 2.09 |
| Methionine | 1.11 | 1.09 | 1.01 | 1.04 | 0.16 |
| Isoleucine | 6.45 | 7.84 | 4.34 | 6.49 | 0.76 |
| Leucine | 8.85 | 7.94 | 8.72 | 8.90 | 0.92 |
| Tyroeine | 2.62 | 2.78 | 2.67 | 2.55 | 0.46 |
| Phenylalanine | 2.86 | 3.15 | 3.09 | 3.04 | 0.72 |

supplementation in the present study. One is that Burroughs and Trenkle (9) were wrong in their postulation concerning M-analog. Another is that some undetermined unfavorable condition of this experiment prevented the conversion of M-analog to methionine. Still another explanation lies in the report published by Linton et al. (31) where it was hypothesized that when incapsulated methionine was fed so as to bypass the rumen, blood plasma levels of free methionine would increase only if methionine was the most limiting amino acid. If methionine were the first limiting amino acid, then it would be immediately absorbed from the blood for utilization in the various body tissues. Thus, there would be no actual measurable increase in plasma free methionine levels. These authors further theorize that any synthesis involving methionine should also utilize some valine and that the plasma free methionine:valine ratios should more nearly reflect the effect of increasing the availability of methionine than would methionine alone. Again, this is not borne out in the present study as there were no significant changes in the methionine: valine ratio as a result of feeding M-analog. It should also be pointed out that Hogan et al. (21) felt that PFAA patterns were of little value due to a high degree of individual variation.

It was noted that values for various free amino acids in blood plasma in the present study did not closely resemble previously published reports (41, 42, 48, 70, 72). It should be pointed out that there was a

great variation between these different PFAA levels reported in various amino acids. Another seemingly well established fact is that PFAA levels are not a true indication of nutritional intake and tissue demand for individual amino acids (21, 27).

The effect of diet upon free amino acids in deproteinized milk is shown in Table 7. The feeding of methionine and M-analog at the lower level caused a significant increase ($P < 0.05$) in the aspartic acid and valine content of milk free amino acids. The feeding of higher level M-analog did not have this effect. No explanation is readily available at this time.

A comparison of the free amino acid content of milk with that of rumen fluid indicated that a direct correlation exists between a number of individual amino acids. High levels of free lysine and glutamic acid in the rumen fluid are reflected in high levels in the milk. Arginine, proline, tyrosine and phenylalanine have similar values in both milk and rumen fluid, while the values for threonine and glycine are not so closely related. The levels of the other free amino acids are markedly different between rumen fluid and milk. This is especially true for aspartic acid which accounts for 18.06 to 19.50% of the total free amino acids in rumen fluid, while it accounts for only 3.58 to 4.12% in milk.

Although Hogan et al. (21) did not analyze for free amino acids, it is of interest to note that they reported an identical amino acid composition between hydrolyzed rumen digesta and milk casein.

Table 7. Mean free amino acids in milk from dairy cows fed different types of experimental concentrates.

| Amino acid | Type of concentrate: Basal | Basal + 0.11% methionine | Basal + o.11% M-analog | Basal + 0.22% M-analog | F value |
|---|---|---|---|---|---|
| | (%) | | | | |
| Lysine | 13.67 | 13.71 | 12.56 | 12.76 | 0.96 |
| Histidine | 0.88 | 0.98 | 0.69 | 1.17 | 0.94 |
| Arginine | 5.64 | 5.32 | 5.03 | 5.42 | 0.46 |
| Aspartic acid | 3.58[a] | 4.12[b] | 4.03[b] | 3.79[a] | 5.69 |
| Threonine | 1.70 | 1.78 | 1.94 | 1.53 | 0.83 |
| Serine | 2.55 | 2.48 | 2.21 | 2.58 | 2.16 |
| Glutamic acid | 40.56 | 39.22 | 40.38 | 41.70 | 0.47 |
| Proline | 4.61 | 5.30 | 4.90 | 4.24 | 1.11 |
| Glycine | 7.65 | 8.57 | 8.32 | 8.74 | 2.21 |
| Alanine | 4.56 | 4.37 | 4.25 | 4.14 | 1.50 |
| Cysteine | 0.56 | 0.57 | 0.60 | 0.71 | 1.64 |
| Valine | 5.03[a] | 6.00[b] | 6.02[b] | 5.36[a] | 5.71 |
| Methionine | 0.39 | 0.49 | 0.29 | 0.34 | 2.31 |
| Isoleucine | 1.50 | 1.64 | 1.67 | 1.50 | 0.48 |
| Leucine | 4.63 | 2.81 | 5.06 | 4.18 | 0.93 |
| Tyrosine | 1.53 | 1.67 | 1.34 | 1.20 | 1.78 |
| Phenylalanine | 0.82 | 0.93 | 0.63 | 0.77 | 3.12 |

