IMPACT OF SULFUR LEVELS AND SOURCES FOR GOATS

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CHAPTER I

INTRODUCTION

Sulfur (S) is an abundant element and is important nutritionally for both plants and animals, where both deficiencies and toxicities can occur (NRC, 1981). Sulfur is an essential nutrient for ruminants (NRC, 1991). Because sulfur interacts with copper, molybdenum and selenium (Spears, 1991), its requirements are difficult to predict. Sulfur is a component of proteins, enzymes, antibodies, some vitamins and several hormones. It is found in three amino acids - methionine, cysteine and cystine - and their derivatives. In addition, sulfate is an important constituent of the chondroitin sulfates in cartilage of the mucins in the gastrointestinal tract, saliva, reproductive tract, and other duct systems.

For ruminants, sulfur is needed at two sites, 1) systemically, to meet the need for specific organic compounds that the body cannot synthesize, and 2) ruminally, to meet the needs of ruminal microbes.

Research concerning the amino acid supply for ruminants has been challenging because of their unique anatomical and physiological features of the digestive tract. Microbes of the rumen buffer the supply of amino acids included in the diet and ferment them as a source of energy. Several studies have indicated that simply adding amino acids to the diet will influence neither animal performance nor plasma amino acid concentrations because dietary amino acids

are degraded in the rumen to ammonia and volatile fatty acids (Chalupa, 1976; Reis, 1978).

To determine whether the quantity of amino acids supplied to tissue limits production of ruminants, supplemental amino acids must be administered by some manner that avoids ruminal destruction and makes them available for absorption in the small intestine. This can be achieved through administering amino acids through a cannula post-ruminally or protecting amino acids or protein from ruminal attack. Several studies have shown that post-ruminal amino acid administration can increase productivity of ruminants (Reis and Schinckel, 1963; Schelling and Hatfield, 1968; Sahlu and Fernandez, 1992; Rulquin et al., 1993). Abomasal infusion of cysteine, methionine, or casein often increases wool production in mature sheep.

Thanks to microbial action in the rumen, inorganic nitrogen and sulfur provided in the diet can be converted to organic compounds to meet a portion or all of the metabolic requirements for these nutrients by the host ruminant. Combined with energy from cellulose sources, diets for ruminants can be formed from substances that are nutritionally inadequate for nonruminant mammals.

Loosli et al. (1949) was able to maintain weight of sheep by feeding a purified diet containing no intact protein. They found that all of the essential amino acids were synthesized in the rumen. Later, Schelling et al. (1967) compared amino acid compositions of protein from whole egg protein with that of microbial protein. Concentrations of essential amino acids were slightly lower in ruminal microbes than in whole egg protein, suggesting that egg protein may be more useful nutritionally than microbial protein for ruminants. Up to 20% of bacterial crude protein (N times 6.25) is nucleic acid, resulting in a higher biological value for egg protein.

An objective assessment of amino acid requirements in ruminants requires detailed knowledge of both the quantity supplied (the sum of the dietary amino acid supply surviving ruminal digestion plus the microbial protein) and the quantity needed (calculated from performance responses to additional extra amino acids supplemented post-ruminally.) Alternatively, the quantity needed can be calculated from amino acid composition of retained tissue. Because amino acid composition of various ruminant products, e.g., wool, milk, meat, will differ, the quantity of individual amino acids needed also must differ. If amino acids are not supplied in the diet, as with a purified diet which contains no amino acids but only non-protein nitrogen as a source of crude protein, the only amino acids available to the animal will be those derived from microbial protein. Even when protein sources are fed, microbial protein will comprise nearly 50% of the total quantity available post-ruminally. Consequently, amino acid content and proportions present in ruminal microbes is important quantitatively and many workers have studied which amino acids in microbial protein may limit production of specific animal products.

Although specific strains of ruminal bacteria may differ slightly in proportions of individual amino acids, amino acid composition of the mixed population found in the rumen is thought to be virtually constant (Storm and Orskov, 1983). Constant composition of protein from various strains of bacteria is not surprising if such bacterial proteins have specific functions and thereby specific structures. However, nutrient deficiencies can alter chemical composition of bacteria. For example, with a shortage of ammonia, polysaccharide storage by bacteria is increased leading to a dilution of other cell components. Similarly, a deficiency of one component of protein, like sulfur, might be expected either to decrease synthesis of microbial protein and limit bacterial growth, or to force selection of ruminal bacteria containing lower

amounts of sulfur-amino acids. To date, changes in amino acid composition due to sulfur deficiency has never been quantified.

Several workers have attempted to determine which amino acid(s) in microbial protein might limit production. Through measuring responses in both plasma concentrations of amino acids and nitrogen retention by lambs infused abomasally with amino acids, Hume et al. (1972) and Schelling et al. (1967) determined that the first limiting amino acids in microbial protein were methionine and lysine. Storm and Ørskov (1983) harvested large amounts of microbial protein and infused it post-ruminally as the only source of protein. They concluded that the most limiting amino acids in the microbial protein for sheep were methionine, lysine, histidine, and arginine. Increases wool and mohair growth in sheep and goats fed typical diets have been noted when supplemental methionine has been supplied either via infusion into the abomasum (Reis, 1967, 1969; Dove and Robards, 1974), injection into the peritoneal cavity (Sahlu and Fernandez, 1992), or incorporated into the diet as chemically or physically protected against ruminal degradation (Wheeler et al., 1979; Oke et al., 1986; Fenn and Leng, 1989) or non-degraded analogues (Radcliffe et al., 1985). Unfortunately, some of these diets may have been deficient in sulfur which in turn might have decrease the potential post-ruminal supply of amino acids containing sulfur.

The major goal of feed formulators is to produce safe, efficient, and economical diets that meet nutrient requirements. Dietary sulfur can be provided in various forms, including the inorganic element, flowers of sulfur, sulfate salts, amino acids containing sulfur or as an analogs, e.g., methionine hydroxyl analog. Often, dietary supplementation with sulfur alone has increased production of milk by cows and fiber production by lambs and goats. Under such circumstances, supplements presumably are correcting a deficiency of sulfur in

the rumen which in turn enhanced ruminal outflow of amino acids containing sulfur.

