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EFFECT OF PROTECTED METHIONINE ON MILK PRODUCTION IN DAIRY COWS

by

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INTRODUCTION

High producing dairy cows require greater amounts of dietary proteins. These proteins need to be of proper amino acid balance and many times high producing dairy cows need additional amounts of certain amino acids that are deficient in the diet. This has stimulated increased interest in the area of amino acid nutrition in ruminant animals. Ruminal microflora are capable of synthesizing all essential amino acids in required amounts (Loosli et. al., 1949; Block and Stekal, 1950; Virtanen, 1966). However, recently (Jacobson et. al., 1967; Hogan, 1975; Schwab et. al., 1976; Kaufman et. al., 1980) suggested that methionine may be a limiting amino acid in many lactating dairy cattle diets. The use of sulfur amino acids has also been shown to increase wool growth in sheep (Reis and Schinckel, 1963). Addition of methionine hydroxy analogs (MHA) has increased milk and milk fat yields (Griel et. al., 1968; Polan et. al., 1970; Bishop, 1971; Bishop and Murphy, 1972; Bhargava et. al., 1975). However, positive response was not always obtained with MHA supplementation (Hutjens and Schultz, 1971; Whiting et. al., 1972; Steele et. al., 1973; Fuquay et. al., 1974; Olson and Grubaugh, 1974; Walleneus and Whitchurch, 1975). The inconsistent results obtained with MHA supplementation may be due to the fact that its stability in the rumen is uncertain. Gil et. al., 1973 and Emery, 1971 believe that MHA may be completely degraded in the rumen.

Unless methionine is supplied to the ruminant animal in a manner protected from microbial attack it will be rapidly deaminated (Salsbury et. al., 1971; Langer et. al., 1978). The ammonia produced in the rumen is either used as a nitrogen source to synthesize amino acids by microorganisms or absorbed and excreted as urea. This has stimulated the discovery of methods to protect proteins and amino acids from microbial attack. Kaufman et. al., 1980, using a fat coated product, noted that 50-60% of the product escapes ruminal degradation. A protected methionine product, Ketionin® has been tested and marketed in Europe. However

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the data supporting the beneficial effects particularly on milk production is inconclusive. Preliminary studies done in our laboratory showed that 71% of methionine in the product escapes ruminal degradation and 52% probably gets absorbed from the intestine (Arambel, et. al., 1983).

The purpose of this study was to determine the effect Ketionin® on milk production in dairy cows.

Ketionin® - DL methionine protected by fatty acids. A product of Rumen Kjemi A/S, Oslo, Norway. U.S. Patent No. 3959493.

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REVIEW OF LITERATURE

Protein Metabolism

Much of the ingested feed protein is hydrolyzed by proteolytic enzymes of microbial origin to amino acids or small peptides (Allison, 1970) and the undegraded part passes out of the rumen. The rate of protein degradation in the rumen is positively correlated with the solubility of the feed protein. The peptides and amino acids are further deaminated to ammonia or incorporated directly to microbial protein (Asplund, 1975). In the ruminant animal, the abomasum is the true site of enzymatic digestion. The partially degraded feed proteins and microbial proteins along with some nonprotein nitrogen are absorbed in the intestine. The ruminant animal is dependent on rumen microorganisms for the balance and supply of amino acids. It has also been stated by Chalmers (1954) that "ruminants are not critically dependent on the amino acid composition of proteins fed to them, does not imply the converse, that all proteins have the same value to them". The microbial protein synthesis rate depends on many factors such as the amount of carbohydrates present, nitrogen source, and proper mineral balance.

The primary nitrogen source for rumen bacteria is ammonia. Ammonia in the ruminal contents is from the deamination as well as deamidation of feed proteins and from urea secreted in saliva (McDonald, 1948). Ammonia that is not utilized by bacteria may diffuse directly through the rumen wall, (Asplund, 1975) and enter the blood. Blood ammonia is converted to urea in the liver and excreted either in urine or returned to the rumen by direct diffusion through the rumen wall or via the saliva (Asplund, 1975). The adult ruminant is capable of secreting large amounts of saliva each day which results in large amounts of available ammonia.

Ammonia absorption is directly related to the concentration of ammonia in the rumen. Ammonia absorption decreases when large amounts of ammonia are produced

(such as from urea). Urea produces considerable amounts of ammonia because urea hydrolysis rate is four times as fast as the ammonia uptake (Preston, 1961). In vitro studies have provided estimates that the rumen microorganisms are capable of directly utilizing 30% of the ammonia formed. A very thin line exists between providing inadequate amounts and providing amounts that will result in large losses across the ruminal wall.