[a, b]Values within a group of means with different subscripts indicate a significant difference ($P < 0.05$). Those with no superscripts indicates no significant differences ($P > 0.05$).

Certain conclusions may be drawn because of the similarities in free amino acid content between rumen fluid and milk and the virtual lack of any such similarity between blood and either rumen fluid or milk. The most obvious inference being that the free amino acid level in milk is probably influenced by free amino acid levels in the rumen. The only available reason for the lack of similarity between unbound amino acid content of blood and the levels in rumen fluid and milk is that turnover rates of the various amino acids must be immensely different. The amino acids are transported via the blood from the digestive system to the mammary gland. These acids such as lysine and glutamine, which are quite high in rumen fluid and milk but proportionally low in blood, must have a relatively high turnover rate in the blood. The mammary gland would exhibit a high demand for these acids and consequently be quite effective in removing them from the blood. The opposite effect would be true in the case of proline, which accounted for 15.42 to 17.54% of the total free amino acids in blood, but only 1.89 to 4.10% in rumen fluid and 4.24 to 5.30% in milk. A low requirement for proline by the mammary gland would cause a decrease in turnover rate for this acid, thus causing a higher blood level of unbound proline. The demand for proline by the other tissues of the body might also be lower than the demand for lysine or glutamine, further compounding the blood proline levels. Plasma free valine would appear to be similar to proline in this respect.

Lysine and glutamine exhibit the property of high turnover rate, while valine and proline display the opposite effect. Doubtless this also takes place between other free amino acids in these biological compounds.

There are no known reports in the literature in which the free amino acid content of milk has been determined. As a result, there is no means of comparing the unbound amino acid levels for milk found in the present study. The values presented herein are probably not atypical as there was similarity between this study and previously reported work in both rumen fluid and blood values for free amino acids.

The results of the analysis for free amino acid content of fecal material are shown in Table 8. Neither methionine nor M-analog supplementation had any significant effect ($P > 0.05$) on the free amino acids in feces. There does not appear to be as close a relationship between the levels of free amino acids in feces and rumen fluid, blood or milk as there was between rumen fluid and milk. Lysine and glutamic acid were present in high levels in rumen fluid and milk, while fecal material was high in proline, leucine and alanine and, consequently, more closely resembled the blood free amino acid pattern. Obviously, by chance alone, certain similarities exist between various substances. It is assumed this is the case with feces, as there are no direct relationships between feces and the other substances studied. The possibility exists

Table 8. Mean free amino acids in feces from dairy cows fed different types of experimental concentrates.

| Amino acid | Type of concentrate | | | | F value |
|---|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | |
| | (%) | | | | |
| Lysine | 10.75 | 11.35 | 11.28 | 11.57 | 0.34 |
| Histidine | 0.82 | 0.87 | 0.89 | 0.71 | 1.79 |
| Arginine | 0.86 | 0.84 | 0.78 | 0.81 | 0.09 |
| Aspartic acid | 8.55 | 9.13 | 8.71 | 8.46 | 1.09 |
| Threonine | 4.37 | 4.68 | 4.29 | 4.90 | 3.64 |
| Serine | 3.69 | 3.97 | 3.68 | 3.87 | 1.09 |
| Glutamic acid | 15.83 | 14.08 | 14.92 | 13.19 | 1.15 |
| Proline | 9.68 | 9.34 | 8.45 | 10.65 | 0.98 |
| Glycine | 4.39 | 3.97 | 4.08 | 4.44 | 0.31 |
| Alanine | 10.72 | 10.89 | 11.73 | 11.45 | 0.30 |
| Cysteine | 1.13 | 1.14 | 1.04 | 0.92 | 0.22 |
| Valine | 6.33 | 6.28 | 6.62 | 6.86 | 0.49 |
| Methionine | 0.82 | 0.82 | 0.75 | 0.75 | 0.42 |
| Isoleucine | 5.40 | 5.61 | 5.66 | 5.42 | 1.17 |
| Leucine | 7.82 | 7.98 | 8.11 | 7.77 | 0.22 |
| Tyrosine | 5.02 | 5.21 | 5.15 | 4.67 | 0.79 |
| Phenylalanine | 3.80 | 3.78 | 3.90 | 3.55 | 0.20 |