This manuscript will examine the roles of sulfur and amino acids containing sulfur in production of ruminants and their importance as an integral components of various proteins.

The objectives of this study were:

1.) to determine if supplementing methionine in drinking water will increase the post-ruminal supply of methionine and enhance mohair growth.

2.) to determine dietary requirements for sulfur in young and adult Angora goats.

3.) to determine if nutrient deficiencies can alter chemical composition of bacteria. This is a test of the concept that: Amino acids composition of the mixed population found in the rumen is constant (Storm and Ørskov, 1983).

Chapters are prepared as manuscripts in the style of the Journal of Animal Science to facilitate publication of results.

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CHAPTER II

REVIEW OF LITERATURE

This chapter will outline literature concerning the role of sulfur in nutrition of the ruminant, and of methionine as the amino acid that may limit rate of mohair growth.

Sulfur, a nonmetallic element, has been placed in the 6a column of the periodic table. Located below oxygen and above selenium, but with properties similar to those, sulfur has an atomic number 16 and an atomic weight of 32.066. Sulfur oxidation states (sulfite, $SO_3^=$ and sulfate, $SO_4^=$) are +4 and +6; in its reduced state, as the sulfide, ($S^=$). sulfur has a valence of -2. Sulfide is the only form of sulfur which bonds with carbon atoms to form the typical sulfur-containing metabolites. Four naturally occurring isotopes are: 32 (95.0%); 33 (0.76%); 34 (4.22%); 36 (0.014%); the radioactive isotopes, 29-31, 35, 37, 38, have been used to study metabolism.

Sulfur occurs in the free state and in combinations, mainly as sulfides and sulfates, and constitutes 0.05% of the crust of the earth. Many of these deposits have a biological action. Organic materials like fossil fuels, formed during anaerobic degradation or putrefaction, also serve as a sources of sulfide. As a plant fertilizer, superphosphates often are used as a source of phosphorus. Typical superphosphates contain 1.4 to 11.9 % sulfur (White and Collins, 1982). Today, other phosphorus sources are replacing superphosphates; this may increase the prevalence of sulfur deficiencies in plants.

People have utilized sulfur since antiquity. Sulfur is recovered from underground deposits, trapped as hydrogen sulfide gas during the processing of petroleum products, and reclaimed following combustion of fossil fuels. Of the total production of sulfur, the primary product (80%) is sulfuric acid.

Sulfur oxides are the second most prevalent environmental pollutant after carbon monoxide. Stationary sources of sulfur oxide production include power stations and industrial processes; these are responsible for 16% of the total sulfur oxide release (Voet and Voet, 1990). As an aerosol, sulfur oxide gases reflect sunlight; models of the greenhouse effect indicate that sulfur pollution of the atmosphere helps to counterbalance the earth-warming effect of carbon dioxide.

Inorganic sulfur enters into biosynthetic pathways at the oxidation levels of sulfate ($SO4^{=}$) and sulfide ($S^{=}$). Other naturally occurring forms of sulfur, e.g., thiosulfate, polythionates, polysulfides, and elemental sulfur, must be either oxidized to sulfate or reduced to sulfide before they can enter biosynthetic reactions (Peck, 1970). Biologically, the interconversion of sulfate and sulfide is a reversible process: sulfate reduction is the reduction of sulfate to sulfide, whereas sulfide oxidation is the oxidation of sulfide to sulfate. Together, these steps are known as the "sulfur cycle".

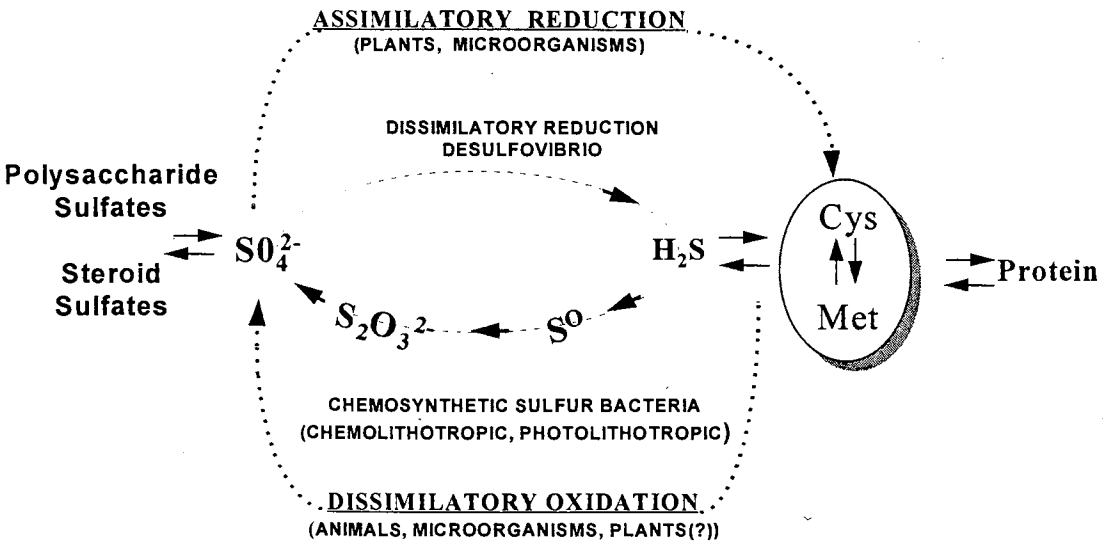

Figure 1. Schematic represetation of flow of sulfur through the biosphere, modified from Cooper et al. (1983).

The sulfur cycle.

Oxidation and reduction: As with others cycles, a variety of organisms play roles in both assimilation and dissimilation reactions (Figure 1). Mammals dissimilate sulfur by catabolizing sulfur amino acids to sulfate and carbon dioxide but cannot assimilate sulfur to fully meet their sulfur requirements from inorganic sulfur; instead, they rely on absorbed methionine and cysteine for their sulfur. In contrast to higher animals, most plants and many microorganisms have evolved mechanisms to assimilate sulfate into all the compounds they need for maintenance and growth. In ruminant nutrition, interest in sulfur has focused primarily on microbial synthesis of sulfur-containing amino acid and B-vitamins in the rumen although these compounds also can be degraded in the rumen.