Amino Acid Metabolism

Whether microbial assimilation or fermentation of amino acids or peptides ensues, proteolysis is always the first step in utilization of proteins (Clarke and Bauchop, 1977). The quantity of amino acids produced depend on several things such as nitrogen intake, microbial population, and nitrogen source (Purser, 1970). The concentration of free amino acids in the rumen is usually low, and free amino acids are rapidly metabolized (Wright and Hungate, 1967). Microbial attack on amino acids is mainly by either decarboxylation or deamination, but in some cases by other reactions (Wright and Hungate, 1967). In the rumen when pH values are near neutrality, deamination is the most predominate route of amino acid catabolism. The major fermentation products from amino acid metabolism in the rumen are carbon dioxide, ammonia, and volatile fatty acids, including the branched chain acids that are growth factors for certain rumen bacteria (Allison, 1970).

Amino acids after absorption are transported to the liver where they may be metabolized to synthesize proteins or glucose (gluconeogenesis) or transported to an amino acid pool (Purser, 1970). Many believe that the ruminant animal is capable of using gluconeogenesis from amino acids to meet their energy requirements. It would seem to be somewhat of a paradox for an animal that desperately needs amino acids for protein synthesis could use large amounts of amino acids to supply energy. The specific amino acids involved in gluconeogenesis are uncertain at the present.

Microbial protein is the major nitrogen containing substance leaving the rumen. Thaysen (1945) estimated that 180 g of microbial protein passes out of the rumen of an adult ruminant each day (McDonald, 1954). Exact amounts of bacterial and protozoal protein is hard to determine because of different rates of flow of material out of the rumen and may also vary extremely with different diets. Bacterial protein provides from 55-85% of the total protein supplied. Hungate (1966), feels that protozoa protein contribute nearly 20% of the total microbial protein produced each day.

Ruminal By-Pass Of Proteins And Amino Acids

Proteins that are protected from microbial degradation in the rumen have obvious advantages particularly for high quality proteins, since they could pass through the rumen without the losses associated with microbial attack (Asplund, 1975). The advantages of by-pass protein and amino acids are numerous. The most obvious would be the greater efficiency achieved by the ruminant animal due to direct absorption of protein and amino acids instead of converting them to microbial protein in the rumen. However, the microbial attack of lower quality proteins in the rumen may actually be an advantage to the animal in that the protein quality may be upgraded. Usually improvements in growth rate, milk production, and wool growth are seen when proteins escape microbial degradation. The improvements are related to the proportion of protein that passes through the rumen undegraded. However, to obtain positive response with by-pass proteins, the protein must be of high quality.

Amino acids can by-pass the rumen in many ways. Researchers have used a variety of methods such as chemical treatments and the use of analogs and their derivatives.

The unique anatomy of the ruminant animal has also increased interest in manipulating the metabolism of the rumen and trying to achieve esophageal groove

closure.The best ruminal by-pass method appears to be the closure of the esophageal groove. Esophageal groove closure is a common function in the young ruminant. In the young animal, sucking milk causes closure of the esophageal groove thereby directing the liquid to the abomasum and by-passing the rumen. Many factors such as age, temperature of the liquid, posture of the animal while drinking, and chemical composition of the liquid affect the esophageal groove closure. Chemicals like copper sulfate also cause the closure of the esophageal groove (Waston and Jarrett, 1941). Esophageal groove closure appears to be a conditioned reflex. Regardless whether the liquid is from a bottle or trough, liquid consumed for quenching the animal's thirst always entered the rumen whereas, liquid that is consumed for "pleasurable anticipation" established during rearing entered the abomasum (Chalupa, 1975). Therefore, it may be difficult to achieve esophageal groove closure in the adult ruminant. Because the esophageal groove closure is the only naturally occurring by-pass mechanism in the ruminant animal, more research is required to fully develop its potential in the area of ruminant nutrition.

Feed processing plays a very important role in digestion of the feed ingredient. There are a variety of processing procedures which can affect the digestion sites of protein. Increased ruminal degradation may be the result of the disruption of the protein matrix whereas, heat applied or generated during grain processing can reduce ruminal degradation of protein (Hale, 1973). However, excessive heating can have a negative effect on the nutritional value of the feed ingredient. Other grain processing procedures can increase the amount of starch released in the rumen and allow for increased microbial protein production. Destruction of certain inhibitors such as trypsin in soybeans can also increase animal performance. The amount of heat damage can be determined by evaluating for acid detergent insoluble nitrogen (ADIN). ADIN can be closely associated with

the irreversible binding or destruction of amino acids (Goering et. al., 1972). Several grain processing procedures provide a potential mechanism for manipulating the quantity of dietary protein that is capable of by-passing the rumen (Chalupa, 1975).

Methods of Protecting Amino Acids

Chemical treatment of proteins forms reversible cross linkages with the amino and amide groups which decrease the solubility of the protein in the rumen. The chemically treated proteins are then degraded by the acidic pH of the abomasum by breaking the cross linkages formed with the amino and amide groups. Many chemicals have been used but aldehydes and tannins are the most predominately used chemicals. Formaldehyde has been used more frequently than any other chemical agent (Chalupa, 1975). Feeding formaldehyde treated casein has increased wool growth, nitrogen retention, and muscle growth; however, formaldehyde treated plant proteins have not always yielded positive results. Because of the inconsistent results, additional research is warranted.