that a similar chance circumstance indicated that levels of some free amino acids in milk are related to levels in rumen fluid. Based on more similarities, however, it is reasonable to assume that these phenomena are not merely chance. There is enough evidence presented to warrant further investigation into the relationship between free amino acids in milk and rumen fluid.

There were no significant differences ($P > 0.05$) in the molar proportions of the VFA in the rumen fluid caused by the supplementation of the diet with methionine or M-analog (Table 9). This was in direct contrast to the work of Patton et al. (47) who found that M-analog supplementation did in fact cause changes in the VFA.

It has been well established that a decrease in acetate:propionate ratio is reflected in a decrease in milk fat percentage (8, 14, 60). VFA analyses were conducted in the present study because of these data and the fact that McCarthy (35) reported the supplementation of methionine or M-analog in preventing ketosis also caused an increase in milk fat percentage. He also states that conditions other than VFA molar ratio changes were responsible for the increased milk fat percentage. McCarthy did not, however, analyze rumen fluid for VFA.

There were no significant differences ($P > 0.05$) in the proportions of any of the various component fatty acids of blood serum cholesterol esters of animals on any of the experimental rations (Table 10).

Table 9. Mean volatile fatty acids in rumen fluid from dairy cows fed different types of experimental concentrates.

| Fatty Acid | Types of concentrate: Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | F value |
|---|---|---|---|---|---|
| | (molar %) | | | | |
| 2:0 | 62.53 | 56.77 | 57.36 | 57.96 | 0.77 |
| 3:0 | 25.38 | 31.35 | 31.18 | 26.94 | 1.58 |
| 4:iso | 0.35 | 0.66 | 0.24 | 0.24 | 2.04 |
| 4:0 | 10.86 | 9.64 | 10.62 | 13.44 | 1.67 |
| 5:iso | 0.06 | 0.16 | 0.17 | 0.27 | 2.81 |
| 5:0 | 0.90 | 1.12 | 0.53 | 1.07 | 1.14 |

Table 10. Mean fatty acids of serum cholesterol esters from dairy cows fed different types of experimental concentrates.

| Fatty acid | Type of concentrate | | | | F value |
|---|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | |
| | (%) | | | | |
| 12:0 | 0.60 | 0.45 | 0.34 | 0.23 | 0.28 |
| 14:0 | 0.79 | 0.93 | 0.71 | 1.22 | 0.57 |
| 14:1 | 0.83 | 1.00 | 0.64 | 0.88 | 0.67 |
| 15:0 | 0.56 | 0.51 | 0.41 | 0.43 | 0.79 |
| 16:iso | 0.26 | 0.20 | 0.22 | 0.15 | 2.36 |
| 16:0 | 3.38 | 3.58 | 2.84 | 2.74 | 0.47 |
| 16:1 | 1.79 | 1.40 | 1.20 | 1.28 | 1.27 |
| 16:2 | 0.16 | 0.21 | 0.18 | 0.14 | 0.25 |
| 18:iso | 0.16 | 0.05 | 0.11 | 0.12 | 0.94 |
| 18:0 | 0.74 | 0.61 | 0.51 | 0.66 | 0.13 |
| 18:1 | 3.41 | 2.87 | 2.30 | 3.37 | 0.94 |
| 18:2 | 82.61 | 81.84 | 85.39 | 83.36 | 0.43 |
| 18:3 | 0.02 | 0.03 | 0.58 | 0.59 | 2.63 |
| 20:1 | 2.44 | 4.47 | 3.43 | 1.84 | 1.65 |
| 18:4 | 0.26 | 0.33 | 0.32 | 0.25 | 0.04 |
| 20:4 | 0.30 | 0.16 | 0.27 | 0.57 | 1.13 |
| 22:1 | 0.22 | 0.32 | 0.16 | 0.16 | 1.58 |
| 20:5 | 0.45 | 0.26 | 0.23 | 0.42 | 1.14 |
| 22:5 | 1.02 | 0.79 | 0.21 | 1.65 | 0.75 |