Inorganic sulfur compounds play more extensive roles in nutrition of microorganisms than of either plants or animals. Certain strains of bacteria utilize sulfate reduction or sulfide oxidation for generating ATP. Reductive dissimilation of sulfate occurs in certain obligate anaerobes; sulfate is used as an electron acceptor and the hydrogen sulfide produced is largely lost into the

environment. Anaerobes can use sulfur, copper or iron exactly as aerobes use oxygen as the terminal electron acceptor (Cooper, 1983).

Other microorganisms oxidize inorganic sulfur compounds using sulfide, elemental sulfur, and thiosulfate as electron donors during aerobic or anaerobic respiration and photosynthesis and form large quantities of sulfate that result in extremely acidic conditions (Peck, 1970). Such compounds are responsible for the acidity of soils which complicates reclaiming mining sites.

Fixing Sulfur to Carbon: Conversion of L-serine to L-cysteine - Serine sulfhydrase (cysteine synthase) is widely distributed in the nature, including mammalian tissues, and has a very wide specificity. In fact, there are similarities between cysteine synthase and cystathione ß-synthase; because vertebrates do not posses a sulfate-reducing system, in vivo incorporation of sulfide (S^{2-}) into cysteine is of minor importance, and equilibrium lies far toward serine formation; the function of the enzyme in vertebrates is probably to synthesize cystathionine. Thus, cysteine synthase may be regarded as a variant cystathionine ß-synthase; both enzimes are C3 specific ß-replacing lyases.

In sheep, the pathway for the biosynthesis of cysteine from serine has been under study (Ward, 1984; Ward et al., 1989). Cysteine is an essential amino acid except by conversion from methionine. There are two key elements in the pathway for cysteine synthesis from serine as showing reaction 1 and 2:

(1) L-Serine + Acetyl-CoA----------> *O*-acetyl-L-serine + CoA. *(serine transacetylase)*

(2) O-acetyl-L-serine + H_2S----------> L-Cysteine + H_20 + Acetate. *(o-acetyl serine sulfhydrylase)*

For a number of years *cysteine synthase* was believed been responsable for the incorporation of sulfide by the synthesis of cysteine from serine and sulfide (Peck, 1970).

(3) L-serine + H_2S <----------> L-cysteine + H_2O. (*cysteine synthase)*

Methionine synthesis: In mammals, methionine serves as a precursor of homocysteine and cysteine. In plants and microbes capable of synthesizing methionine, the major route of methionine synthesis is reversal of this process and involves cysteine and homocysteine. In microorganisms, ß-cystathionase splits the C-S bond resulting in formation of homocysteine. In mammalian tissue, Y-cystathionase splits the other C-S bond forming cysteine.

Mammalian enzymes cannot reduce sulfate to sulfide to use for biosynthesis of sulfur-containing amino acids, biotin, thiamin or coenzyme A. Nevertheless, mammals can incorporate sulfate into various lipids, carbohydrates and phenols. In contrast, plants and most bacteria readily reduce sulfate to sulfide and thereby can survive with sulfate as their sole source of sulfur. In ruminants, the microbial population of the rumen has the ability to convert inorganic sulfur to organic sulfur compounds that can be utilized by the animal. Block et al. (1951) cited by NRC.(1991) concluded that sodium sulfate was converted in the rumen to cystine and methionine and then incorporated into microbial protein.

Sulfur is required for the formation of all sulfur-containing compounds found in all body cells as illustrated in Figure 2. This makes sulfur an essential nutrient (NRC, 1991). Sulfur comprises about 0.15% of wet weight of the body of mammals. Important compounds in the body containing sulfur include the sulfur amino acids (methionine, cysteine, cystine, homocysteine, cystathionine, taurine, and cysteic acid), thiamin (essential for carbohydrate metabolism), biotin (important in lipid metabolism), lipoic acid and coenzyme A (essential for aerobic energy metabolism), chondroitin sulfate (a component of collagen and connective tissue metabolism), glutathione (involved in peroxide reduction), fibrinogen and heparin (essential for blood clotting or fluidity) and certain hormones including estrogens. All these compounds containing sulfur except for

thiamin and biotin can be synthesized in the body from methionine. About 50% of the total requirement for sulfur amino acids can be provided by cysteine.