Several new products have been devised to protect the free amino acids from ruminal degradation. Methionine coated with kaolin and triglycerides, when fed to ruminants allowed methionine for absorption in the small intestine (Sibbald et. al., 1968) as evidenced by increased free methionine level in the blood. Neudoerffer et. al.(1971) have used a similar approach to encapsulate amino acids. The capsule was produced by extruding a mixture of saturated fat and DL-methionine. The methionine was liberated by either enzymatic hydrolysis of the triglycerides or by physical abrasion caused by peristalsis (Neudoerffer et. al., 1971). They noted that nearly 60-65% saturated fat encapsulated methionine in the kaolin product would be available for absorption in the lower gastro intestinal tract. Apparently,the capsule was not completely resistant to ruminal degradation as approximately 30% was broken down in the rumen.

Kaufman et. al. (1980) investigated various methods of protection - fromaldehyde treated (HMM-Ca) and two types of fat coating on methionine degradation in the rumen. Cows that were fed 10-18 g/day of unprotected or protected methionine showed the following amounts of undegraded methionine reaching the small intestine - 0-5% with unprotected DL-methionine, 15-20% with fat coated I, 20-35% with HMM-Ca, and 50-60% with fat coated II methionine product. Arambel et. al. (1983) reported that 71.9% of methionine contained in ketionin escaped ruminal degradation. Because fecal samples had only 19.2% of methionine, it was concluded that 52% of methionine was probably absorbed from the intestinal tract.

Another method of protecting amino acids is to rearrange the structure of the amino acid in order to make it more resistant to microbial attack in the rumen. However, such structurally rearranged amino acids must be absorbable in the small intestines and also be metabolized in body tissues. Today many researchers have used the hydroxy analog of methionine. This form seems to have longer stability in rumen fluid in vitro than methionine. Belasco (1980) found that methionine was completely degraded but, only one-half of the MHA had been degraded in the rumen fluid. In cows fed carbon-14 labeled MHA and methionine, it was determined that MHA was more resistant to ruminal degradation than methionine (Belasco, 1980). However, others concluded that MHA is degraded in the rumen. Lewis and Emery (1962) assigned amino acids into three classes according to their rates of deamination. Methionine was placed in the third group, in which deamination was less pronounced. Cystine, another sulfur amino acid, has a fast deamination rate and seldon leaves the rumen undegraded. Researchers conclude that certain ruminal bacteria can actively utilize cystine but are only passively able to use methionine. It has been reported that protozoa are capable of ingesting preformed amino acids (Allison, 1970). It is suggested that increased protozoal numbers associated with

prolonged methionine supplementation may enhance the methionine protection (Bird, 1972). Langer et. al.(1978) have noted that MHA can be converted to methionine in the tissues of ruminants; however, little if any escaped the rumen undegraded.The use of analogs still needs to undergo further research to determine their value in ruminant nutrition.

Amino Acid Availability

An important aspect of amino acids is the methods of determining their availability. Today, with the use of ion-exchange chromatography, amino acid levels in most feedstuffs can be obtained easily. The levels obtained by ion-exchange are only potential levels that can be used by the nutritionist. It is a commonly known fact that amino acid levels in many feeds fall far short of its predicted potential values. This can be attributed to the fact that not all amino acids are made available to the animal during digestion. Amino acid availabilities can be reduced due to processing and storage. The introduction of an unfamiliar by-product or protein rich feedstuff triggers the need for obtaining a rapid and meaningful means of assessment (McNab, 1979).

The method of determining amino acid availability depends on two quantities; first, the extent to which the protein is broken down into smaller units and made available to be absorbed by the animal and secondly, the extent to which these units can be usefully assimilated (the biological value). Normally amino acid digestibility studies are done in vitro whereas, biological values are assessed in vivo.

Probably the most commonly used method of expressing dietary amino acid availability is based on animal performance such as growth and production. Normally, when amino acids are added to the diet they are considered to be 100% available. However, the most negative drawback of animal performance studies are that they are not solely dependent on amino acid availability. Consideration must

be given to such factors as environmental conditions, level of dietary protein, interactions with other amino acids, and the amount of feed consumption. Regardless of the drawbacks, some form of animal performance studies is required to fully determine the amino acid availability.

Another method to determine amino acid availability is based on enzymatic assays. The feed is digested with proteolytic enzymes under controlled conditions and the undigested materials are separated and the peptides and amino acids are examined by a variety of techniques. Enzymes that have been used include pancreatin, pepsin, and papain.

Microbiological procedures are available to determine protein and amino acid quality of many feed ingredients. Streptococcus zymogenes, a very active proteolytic microorganism, requires certain amino acids (arginine, histidine, isoleucine, leucine, valine, methionine, and tryptophan) for optimum growth. The amount of bacterial growth can determine the amount of amino acids that can become available to the animal during digestion and metabolism (McNab, 1979).