This is in direct contradiction to McCarthy (35) who found that I-V infusion of L-methionine within 43 hours increased the 18:2 fraction of total serum lipids from 12.9% of the total to 42.0% of the total. Fatty acids of serum cholesterol esters accounted for a major portion of the total fatty acids present in the total serum lipids (7). Thus, the values for total free fatty acids and cholesterol ester fatty acids are similar.

The present study was, obviously, incapable of showing the effect of methionine or M-analog on the 18:2 fraction of serum cholesterol esters because on the control diet 18:2 accounts for 82.61% of the total fatty acids in the cholesterol ester fraction. This compares with normal values reported by others (6, 7, 35). It can be concluded that the methionine level of the basal diet was high enough to prevent a methionine deficiency.

The supplementation of methionine in the diet resulted in an apparent significant increase ($P < 0.05$) in 18:iso in serum triglycerides from 0.32 to 0.69% of the total triglycerides (Table 11). Conversely, M-analog caused an apparent depression of 18:iso. There is no apparent explanation for these findings. The low level of 18:iso in serum triglycerides and small errors in analytical procedures could account for significant changes, thus rendering the value of this finding questionable.

There were no other significant changes ($P > 0.05$) detected in the serum triglycerides attributable to methionine or M-analog

Table 11. Mean fatty acids of serum triglycerides from dairy cows fed different types of experimental concentrates.

| Fatty acid | Type of concentrate | | | | F value |
|---|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | |
| | (%) | | | | |
| 12:0 | 0.92 | 0.70 | 0.92 | 1.27 | 0.46 |
| 14:0 | 1.72 | 1.79 | 1.32 | 2.04 | 0.70 |
| 14:1 | 1.19 | 1.85 | 1.18 | 1.91 | 1.53 |
| 15:0 | 0.94 | 1.23 | 1.09 | 1.55 | 0.49 |
| 16:iso | 0.54 | 0.61 | 0.57 | 0.99 | 0.60 |
| 16:0 | 34.24 | 28.33 | 35.32 | 35.66 | 0.78 |
| 16:1 | 3.30 | 3.16 | 2.24 | 1.74 | 0.73 |
| 16:2 | 0.74 | 0.18 | 0.27 | 0.45 | 0.57 |
| 18:iso | 0.32[a] | 0.69[b] | 0.01[c] | 0.23[a] | 6.81 |
| 18:0 | 22.96 | 19.65 | 24.46 | 21.02 | 0.44 |
| 18:1 | 19.47 | 19.05 | 16.19 | 15.49 | 0.37 |
| 18:2 | 9.11 | 12.36 | 3.34 | 10.53 | 2.44 |
| 18:3 | 0.07 | 0.65 | 0.96 | 0.09 | 3.24 |
| 20:1 | 0.11 | 1.96 | 1.48 | 1.94 | 2.23 |
| 18:4 | 0.18 | 1.34 | 1.33 | 0.92 | 0.83 |
| 20:4 | 0.34 | 1.08 | 1.65 | 0.38 | 1.90 |
| 22:1 | 0.55 | 0.84 | 1.79 | 0.87 | 3.01 |
| 20:5 | 1.26 | 0.78 | 2.13 | 0.15 | 2.00 |
| 22: 5 | 2.63 | 3.77 | 3.74 | 2.75 | 0.20 |

[a], [b], [c]Values within a group of means with different superscripts indicate a significant difference ($P < 0.05$). Those with no superscript indicate no significant differences ($P > 0.05$).

supplementation. The values detected were similar to those previously reported (8) and are considered to be normal.

Both levels of M-analog supplementation caused a significant depression ($P < 0.05$) of 15:0 serum free fatty acid in the blood of animals on those rations (Table 12). The ration containing methionine and the diet with the higher level of M-analog caused a significant increase in the 22:5 fatty acid. The significance of these two blood serum acids has never been established, consequently, the significance of the changes caused by the experimental diets remains unclear. No other important changes were noted. The levels of the major serum free fatty acie components were similar to previously published work (6, 35) and are accepted as being normal.