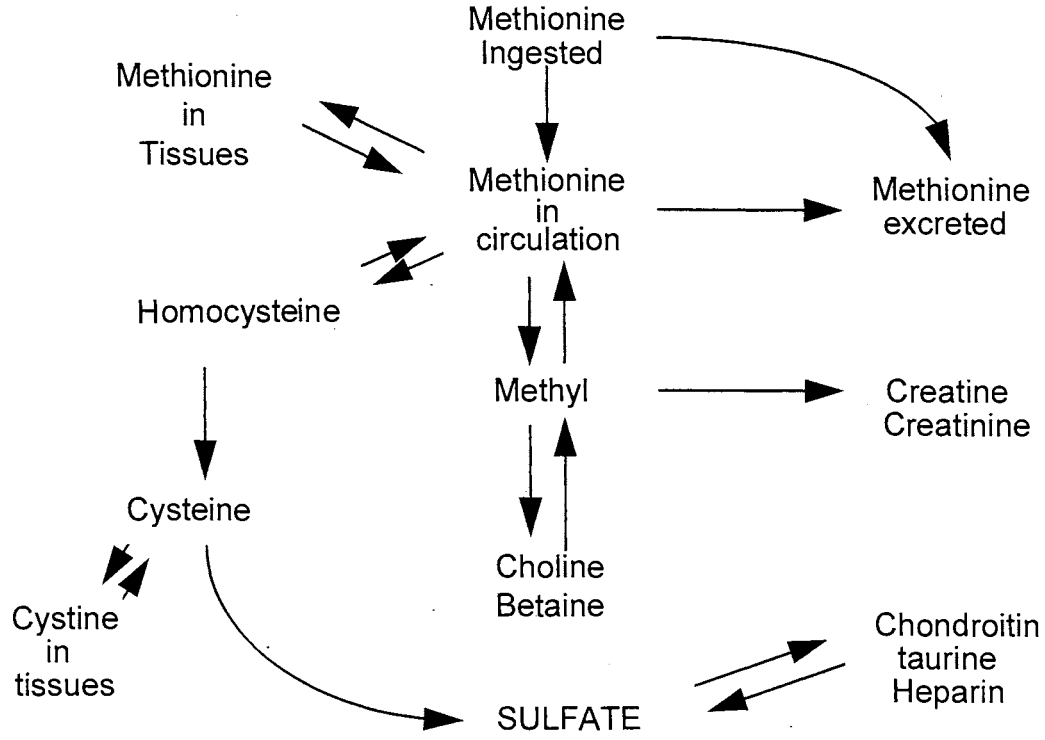

Figure 2. Nutritional Interrelations of sulfur compounds.

Cystine, a component of glutathione, also is closely involved in acid-base balance of intra- and extracellular fluids. Protein structure is dependent on sulfur amino acids with disulfide bonds between and within polypeptide chains (NRC, 1991). Besides being essential for life, supplementation with certain forms of sulfur has increased production by ruminant animals.

Absorption of sulfur

The form of sulfur in the diet can influence (1) absorption from the intestinal tract; (2) degree of association with certain blood components, (transport to sites within the body where it is to be used); and (3) utilization by tissues. Sulfate and L-amino acids containing sulfur are absorbed actively from the small intestine, particularly in the ileum (Dziewiatkowski, 1970). Excesses are catabolized with sulfur being excreted as sulfate, largely in urine. Urinary S

is present primarily as inorganic SO_4, although thiosulfate, taurine, cystine, and other organic compounds also may be present. Endogenous fecal S also enters in the gastrointestinal tract via bile as a component of taurocholic acid. Because the bulk of dietary S comes from dietary protein (and thereby amino acids), urinary S excretion tends to parallel urinary N excretion. High protein diets are associated with large amounts of urinary S and N and low urinary pH. Urine with a low pH typically carries more calcium; in humans, high protein diets have been implicated in enhanced calcium loss and an increased incidence of osteoporosis.

When non-protein N sources are substituted for protein in diets for ruminants, a growth response often is obtained when supplemental inorganic S is provided. This is because less dietary sulfur is being provided than ruminal bacteria need S for synthesis of protein. Synthesis of microbial protein is limited when S supply is inadequate for the formation of S-amino acids. This makes inorganic S important nutritionally. Sheep and goats, which produce wool or mohair which both are high in S-containing amino acids, will have less excess sulfur to recycle to the rumen via saliva; thereby, they need a higher dietary S:N ratio than ruminants not producing wool or mohair fiber. Ruminants can utilize dietary sulfate for amino acid synthesis through the reduction of sulfate to sulfide by rumen bacteria (maximum reduction occurs at pH 6.5). See Figure 3. Sulfate incorporation by bacteria into cysteine is faster than into methionine.

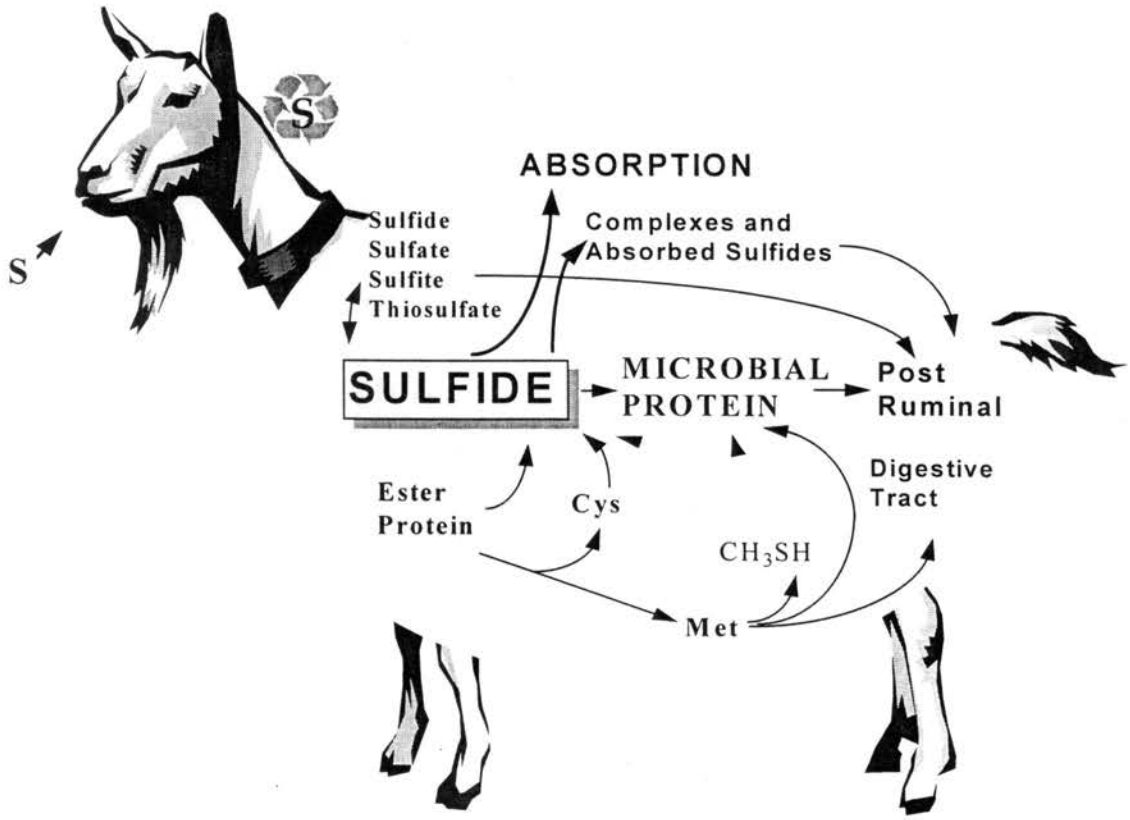

Figure 3. Sulfur metabolism in ruminants. Adapted from Qi et al. (1992).