Methods with fecal analysis have been developed to determine the amount of amino acids in proteins. The major drawback with these assays are that endogenous amino acids are difficult to determine. It is strongly believed that dietary factors, especially protein, may influence the secretion and excretion of endogenous amino acids (McNab, 1979). It may be possible that the microflora of the intestine may deaminate the amino acids of the undigested protein residues. Results obtained with fecal analysis have therefore tended to overestimate amino acid availabilities for the animal.

Because dietary proteins are carried to the tissues as free amino acids, plasma free amino acid levels would be a good indication of available amino acids for digestion in the animal. However, the plasma free amino acid levels have not been good indicators of amino acid availability due to the fact that the plasma

system is a very dynamic system and keeps a constant balance between supply and demand (McNab, 1979).

More research is needed to provide accurate information on the amino acid availability to the animal. At present, the incorporation of more than one of the assays mentioned above can supply beneficial information to the nutritionist.

Methionine Discovery And Structure

Methionine was discovered in 1923 by Muller as a sulfur containing amino acid contained in casein hydrolysate that was unlike cystine or cysteine. Later, Borger and Coyne, determined the structure and named the amino acid, methionine, (Degussa,1982). The name methionine was given because the amino acid is actually a methyl-thioether. Methionine has a molecular weight of 149.2, is highly stable, and decomposes at 270-273 C. Methionine is colorless to a weakly yellowish in color and its odor is somewhat similar to an organic sulfur compound.

Unlike most amino acids, both the D and L forms of methionine can be utilized in the body. Therefore, during synthetic production of methionine, both D and L forms are produced in equal proportions. When D and L forms are presented together the D-form cannot be used as such for the biosynthesis of proteins, but can be utilized by the organism, (Degussa,1982). All metabolic systems have an enzyme network that attack the D-form and through transformations convert the D-form to the active L-form. First the D-form of methionine is oxidized enzymatically to an inactive α-keto acid and then converted to the L-methionine by transamination, (Degussa,1982). The reactions occur very rapidly and efficiently. The D-form of methionine in the body is thus converted to the L-form where it can be used for protein synthesis and methylation. Studies with in vitro experiments have shown that the L-form of methionine is absorbed from the intestines and in the kidneys better than the D-form of methionine. The difference in absorption

rate may be due to the fact that the L-form is actively transported while the D-form of methionine is passively transported.

One form of methionine that has predominantly been used in swine, poultry, and lactating dairy cattle studies is methionine hydroxy analog (MHA). In MHA, the amino group has been replaced with a hydroxy group. Like methionine, MHA can be represented with and D and L-form and is produced technically as 50% D and 50% L-form. The hydroxy analog itself is relatively viscous, malodorous, and because of these disadvantages, is produced commercially as a calcium salt to add stability. Biochemically the MHA product is converted to the L-form of methionine. However, both the D-form of MHA and the L-form of MHA must be converted to the L-form of methionine.

Role Of Methionine In Metabolism

Methionine, is involved in many different roles and functions. It is an essential amino acid of all naturally occurring proteins. Most researchers tend to agree that methionine is an essential amino acid for monogastric animals. The ruminant animal is only slightly dependent on the amino acid composition entering the body because the microflora is capable of synthesizing enough of the essential amino acids to maintain proper body functions. Loosli and his co-workers (1949) have shown that all ten essential amino acids are synthesized in large amounts in the ruminant animal with urea as the main nitrogen source.

Methionine, is involved in many metabolic systems. One very important reaction involves the transmethylation where it serves as the primary methyl donor to many body reactions. Another reaction involving methionine is the transsulfuration. In this reaction, the sulfur portion of methionine is incorporated into cysteine and the carbon skeleton is converted into alpha-ketobutyrate (Schepartz, 1973). This pathway has been known to cause the greatest turnover of methionine. Because ketobutyrate can be decarboxylated to form propionate,

methionine is considered to be a glucogenic compound. However, under practical conditions, the conversion of methionine (rather its demethylated derative homocysteine) to ketobutyrate may be too slow to form glucose rapidly enough to meet the animal's energy needs (Schepartz, 1973).

Methionine has also been classified as a lipotropic factor. As a methyl donor, methionine is closely related to the synthesis of lipoproteins from lipids and apoproteins (Harper et. al., 1979). In vitro experiments have shown that methionine stimulated lipid synthesis (Patton et. al., 1968). Many researchers believe that lipids may be stored by rumen microorganisms for energy sources. The microorganisms in the rumen that have the greatest lipid association are the protozoa. Supplementation of methionine or hydroxy methionine increased the synthesis of both the polar lipids and other lipid precursors and rumen microorganisms could convert both acetate and glucose to long chain fatty acids (Patton et. al., 1968).

Gil et. al. (1973) noted that rumen microorganisms depleted of energy substrates were unable to utilize MHA as an energy source for growth. Rumen inocula from animals receiving cellulose, corn starch, urea, and water in vitro resulted in decreased cellulose digestion without the addition of methionine. However, the addition of methionine appeared more effective than MHA (Salsbury et. al., 1971).