Methionine and the higher level of M-analog caused a significant depression ($P < 0.05$) in the serum phospholipid 18:4 (Table 13). Again, there is no readily available explanation of this finding. There were no other meaningful changes noted. The values obtained from this study are comparable to other published values (6, 7).

Both the 15:0 and 16:2 fatty acid components of milk fat were significantly depressed ($P < 0.05$) by the addition of the higher amount of M-analog (Table 14). Methionine and the lower level of M-analog also reduced 15:0 and 16:2 below their respective levels as a result of the basal diet, however, these depressions were not significant

Table 12. Mean fatty acids of serum free fatty acids from dairy cows fed different experimental concentrates.

| Fatty acids | Type of concentrate | | | | F value |
|---|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | |
| | (%) | | | | |
| 12:0 | 0.96 | 1.62 | 0.50 | 0.69 | 1.84 |
| 14:0 | 2.27 | 1.69 | 0.36 | 1.17 | 1.24 |
| 14:1 | 0.77 | 1.52 | 0.29 | 0.89 | 2.49 |
| 15:0 | 0.71[a] | 0.48[a,b] | 0.28[b] | 0.32[b] | 4.79 |
| 16:iso | 0.22 | 0.01 | 0.36 | 0.16 | 1.22 |
| 16:0 | 37.46 | 49.50 | 43.68 | 39.85 | 1.54 |
| 16:1 | 0.92 | 1.34 | 0.13 | 0.36 | 1.14 |
| 16:2 | 0.76 | 1.33 | 0.02 | 0.61 | 1.40 |
| 18:iso | 7.09 | 0.63 | 9.34 | 5.23 | 1.54 |
| 18:0 | 19.54 | 17.84 | 24.67 | 25.16 | 1.86 |
| 18:1 | 12.68 | 8.06 | 7.86 | 10.04 | 0.77 |
| 18:2 | 7.95 | 6.15 | 4.75 | 5.45 | 1.02 |
| 18:3 | 1.15 | 0.42 | 0.45 | 0.47 | 0.68 |
| 20"1 | 0.47 | 0.68 | 0.58 | 0.39 | 0.42 |
| 18:4 | 1.19 | 1.33 | 0.93 | 0.61 | 1.08 |
| 20:4 | 1.26 | 1.29 | 2.13 | 1.41 | 0.45 |
| 22:1 | 1.19 | 1.41 | 0.85 | 0.58 | 0.32 |
| 20:5 | 0.75 | 1.01 | 0.62 | 0.75 | 0.27 |
| 22:5 | 2.20[a] | 5.02[b] | 2.40[a] | 6.13[b] | 5.37 |

[a,b,c]Values within a group of means with different superscripts indicate a significant difference ($P < 0.05$). Those with no superscript indicate no significant differences ($P > 0.05$).

Table 13. Mean fatty acids of serum phospholipids from dairy cows fed different experimental concentrates.

| Fatty acid | Type of concentrate: Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | F value |
|---|---|---|---|---|---|
| | (%) | | | | |
| 12:0 | 2.60 | 2.53 | 2.08 | 0.53 | 0.32 |
| 14:0 | 2.95 | 4.43 | 2.95 | 0.35 | 2.13 |
| 14:1 | 0.88 | 1.86 | 0.95 | 1.83 | 0.73 |
| 15:0 | 0.90 | 1.42 | 1.59 | 0.85 | 2.04 |
| 16:iso | 0.21 | 0.22 | 0.17 | 0.07 | 1.20 |
| 16:0 | 20.61 | 24.80 | 28.10 | 28.62 | 0.97 |
| 16:1 | 3.01 | 4.35 | 5.35 | 6.15 | 0.87 |
| 16:2 | 0.25 | 0.03 | 0.79 | 0.40 | 2.36 |
| 18:iso | 0.45 | 1.41 | 0.67 | 0.26 | 3.39 |
| 18:0 | 19.35 | 15.93 | 19.08 | 18.08 | 0.61 |
| 18:1 | 18.16 | 16.04 | 12.20 | 14.99 | 0.80 |
| 18:2 | 9.81 | 4.38 | 5.60 | 2.65 | 1.00 |
| 18:3 | 0.30 | 0.31 | 0.75 | 0.64 | 0.73 |
| 20:1 | 2.25 | 2.92 | 0.36 | 1.48 | 1.18 |
| 18:4 | 2.17[a] | 0.77[b] | 2.89[a] | 0.44[b] | 18.36 |
| 20:4 | 1.61 | 1.09 | 1.67 | 2.00 | 0.18 |
| 22:1 | 3.37 | 3.01 | 0.16 | 4.44 | 1.75 |
| 20:5 | 0.66 | 2.19 | 1.93 | 2.19 | 0.77 |
| 22:5 | 10.54 | 12.47 | 12.95 | 14.06 | 0.13 |