Excretion of sulfur.

Fecal excretion of sulfur per unit of fecal dry matter by ruminants is fairly constant. In contrast, urinary excretion tends to increase as feed intake increases. Sulfate is reduced to sulfide by microbes in the rumen or the gut. Absorbed sulfide is oxidized to sulfate before being excreted.

Interrelationship of Sulfur with Copper and Molybdenum

A three-way interaction of copper, molybdenum, and sulfur decreases the bioavailability of copper in ruminants. This interaction has been recognized for more than 30 years. In the rumen, sulfur is reduced to sulfide; sulfide reacts with ingested molybdenum to form tetrathiomolybdate or oxythiomolybdates. These products in turn react with copper to yield a physiologically unavailable form of copper. Low concentrations of $(MoSO_4)^{-2}$ in the diet of rats induces clinical signs of copper deficiency by inhibiting absorption and modifying copper metabolism.

Absorbed $(MoSO_4)^{-2}$ forms an insoluble complex with copper that is found in blood. Hence, even though the concentration of total copper in the plasma is increased, such copper is not available for metabolism. This complex will bind to plasma proteins even in the presence of trichloroacetic acid. In sheep, the solid phase of ruminal contents plays the major role in the copper-molybdenum-sulfur interaction (O'Dell, 1990).

Interrelationship of Sulfur with Selenium.

The interrelationship between selenium and sulfur is due in part to their similarity in structure. Selenium can replace sulfur in some organic compounds, but the metabolic activity of the seleno-compound usually is much lower than that of the compound containing sulfur. Selenium toxicity can be reduced by sulfur supplementation, but the form of dietary sulfur influences its prophylactic usefulness (Levander and Burk, 1990).

Requirements for Sulfur

Dietary S requirements (NRC, 1981, 1984, 1985, 1989) as a percent of ration dry matter are shown in the table 1.

Table 1. Sulfur requirements in Sheep, Goats, Beef and Dairy Cattle.

Animal Class		Requirement % of diet DM	Ratio N:S
Sheep	(NRC, 1985)		
	Young lambs	.18 to .26	10:1
	Mature ewes	.14 to .18	
Goats	(NRC, 1981)	.18 to .26	10:1
	Qi et al. (1992)	.26	
Beef Cattle	(NRC, 1984)	.08 to .15	15.3:1
Dairy Cattle	(NRC, 1989)		
	Growing heifers and bulls	.16	15:1
	Dry pregnant cows	.17	
	Lactating cows	.20	12:1
	Young calves(Huber, 1984)	.15	
	Mature bulls	.11	

*N:S - The metabolism of sulfur and nitrogen is closely related in ruminants as in other animals. Consequently, requirements often are expressed as a ratio of nitrogen to sulfur (N:S). (Huber, 1984,cited by church 1989

Sulfur in Ruminants Products

Beef, Lamb and Milk.

Various tissues (beef, lamb and milk) have reasonably similar ratios of nitrogen to sulfur (N:S) of about 15:1. The composition of these products remains homeostatic in both N:S and amino acid ratios despite wide differences in the ratio of N:S in the diet (Garrigus, 1970). Sulfur amino acid comprise about 3.9% of muscle protein and 3.6% of milk protein (Block & Weiss, 1956).

Most diets fed to beef cattle contain amounts of sulfur adequate to meet the animal's needs, but in certain feeding regimes, supplementation with sulfur has proven beneficial. Cattle fed high amounts of grain or urea in the ration often have responded to addition of elemental sulfur (NRC, 1984).

Insufficient sulfur in the diet of dairy cattle reduces feed intake, lowers digestibility, reduces rate of gain, and depresses milk production. On the other hand, sulfur excesses can decrease feed intake and interfere with the metabolism of other minerals (NRC, 1989)

Products of Conception.

Sulfur is not distributed differentially in the products of conception, except for being high in all keratin proteins.

Wool, Hair and Mohair.

Wool is a mixture of proteins with a high content of cystine; These proteins can be fractionated into three main groups: low-sulfur proteins (which contain all the methionine and most of the lysine in wool) , high-sulfur proteins (which are rich in cystine, proline and serine and ultra-high-sulfur proteins) and high-tyrosine proteins (which are rich in tyrosine and glycine). Wool also is rich in glutamic acid, serine and glycine (Reis, 1979). Amino acid availability can change wool composition, either stimulating or reducing the rate of wool growth. Sulfur or SAA can play a major role in regulating wool growth. Under most

situations, sulfur-amino acid supply limits wool growth (Reis, 1979). In sheep fed roughage diets, wool growth rate was increased when the supply of sulfur-amino acids was increased from 0.2 to 0.45 g/MJ of ME. Wool growth (about 13 g clean wool/d) was maximum with 0.45 g sulfur-amino acids/MJ of ME for wethers given an abomasal infusion of casein as the sole source of amino acids (Kempton, 1979).

Hair, wool, and mohair are the primary ruminant products that deviate widely from the N:S ratio of about 15:1, having N:S ratios of 10:1 to 53:1. These products are keratins and contain about 13% cystine (Reis, 1979). The sulfur content of wool can vary from 2.7 to 4.2% depending on the diet. Growth of wool has a higher priority than growth of muscle and tissues. An increased supply of SAA increases fiber growth because SAA comprise about 10.1% of the amino acid in keratin (wool and mohair). Wool growth response has been used to estimate of protein supplementation from the rumen by Reis & Schinckel (1963), Reis, (1967; 1979; 1990) and Doyle & Bird (1975). In goats, addition of of protein to the diet also has increased mohair growth (Sahlu and Fernandez, 1992)

Sheep in negative S and energy balance will mobilize body tissue in order to maintain wool growth (Qi, 1993). Studies of the variability of sulfur content of wool are numerous. Hair differs little from wool in sulfur content (Reis, 1979). But species difference in total keratin production. Because rates of sulfur deposition are higher, and sulfur requirements are much higher for sheep and goats than for cattle as shown in Table 1.