MHA seems to increase protozoal numbers in the rumen on an all grain diet (Patton et. al., 1970). Both MHA and DL-methionine have increased the incorporation of bacterial nitrogen and stimulated substrate digestion rates (Polan et. al., 1970; Gil et. al., 1973). Patton et. al.(1968) noted stimulation of metabolic activity of the microorganisms in rumen fluid when methionine and its analog (DL-hydroxy methyl mercapto butyrate-calcium) were used.

It has been postulated that the shortage of methionine during early stages of lactation may be important in the development of bovine ketosis. Methionine may be connected in preventing primary ketosis from going into secondary ketosis (McCarthy, 1968). Thus it is believed that methionine may play a very important role in the prevention of ketosis (Griel et. al., 1968).

Also, feeding of MHA has improved reproduction efficiency in beef cattle (Clanton and England, 1980) and in lactating dairy cows (Fuquay et. al., 1978).

Role Of Methionine In Milk Production

In dairy cows, amino acids serve four fundamental functions - maintenance, precursor for glucose synthesis (gluconeogenesis), for protein in muscle or associated with fetal growth, and for the synthesis of milk protein (Tamminga and Oldham, 1980). The ruminant is very much like the nonruminant in that essential amino acids must be supplied from either the digestive system or from the catabolism of body proteins. Also, most ruminants receive adequate amounts of essential amino acids from the diet except the lactating dairy cows in the early part of lactation (Hogan, 1975).

Methionine has been suggested to be a limiting amino acid during early stages of lactation in the dairy cow (Jacobson et. al., 1967; Hogan, 1975; Schwab et. al., 1976; Kaufman et. al., 1980). The limiting amino acids for meat and wool production are lysine and cysteine, respectively, (Hogan, 1975).

However, supplementing unprotected methionine (free amino acid) resulted in very rapid conversion to ammonia by the rumen microorganisms (Salsbury et. al., 1971). Since MHA was more resistant to the microorganisms, many studies have been conducted with MHA. Feeding MHA (25-40 g) has increased total milk production (Jacobson et. al., 1967; Griel et. al., 1968; Hutjens and Schultz, 1970; Patton et. al., 1970; Bishop, 1971; Bhargava et. al., 1977). In certain studies, MHA has increased milk fat (Hutjens and Schultz, 1970; Polan et. al., 1970; Bishop, 1971;

Kim et. al., 1971; Rosser et. al., 1971; Holter et. al., 1972; Fosgate, 1973; Steele et. al., 1973; Bhargava et. al., 1975; Chandler et. al., 1976; Bhargava et. al., 1977). The milk fat increase was usually noted in younger cows in the first twelve weeks of lactation. However other researchers (Fisher, 1969; Teichman et. al., 1969; Burgos and Olson, 1970; Hutjens and Schultz, 1971; Fisher, 1972; Whiting et. al., 1972; Fuquay et. al., 1974; Olson and Grubaugh, 1974; Wallenius and Whitchurch, 1975; Schwab et. al., 1976; Kenna and Schwab, 1981; Stokes et. al., 1981) have not been able to show a positive response from feeding MHA or MHA like products. Olson and Grubaugh (1974) state that the age of the cow and stage of lactation may be the greatest determining factors on the demands for additional methionine.

Milk Fat Production

In the lactating animal, the largest demand for methionine is for the synthesis of milk proteins and as the principal methyl donor in the formation of phospholipids.

McCarthy et. al. (1968) have suggested that methionine may have a special function in binding lipid and protein moieties in the formation of serum lipoproteins.

Under normal conditions during early lactation, fiber intake is decreased resulting in depressed milk fat. Feeding MHA during this period may increase the microbial activity in the rumen which might further stimulate fiber digestibility (Holter et. al., 1972). The increased fiber digestibility may help alleviate the milk fat depression by correcting the ruminal acetate-to-propionate ratio.

In a trial conducted by Rosser et. al., (1971) with 40 g of MHA added to the ration, an increase in ruminal acetate and butyrate values and a decrease of ruminal propionate were observed. MHA plays a very important role in milk fat synthesis by providing better availability of fatty acids (McCarthy et. al., 1968; Hutjens and Schultz, 1971).

Patton et. al., (1968) demonstrated methionine stimulated lipid synthesis in vitro with the rumen microorganisms. After feeding methionine, Patton et. al. (1970) noted an increase of polar lipids formed which is directly related to microbial growth. Greatest proportion of lipids in the rumen were associated with the protozoa. Increased numbers of protozoa, which are highly digestible and contain large amounts of lipids (Hungate, 1966), may explain the increased fat production associated with feeding MHA. Free fatty acids and triglycerides in the α -serum lipoproteins were reduced in cows receiving MHA compared to cows receiving no MHA (Polan et. al., 1970).

One can conclude that the increased synthesis of polar lipids and the better utilization of fatty acids as well as other lipid precursors with methionine or MHA supplementation indicates that this amino acid may be limiting in the nutrition of certain rumen microorganisms, mainly the rumen protozoa (Patton et. al., 1970).