[a,b]Values within a group of means with different superscripts indicate a significant difference ($P < 0.05$). Those with no superscript indicate no significant differences ($P > 0.05$).

Table 14. Mean fatty acids of milk fat from dairy cows fed different experimental concentrates.

| Fatty acid | Type of concentrate: Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | F value |
|---|---|---|---|---|---|
| | (%) | | | | |
| 6:0 | 3.43 | 3.56 | 2.45 | 3.83 | 1.79 |
| 8:0 | 2.92 | 2.82 | 2.16 | 2.97 | 1.23 |
| 10:0 | 5.78 | 6.48 | 5.00 | 6.33 | 1.81 |
| 10:1 | 0.50 | 0.48 | 0.29 | 0.37 | 1.35 |
| 12:0 | 5.43 | 5.82 | 4.72 | 5.21 | 2.85 |
| 12:1 | 0.02 | 0.02 | 0.02 | 0.01 | 1.64 |
| 13:0 | 0.09 | 0.08 | 0.08 | 0.07 | 0.68 |
| 14:iso | 0.07 | 0.05 | 0.05 | 0.04 | 0.40 |
| 14:0 | 14.64 | 14.41 | 14.53 | 14.23 | 0.16 |
| 14:1 | 1.67 | 1.69 | 1.50 | 1.03 | 1.40 |
| 15:0 | 0.97[a] | 0.78[a,b] | 0.81[a,b] | 0.69[b,c] | 5.01 |
| 16:iso | 0.26 | 0.19 | 0.19 | 0.09 | 1.43 |
| 16:0 | 34.25 | 35.32 | 39.52 | 35.99 | 3.15 |
| 16:1 | 2.14 | 2.06 | 1.81 | 1.32 | 3.91 |
| 16:2 | 0.17[a] | 0.14[a] | 0.13[a] | 0.05[b] | 10.52 |
| 18:iso | 0.08 | 0.14 | 0.08 | 0.11 | 1.35 |
| 18:0 | 5.79 | 5.10 | 5.62 | 5.61 | 0.67 |
| 18:1 | 18.60 | 18.41 | 18.28 | 19.25 | 0.14 |
| 18:2 | 1.82 | 1.62 | 1.54 | 1.64 | 3.04 |
| 18:3 | 1.08 | 0.95 | 1.06 | 1.05 | 0.45 |

[a,b,c]Values within a group of means with different superscripts indicate a significant difference ($P < 0.05$). Those with no superscripts indicate no significant differences ($P > 0.05$).

($P > 0.05$). As in the case of the 15:0 and 22:5 free fatty acids in serum, the depression of these acids has no readily available explanation. The low level in which these components were found questions the biological significance and tends to negate the mathematical differences found. The experimental concentrates effected no other significant ($P < 0.05$) differences in the milk fatty acids.

The only significant differences ($P < 0.05$) that were noted in either blood serum or in milk were in the minor fatty acid components. As there were no significant differences ($P > 0.05$) noted in the major fatty acids of rumen fluid or blood serum, it may be concluded that there probably would not be any differences in milk fat. All significant effects in milk were found in fatty acids which were presented in very small amounts and whose biological significance has never been established. It is safe to conclude that the feeding of methionine or M-analog under the conditions of this experiment had no significant biological effect on rumen fluid, blood serum or milk lipids.