Supplemental Forms of Sulfur

Methionine and Cystine. All animals readily convert methionine to cystine; the reverse reaction is not possible in mammals. Hence, an animal's tissue requirement for methionine can be met only by absorbed methionine. The

requirement for cystine, in contrast, can be met by either cystine or methionine. Methionine also may compensate partly for deficiencies of choline or vitamin B12 by providing methyl groups (NRC, 1984). Unless protected from deamination in the rumen, supplemental dietary amino acids are largely catabolized. Following degradation, dietary amino acids yield sulfur and nitrogen (ammonia) for use by ruminal microorganisms, but some dietary amino acids and protein will escape ruminal fermentation. To estimate ruminal escape of individual dietary amino acids, researchers have subtracted bacterial amino acid flow from total amino acid flow at the duodenum. Some 50 to 85% of the postruminal protein normally is microbial protein; the remainder consists of dietary protein that escapes ruminal fermentation. Bypassing the rumen will alter the site of nutrient digestion which in turn will reduce ruminal losses. The large increases in wool and mohair production that are observed with rumen-escape methionine reflect the high need for sulfur amino acids for synthesis of wool and mohair relative to that of microbial protein.

When amino acids supplied in excess, catabolism is enhanced and food intake and deposition of certain proteins including wool may be depressed.

Hydroxy analog of Methionine. This synthetic analog of methionine appears less extensively degraded than DL-methionine in the rumen. It is converted to methionine by the liver. The degree to which SAA or their derivatives, e.g., methionine-hydroxy-analog, will escape ruminal destruction has been debated by Whiting et al.(1972), Bird and Moir (1972), Papas et al. (1974) and Chalupa (1976).

Sulfur and Urea

Substitution of urea for true protein in the diet may increases the need for supplemental sulfur. Urea has been used widely as a protein supplement for ruminants. For the most efficient utilization of urea, NRC (1989) suggested that

the dietary nitrogen-to-sulfur ratio should be at 10:1 or below (more sulfur per unit of nitrogen) based on studies with sheep. In contrast, Bouchard and Conrad (1973) concluded that a nitrogen-to sulfur ratio of 12:1 was adequate to maximize feed intake by lactating dairy cows.

Sulfur in Natural or Metabolic Products Supplied to the Ruminant.

Plant products. Forages generally contain 0.1 to 0.3% of sulfur (NRC, 1984) as shown in Table 2. The nitrogen-sulfur ratio has been used as an index of the biological value of feedstuffs. The sulfur requirement of a given plant species depends largely upon the amount of amino acids containing sulfur found in the protein.

Table 2. Protein, nitrogen and sulfur content and N:S ratios of some common feedstuffs.

Feed name	Protein	Nitrogen	Sulfur	N:S
		%		Ratio
Alfalfa meal	18.90	3.02	0.24	12.60
Alfalfa hay	23.00	3.68	0.33	11.15
Corn silage	6.30	1.01	0.11	9.16
Cotton seed meal	48.90	7.82	0.34	23.01
Fish meal	66.70	10.67	0.49	21.78
Blood & bone meal	50.20	8.03	0.28	28.69
Meat bone meal	54.10	8.66	0.27	32.06
Peanunt hulls	7.80	1.25	0.10	12.48
Peanut meal	52.00	8.32	0.29	28.69
Sunflower	46.90	7.50	0.22	34.11
Soybean seeds	42.80	6.85	0.24	21.53
Soybean meal	49.90	7.98	0.37	21.58

Therefore, the need for sulfur fertilization by plants is dictated by the factors which affect protein production. The proportion of sulfur amino acids (cysteine and methionine) in any protein is under genetic control. Hence, the nitrogen to sulfur ratio of plant protein is relatively constant (Thompson et al.,

1970) For this reason, research about sulfur metabolism in plants has been limited largely to the transformation of sulfate sulfur to cysteine and methionine. In meat and fish samples, the N:S ratios also are quite constant permitting one to predict sulfur content from analysis for nitrogen content. The mean N:S ratio is 15.2:1 for muscle tissue and 13.8:1 for fish tissue.

Water. Because of their solubility, water often contains a sizable amount of sulfates, nitrates, and various trace minerals (NRC, 1984). Sulfate in water can sometimes meet S requirements for animals.

Recycled S. The amount of sulfur recycled to the rumen via saliva or diffusion through the ruminal wall is negligible relative to the amount of nitrogen that is recycled. Yet, relative to requirements, recycling can provide a substantial amount of sulfur for ruminal microbes.

Microbial Mater. The microbial protein passing to the abomasum of ruminant differs from the form consumed by the ruminant. Many studies of the biological value (amino acid balance) of microbial proteins indicate that high-quality dietary proteins are reduced in nutritive value by conversion to microbial protein in the rumen. In contrast, low-quality proteins and inorganic nitrogen and sulfur content are upgraded by partial conversion to microbial protein. Postruminal protein has a relatively constant composition and biological value due to partial conversion of dietary protein to and dilution by microbial protein. Provided that the supply of digestible nutrients (supplying ATP for microbes), nitrogen and sulfur in the rumen all are adequate, microbial outflow from the rumen will provide about 6.6 g digestible protein per MJ or (4.184J= 1cal) 27.6 g digestive protein per Mcal of ruminally digested ME. Presumably, protozoal protein contributes little to the total supply of microbial protein at the duodenum. Most researchers have found that bacterial crude protein contains about 3.6% sulfur-amino acids and .3% S. This is impossible since met and cys both contain over 20% of their weight as

sulfur, so that at least .72% S must be present in protein to yield 3.6% sulfur amino acids. See Table 3.