Milk Protein Production

It has been firmly established that the free amino acids of the blood are the principal precursors for the milk proteins synthesized in the mammary glands. From tracer experiments done by Campbell and Work (1952) it was noted that valine, lysine, and methionine of casein are obtained from the free amino acids circulating in the plasma and not from the plasma proteins.

Verbeke and Peeters (1965) observed large concentrations of free amino acids in both arterial and venous plasma in the lactating cows. In their studies, glycine represented the greatest amount, making up nearly 25% of the total α-amino N. Very low concentrations of aspartic acid, cystine, and methionine were found. They have also shown that concentrations of most amino acids of the blood decrease during passage across the lactating mammary gland. The amino acids in the blood are thus incorporated into milk protein. Results obtained in perfusion experiment on the

isolated cow's udder have suggested that part of the non-essential amino acids may be synthesized in the udder from volatile fatty acids present in the blood.

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INTRODUCTION

Although ruminal microflora are capable of synthesizing all essential amino acids in required amounts (Loosli et. al., 1949; Block and Stekal, 1950; Virtanen, 1966), methionine has been indicated as a limiting amino acid in dairy cattle diets (Jacobson et. al., 1967; Hogan, 1975; Schwab et. al., 1976; Kaufman et. al., 1980).

Feeding methionine hydroxy analogs (MHA) has increased milk and milk fat yields (Griel et. al., 1968; Polan et. al., 1970; Bishop, 1971; Bishop and Murphy, 1972; Bhargava et. al., 1977). However, positive response was not always obtained with MHA supplementation (Hutjens and Schultz, 1971; Whiting et. al., 1972; Steele et. al., 1973; Fuquay et. al., 1974; Olson and Grubaugh, 1974; Walleneus and Whitchurch, 1975). Such inconsistent results may in part be due to the fact that MHA may be somewhat unstable in the rumen.

Unless methionine is supplied to the ruminant animal in a manner protected from microbial attack, it will be rapidly deaminated (Salsbury et. al., 1971; Langer et. al., 1978). The ammonia produced in the rumen is either used as a nitrogen source to synthesize amino acids by microorganisms or absorbed and excreted as urea. Recently, a protected methionine product called Ketionin has been tested and marketed for dairy cattle in Europe. Previous work done in this laboratory with the product suggested that 71% of the methionine escaped ruminal degradation and 52% gets absorbed from the intestinal tract (Arambel et. al., 1983).

The purpose of this study was to determine the effects of Ketionin on milk production in dairy cows.

MATERIALS AND METHODS

Forty-eight multiparous Holstein cows were used. The cows were fed 9 kg of a 14% crude protein grain mixture and prairie hay ad libitum during the last three weeks of the dry period. After calving, they were fed alfalfa hay and a control

concentrate mixture ad libitum (Table 1), and ca. 9 kg corn silage daily. The cows were fed twice daily and daily feed intakes were recorded.

Two weeks after calving, the cows were divided into three treatment groups. The groups were balanced according to milk produced during the second week of lactation. One group served as control and the other two received Ketionin at 50 g and 80 g of the grain diet for 8 weeks. The cows were housed in free-stall barns with water and a 50:50 mixture of dicalcium phosphate and salt available at all times. The treatment groups were housed in separate but adjacent barns.

Individual milk weights were recorded at each milking. Milk composition was determined on 24 hour composite samples collected at 2, 4, 6, 8, and 10 weeks. Milk was analyzed for fat content by the Milko-tester (AOAC, 1975), for protein by the dye-bind method (Udy, 1956), and for solids-not-fat by the method of Golding (Golding, 1959). Cows were weighed at two-week intervals before the morning feeding. All data were analyzed by a randomized complete block design.

RESULTS

The milk production results are shown in Table 2. It is apparent that most of the response to Ketionin was between week 3 (the initiation of Ketionin feeding) and week 5. Consequently the data were analyzed for two periods - weeks 3 through 5 and 6 through 10 (Table 2). During weeks 3 through 5, the cows receiving both levels of Ketionin produced more milk than did the controls. However, only the milk production of the cows receiving 50 g Ketionin was significantly different from controls ($P<.05$). Ketionin had no effect on milk production during weeks 6 through 10, ($P>.05$).

Milk fat percentage was significantly ($P<.05$) lower in cows receiving 50 g Ketionin than those in the control or 80 g group (Table 2). This was surprising because methionine supplementation was expected to increase milk fat percentage. Milk fat percentage is usually inversely correlated with milk production, and the

Table 1. Percentage composition and analysis of feeds.