Table 15 shows that the experimental concentrates caused no significant variation ($P > 0.05$) in milk production or in percent fat or total solids in the milk. Production was neither increased nor depressed by the experimental concentrates. This is in agreement with Williams et al. (75) who reported that M-analog caused no changes in 4% fat corrected milk. Therefore, the addition of methionine and M-analog

Table 15. Mean yield and composition of milk and body weights of dairy cows fed different experimental concentrates.

| | Type of concentrate | | | | |
|---|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | F value |
| | (%) | | | | |
| Milk | 25.21 | 26.21 | 26.10 | 25.24 | 1.03 |
| Milk Fat (%) | 2.88 | 2.78 | 2.78 | 3.00 | 0.54 |
| Milk Solids (%) | 8.41 | 8.46 | 8.37 | 8.37 | 2.30 |
| Body weight (kg) | 656.9 | 655.6 | 652.4 | 651.0 | 1.03 |

caused no adverse effects in milk production or gross composition. These facts are substantiated by the constancy of body weight of animals on all diets. When McCarthy (35) fed 40 and 80 g of M-analog to 44 cows from two weeks prepartum to eight weeks postpartum none of the animals developed ketosis. Milk production in animals fed 40 g of the daily analog was increased by 33.2 pounds/week. Cows receiving 80 g analog/day increased production by 53.5 pounds/week. Jersey cows showed a better response than did Holsteins. This research utilized cows in early lactation while the data for comparison came from cows in mid-lactation. This difference could explain the lack of response in increased milk production in the present study: however, it is plausible to conclude that the control diet was not low enough in methionine to allow supplementation to produce favorable effects.

The data included in Table 16 indicate that the addition of methionine or M-analog to the diet had no significant effect ($P > 0.05$) on the digestibility of protein, fiber, fat, or total feed.

Table 16. Mean digestibility of total rations fed to dairy cows receiving different experimental concentrates.

| | Type of concentrate | | | | |
|---|---|---|---|---|---|
| | Basal | Basal + 0.11% methionine | Basal + 0.11% M-analog | Basal + 0.22% M-analog | F value |
| | (%) | | | | |
| Digestibility of combustible energy | 60.92 | 60.76 | 59.96 | 62.29 | 1.20 |
| Digestibility of protein | 61.81 | 65.58 | 65.45 | 64.16 | 1.51 |
| Digestibility of fiber | 37.65 | 38.45 | 37.51 | 41.17 | 2.97 |
| Digestibility of fat | 49.63 | 37.68 | 44.16 | 50.81 | 0.39 |

## SUMMARY

All experimental concentrates were rejected early in the first feeding period, however, the cows adjusted to the feed with insignificant refusal occurring in the last two periods. Supplementation of the basal concentrate with the higher level of M-analog increased the level of free threonine in rumen fluid. The levels of other free amino acids in rumen fluid were not influenced by the experimental concentrates. The addition of methionine or M-analog to the basal concentrate caused no changes in the free amino acid patterns of blood plasma. Methionine and the lower level of M-analog supplementation increased aspartic acid and valine levels in milk free amino acid content.

There appeared to be a close relationship between rumen fluid and milk in their content of free lysine, glutamic acid, arginine, proline, tyrosine and phenylalanine. Levels of the other free amino acids in these substances did not appear to be related. No relationship between the proportions of free amino acids in blood with those in rumen fluid or milk was shown.

The experimental concentrates had no effect on the free amino acid content of feces, nor was there any apparent significant relationship between free amino acids in feces, rumen fluid, blood plasma or milk.

Supplementation with methionine or M-analog had no significant effect on rumen VFA or fatty acids of blood serum cholesterol. The proportion of 18:iso in serum triglycerides was raised by addition of methionine to the basal concentrate and depressed by the addition of the lower level of M-analog. Both levels of M-analog supplementation depressed the 15:0 level in serum free fatty acids while methionine and the higher level of M-analog increased 22:5. A decrease in 18:4 in serum phospholipids resulted from supplementation with methionineor the higher level of M-analog. The higher level of M-analog depressed the 15:0 and 16:2 fractions of milk fat. All significant effects were found in fatty acids which were present in very small amounts and whose biological significance has never been established. The experimental concentrates caused no significant changes in the total production or composition of milk, body weights, or the digestibility of feed.

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