Table 3. Elemental Composition of three species (%)*

Element	Mammals	Alfalfa	Bacteria
Oxygen	62.8	77.9	73.7
Carbon	19.4	11.3	12.1
Hydrogen	9.3	8.7	9.9
Nitrogen	5.1	.8	3.0
Phosphorus	.6	.7	.6
Sulfur	.6	.1	.3
N/S	8.0	8.3	9.5
Total	97.9	99.6	99.7

Adapted from Moran and Scrimgeour, 1994

*Composition is given as percentage by weight.

The composition of microbial bacteria isolated from the rumen of ruminants can vary with diet and between samples taken before and after feeding. Much of this variation is due to variable amounts of accumulated polysaccharide. Nevertheless, composition between non-polysaccharide composition is surprisingly constant between large and small bacteria and between protozoa and bacteria (differences only in five out of 17 amino acids). The concentration of sulfur and nitrogen and protein in microbial fractions isolated from the rumen of sheep is shown in table 4.

Table 4. The values are means of six determinations on samples taken before and after feeding, three different diets and the results are expressed as mg/100 mg of polysaccharide-free air-dry weight.

Component	Protozoa	SD	Large Bacteria	SD	Small Bacteria	SD
Total S	.29	.04	.26	.03	.42	.03
Total N	9.80	.54	9.66	.13	7.44	.29
N/S	33.79		37.15		17.71	

Adapted from J.W.Czerkawski, 1976

*Composition is given as percentage mg/100 mg of polysaccharide-free air-dry weight.

Bacterial protein synthesis would require about 12 mg sulfur/g of true protein. Assuming that one has 16 g bacteria protein synthesized per 100 g of organic matter fermented in the rumen, this equals 192 mg Sulfur per 100 g OM fermented in the rumen. If half of the ME is fermented in the rumen and all the S is used in the rumen, then 86 mg sulfur is needed per OM digested. In the diet, this equals .09% S. If ME is 4 Mcal/kg OM digested, then the sulfur need equals 21 g/Mcal ME. In the absence of unfermented dietary protein in duodenal digesta, the supply of sulfur-amino acids from microbial protein can limit wool growth. To meet the sulfur-amino acid requirement for maximum wool growth, the supply of sulfur-amino acids must be approximately twice that supplied from microbial protein alone (Kempton, 1979). Dietary nutrients that escape ruminal degradation can help fill this gap.

Diagnostics

Ruminal metabolism. Whanger and Matrone (1970) reported that in earleir studies (Whanger, 1965; Whanger and Matrone, 1965) ruminal microorganisms in sheep fed sulfur-supplemented diets were predominantly gram positive whereas those from the sulfur-deficient sheep were mostly gram negative. The viscosity of ruminal fluid was 2 to 3 times greater in sheep fed the sulfur deficient diet. The lactate concentration was higher and butyrate was lower in ruminal fluid from sulfur-deficient sheep. Lactate concentration in ruminal fluid of the sulfur-deficient animals peaked at 78 mM about 2 hr after feeding and then declined gradually. In contrast, the concentration of lactate in the rumen of sulfur-fed sheep reached only a small peak (12 mM) half an hour after feeding and declined to zero by two hr.

Blood concentration. The sulfur status of animals has been evaluated by balance studies and by blood serum concentrations of sulfate and amino acids (NRC, 1991). Signs of a severe sulfur deficiency include a poor appetite,

listlessness and emaciation of the animal. Wool loss can occur in sheep due to thinning of the wool. A deficiency normally is diagnosed by measuring S content of the diet. In blood, concentrations of various nutrients may change with a sulfur deficiency. Decreases in plasma serine, citrulline, alanine, cysteine, glycine, tyrosine, and non-essential amino acids have been reported by Church (1989). When sulfur is deficient, microbial fermentation and growth can be altered and lactate utilization may decrease which results in lactate accumulation in the rumen and in blood. Cellulose digestion also can be depressed, probably because of depressed microbial growth.

An excess supply of sulfur also can alter requirements for various trace minerals including copper. In addition, a large amount of H_2S can be produced in the rumen which can affect microbial and ruminal motility. Ultimately, the toxicity of S depends on the amount of hydrogen sulfude formed. Elemental sulfur is considered to be the least toxic elements whereas H_2S rivals cyanide in toxicity.

Selenium and sulfur are structural analogs. Sulfur cannot replace selenium in essential compounds, but an excess of sulfur can reduce selenium absorption.

Toxicity usually is a results from excess dietary sulfate, either when $(NH_4)_2SO_4$ is used as a source of nitrogen, when $CaSO_4$ is used as a Ca source, or drinking water contains an excessive amount of sulfur (usually as calcium sulfate or "gippy" water).

Sulfur and Disease

Sulfur has been used to treat certain digestive disorders in lambs. Elemental sulfur can be effective for control of enterotoxemia in feed lambs (Garrigus, 1979).

Conclusions

1. The ratio of sulfur-amino acids to protein is lower in microbial protein than in milk, wool or mohair. Therefore, total quantity of sulfur-amino acids may be inadequate for maximum production of these proteins. Increasing the sulfur amino acid supply by feeding non-fermentable protein or ruminally bypassed methionine often will increase yields of these products.

2. Postruminally, the only form of sulfur that can limit production is the amount of sulfur-amino acids.

3. The sulfur status of the animal body depends on the plane of nutrition of the animal, and its mineral (copper, molybdenum) intake. The nutritional plane for sulfur also may vary with season of the year.

4. The ruminant will respond to additional sulfur-amino acid given abomasally, intravenously or intraperitoneally when sulfur-amino acids are deficient.