Ingredients (as fed basis)	Ketionin concentration (g) 0	50	80
	(%)		
Rolled corn	83.4	83.10	82.9
Soybean meal	10.0	9.96	9.94
Starea (70%)[a]	4.0	3.98	3.98
Dicalcium phosphate	2.0	1.99	1.99
Trace mineral salt	.5	.50	.50
A&D vitamin supplement[b,c]	.1	.10	.10
Ketionin		.36	.57
Crude protein (Dry matter basis)			
Grain diet	17.5	16.0	17.5
Alfalfa hay	18.6		
Corn silage	8.3		
Dry matter			
Grain diet	88.4	88.7	88.5
Alfalfa hay	89.2		
Corn silage	41.5		

[a]Starea is an extrusion processed mixture of grain and urea.

[b]1000 IU vitamin A/lb grain diet.

[c]500 IU vitamin D_3/lb grain diet.

TABLE 2. EFFECT OF KETIONIN ON AVERAGE MILK PRODUCTION, MILK COMPOSITION, BODY WEIGHT CHANGE, AND PROTEIN EFFICIENCY

	Weeks 3-5 Ketionin (g/day)			Weeks 6-10 Ketionin (g/day)		
	0	50	80	0	50	80
Milk production (kg/day)	30.0[a]	31.6[b]	30.5[a]	30.2[a]	31.2[a]	30.7[a]
Milk fat (%)	3.55[a]	3.11[b]	3.45[a]	3.17[a]	2.94[a]	3.33[a]
Milk protein (%)	3.03[a]	2.99[a]	3.03[a]	3.04[a]	3.06[a]	3.08[a]
Milk protein (kg/day)	.91	.95	.92	.92	.95	.95
Milk SNF (%)	8.82[a]	8.68[a]	8.65[a]	8.62[a]	8.53[a]	8.69[a]
Weight change (kg/period)	-16.73[a]	-6.64[a]	-14.23[a]	-9.09[a]	+2.05[a]	-7.36[a]
Protein adjustment for weight change	.25	.10	.22	.08	+.03	.07
Maintenance protein (g/day)	459	459	459	459	459	459
Feed protein (kg/day)	3.06	2.98	3.13	3.19	3.11	3.27
Net protein efficiency (g/kg milk)	78.2	76.7	80.5	87.4	85.9	89.1
(g/g milk protein)	2.59	2.56	2.66	2.88	2.80	3.00

[a,b]Values within columns with different superscripts differ ($P < .05$).

lower fat percentage for the group receiving 50 g Ketionin may reflect the increased milk production for this group. Milk protein and SNF percentage were not affected by treatments (P>.05).

While cows receiving 50 g Ketionin produced the most milk, they also gained weight from 3 through 10 weeks while cows in the other groups lost weight (Table 3). This suggests that body fat reserves for the cows receiving 50 g Ketionin were not depleted as rapidly during peak production as is expected for high producing cows and as occurred in the control and 80 g Ketionin groups.

Feed intake (Table 3) was similar among groups and it would not be possible to ascribe the significant increase in milk production for the 50 g Ketionin group to the slightly greater grain intake for this group. The concentrations of Ketionin used in this study did not in any way reduce feed intake as it was once feared that it might do. Also, the protein content of the grain diet containing .36% Ketionin was consistently lower than those of the other two diets. This may have been due to sampling variation. In any event the group that received the least protein (50g Ketionin group) produced the most milk.

There were no significant differences in reproductive efficiency between the controls or 50 g and 80 g groups based on number of services per conception (Table 4). However, three cows were removed from the 80 g group and one cow from the control group because they were classified as problem breeders (requiring more than three services). One cow in the 50 g group was palpated as having cystic ovary and was not included in calculating the reproductive efficiency.

DISCUSSION

Methionine has been suggested as being the first limiting amino acid for lactating cattle. Apparently microbial synthesis of methionine in the rumen is inadequate. Also, because milk protein contains large amounts of sulfur containing amino acids. High producing dairy cows need additional methionine to meet the

TABLE 3. EFFECT OF KETIONIN ON AVERAGE DAILY FEED INTAKES

	Weeks 3-5			Weeks 6-10		
	Ketionin (g/day)			Ketionin (g/day)		
	0	50	80	0	50	80
Consumption (kg/day)						
Grain	11.77	12.22	12.03	12.18	12.86	12.75
Alfalfa hay	3.77	3.89	3.89	3.97	4.01	3.89
Corn silage	3.70	3.66	3.68	3.77	3.72	3.79
Feed protein	3.06	2.98	3.13	3.19	3.11	3.27

Table 4. Effect of ketionin on reproductive efficiency (service per conception).

Control (0 g/day)		Ketionin (50 g/day)		Ketionin (80 g/day)	
Cow No.	No. of services	Cow No.	No. of services	Cow No.	No. of services
K56	3	421	2	312	2
K16	1	K14	3	392	3
K25	1	K3	3	K10	1
593	2	601	1	513	2
K27	3	529	1	K35	3
K8	1	550	1	597	1
411	2	618	1	320	2
605	1	609	1	273	2
577	1	619	3	606	2
K28	2	612	2	537	2
K30	2	584	1	616	2
490	1	450	1	456	3
603	1	K19	1	579	1
641	1	545	1		
494	3	K52	2		
$\bar{x}$ = 1.667		$\bar{x}$ = 1.600		$\bar{x}$ = 2.000	

demand. Inclusion of MHA has improved milk and milk fat yield (Griel et. al., 1968; Polen et. al., 1970; Bishop et. al., 1971; Bhargava et. al., 1977). However, several have failed to show a positive response (Fisher, 1972; Fuquay et. al., 1974; Kenna Schwab. 1981; Stokes et. al., 1981). Probably because of the instability of the analog in the rumen (Emery, 1971; Gil et. al., 1973; Langer et. al., 1978). Ketionin appeared to be a stable product because 71% methionine in the product escaped ruminal degredation (Arambel et. al., 1983).

Supplementation of 50 g Ketionin per day per animal increased the total milk production during early lactation (3-5 weeks). Surprisingly the 80 g group showed no response in milk yield compared to the control group. This difference in milk production between 50 g and 80 g groups is difficult to explain. Olson and Grubaugh (1974) reported that the age of the cow and stage of lactation may be the greatest determining factors on the demands for additional methionine. The demand is greatest in young cows in the first twelve weeks of lactation. Cows used in our study were multiparous and the only positive response in milk production with 50 g Ketionin was during the initial lactation period (3-5 weeks).

Milk fat content in Ketionin-fed cows was either depressed (50 g group) or remained unchanged (80g group). In most studies a significant increase in milk fat content has been reported with MHA supplementation (Griel et. al., 1968; Polan et. al., 1970; Bhargava et. al., 1977). MHA was usually fed at 25-40 g per head per day. Assuming that most of the MHA is resistant to microbial attack, the dose was substantially higher than the amount of methionine fed in our study. Methionine concentration in Ketionin was 28.7 % which amounts to 14.35 and 22.96 g of true methionine in 50 g and 80 g Ketionin respectively. Arambel et. al. (1983) observed that about 52% of methionine contained in Ketionin was available for intestinal absorption. So cows fed 50 g or 80 g Ketionin received only 7.5 g and 11.9 g true methionine respectively for absorption. The low level of methionine fed in the

study may account for the lack of response in milk fat yield. In most studies where a significant response in milk yield was shown ,the dietary crude protein content ranged from 12.5% to 15.5% (Griel et. al., 1968; Chandler et. al., 1976; Bhargava et. al., 1977). Crude protein of the grain concentrate fed in our study was 16.0% and 17.5% for the 50g and 80 g Ketionin groups respectively. Such high protein levels are close to the NRC recommended values for high-producing dairy cows. It is possible that we may have approached a protein sparing effect during the initiation of lactation and exceeded the requirement during late lactation (weeks 6-10). Apparently dietary crude protein content is a critical factor in determining the advantage of amino acid supplementation. It is logical to expect a significant response with methionine supplementation under conditions where the protein content is limited. This may explain the commercial exceptance of Ketionin in Europe where cattle , unlike in the United States , are traditionally raised on high roughage low grain diets.

Also, in our study possibly influence of other limiting amino acids besides methionine was not taken into consideration. Chalupa (1981) stated that with corn and corn silage diets, lysine may be more limiting than methionine to dairy cows. The diet fed in our study consisted of corn and corn silage.

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EFFECT OF PROTECTED METHIONINE ON MILK PRODUCTION IN DAIRY COWS

by

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Methionine is considered to be a limiting amino acid for lactating dairy cattle. However, supplementation of free dietary methionine is of no benefit because it is readily degraded in the rumen. Hence, methods have been devised to protect methionine from microbial attack in the rumen. A protected methionine product called Ketionin is widely used in Europe. Ketionin contains DL methionine (30%) protected with saturated and unsaturated fatty acids and calcium carbonate. Preliminary studies indicated that 71% of methionine contained in the product escaped ruminal degradation. Because only 19% of methionine was excreted in the feces it was assumed that 52% was probably absorbed from the intestine. The objective of this study was to test the effect of Ketionin on milk production in dairy cows.

Forty-eight multiparous dairy cows in their third week of lactation were divided into three groups. Groups were balanced according to the milk production recorded in the second week of lactation. One group served as the control and the other two groups received in the diet 50 g and 80 g Ketionin per head per day, respectively. The diet consisted of alfalfa hay and a corn grain and soybean meal concentrate mixture. Milk production was recorded dairy and milk samples collected at the middle of weeks 2, 4, 6, 8 and 10 were analyzed for fat, protein and solids not fat. Cows were weighed at two-week intervals. Daily feed intake was recorded. The treatment period lasted for 8 weeks.

Cows receiving Ketionin generally produced more milk than the controls during 3 to 5 weeks. However, the difference was significant ($P<.05$) only in cows receiving 50 g dose. Ketionin had no effect on milk production during 6 and 10 weeks. Milk fat was lower ($p<.05$) in cows fed 50 g Ketionin than those in control or 80 g group. Milk protein and solids not fat were unaffected by Ketionin. Feed intake was similar in all three groups. Cows in 50 g group gained weight during the treatment period while cows in the other two groups lost weight.