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CHAPTER III

IMPACT OF INCLUDING DL-METHIONINE IN THE DRINKING WATER 0F ADULT AND YOUNG ANGORA GOATS

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ABSTRACT. Four trials were conducted to examine production and metabolic responses to supplemental DL-methionine (met) in drinking water or feed of angora goats. In trial 1, 14 three-year old wethers weighing 33 kg were given ad libitum access to a 40% roughage diet (13.8% protein; .15% S) for 60 days. Three or four adult goats each received either 0 (basal), 2.5, 5.0, or 7.5 g DL-met daily in their drinking water. Feed intake, shorn body weight gain and greasy fleece weight tended to increase when met was included in the water. Providing 2.5 g met daily increased clean mohair production by 37.5%. Mohair diameter and length tended to be lowest for goats receiving 5 g met daily. The concentration of met in plasma exhibited a quadratic increase while ser, gly, thr, ala, val, phe, ile and leu exhibited linear decreases when met was added to drinking water; his decreased quadratically. Lys responded cubically ($P < .01$) to added DL-met in the drinking water. Total, essential, and nonessential amino acid concentrations in plasma were higher with .15% of sulfur in the diet and

decreased with met supplementation. Methionine as a fraction of total essential amino acids and of total amino acids were highest with .21% of dietary sulfur. Most of amino acids in the plasma were lower numerically at 4 hr than at 1 hr post-feeding. Maximum mohair production by these adult wethers was with .18% dietary sulfur (17 g/d) when the nitrogen: sulfur ratio was 12.3. In the second trial, 14, one-year old wethers weighing 29 kg were given ad libitum access to the same diet as experiment one for 60 days. Three or four kids received either treatment 1) the basal diet only, 2) the basal diet plus 2.5g/d of DL-met in their drinking water, 3) the basal diet supplemented with sodium sulfate to equal .20% sulfur, or 4) treatment 3 plus 2.5 g of met daily in the feed. Sulfur intakes provided the dietary equivalents of .15, .18, .20, and .23% S. Feed intake tended to increase when met was provided either in drinking water (1077 vs 1020) or in the feed (1066 vs 1012). Top-dressing the .20% S diet with DL-met increased clean mohair production by 35% (.74, .76, .87, and 1.00 kg per animal per treatment). Mohair diameter, length, and percentages of undesirable (med and kemp) fibers were not altered by met supplementation. L-lactate in the plasma was quadratically increased ($P < .09$) by sulfur supplementation. D and L-lactate in the rumen were not altered by sulfur levels. The concentrations of met, thr, ala, arg, tyr, cys, ile, leu, lys in the plasma were increased by higher S levels; ser was decreased with .18% sulfur but increased at higher sulfur levels. Glu, gly, thr, val, ile and lys reached their peak values with .20% sulfur. Almost all amino acids increased linearly with postprandial time. For these young wethers, mohair production (g/d) peaked at 17 g/d with .23% of sulfur and a nitrogen:sulfur ratio of 9.6. In the third trial, we used three adult fistulated wethers. These goats received either 1) the basal diet containing .15% of sulfur, 2) the basal diet plus 2.5 g of DL-met daily in their drinking water for a sulfur intake equivalent to .18% dietary sulfur), or 3) the basal diet plus 2.5 g of DL-met

daily top-dressed on the feed (.18% dietary sulfur). Methionine supplemented in water or feed increased plasma concentrations of glu, arg, met, met as a percentage of essential or of total amino acids. Plasma met concentrations were highest when met was added in the top of the feed; val, met, and met as a percentage of essential or total amino acids were increased by met supplementation while phe was higher when met was placed in drinking water. Plasma concentrations of ser, thr, ala, tyr, val, leu, total, essential and nonessential amino acids exhibited a quadratic response over time peaking at 1 hr post-feeding. Only met as a percentage of total amino acids was increased linearly by met supplementation. Ruminal ammonia decreased when DL-met was added to the diet. VFA ratios, protozoal numbers, and pH were not altered by added met. In the fourth trial, two wethers 12-month old Angora goats weighing 29 and 32 kg were used. Goats were fed the .15% sulfur basal diet for 21 days. One goat received only the control diet while the other received supplemental DL-met (1.4 g/d) in its drinking water. Blood samples were taken from the jugular vein of both animals before feeding and from the mesenteric vein when the animals were slaughtered 1 hour post-feeding. For the goat receiving supplemental met, plasma cys was 7% greater 1 h post-feeding than before feeding whereas in the goat not receiving met, plasma cys was 64% lower 1 h post-feeding. The increase in plasma met at 1 h post-feeding was 39% for the supplemented versus 24% for unsupplemented animal. Compared with prefeeding concentrations, plasma val decreased and leu increased in both animals. Plasma free lys decreased in the control animal but increased in the supplemented animal. In summary, met as a water or feed supplement increased mohair production without increasing mohair diameter. Responses to met in water need to be tested with goats fed a higher dietary sulfur level to

determine if the response can be attributed merely to an increased supply of sulfur.

KEY WORDS: Methionine, Goats, Sulfur, Amino acids, Mohair.

Introduction

The sulfur amino acid cysteine or its precursor, met, has been recognized as the primary factor that limits wool growth in sheep and goats. However, oral administration of cysteine and met has been reported to have no effect on wool growth (Reis, 1979). This is because these amino acid are presumed to be fermented in the rumen with sulfur being released as sulfide. Only a portion of this sulfide is used by the microbial flora of the rumen and incorporated into sulfur amino acids of microbial protein. Postruminal administration of either intact protein (Cottle, 1988a) or met (Reis, 1967; Cottle, 1988bc; Sahlu and Fernandez, 1992) has markedly increased the rate of wool and mohair growth. In general, researchers have concluded that increasing the supply of met will increase wool growth rate. Growth of wool or mohair can be divided between an increase in length and an increase in diameter, part of the latter being associated with an increase in the amount of ultra-high-sulfur keratin protein in wool. Physical properties of wool are not altered.

An increased supply of sulfur amino acids may increase growth of wool or mohair simply because the concentration of sulfur amino acids in wool or hair is very high. Sulfur amino acids comprise over 10% of the amino acid in keratinous (wool and mohair) protein versus only about 3.6% of milk protein, 3.9% of muscle protein (Block and Weiss, 1956) and 3.9% of bacterial protein synthesized in the rumen (Hungate, 1966). Of the sulfur amino acids in mohair, about 92% is cystine (Qi et al., 1991). Reis et al. (1989) found that two-thirds of the postruminally infused met could be replaced by cystine without depressing

wool growth. Sulfur in the rumen is derived from both the diet and via recycling in saliva; less sulfur is recycled by sheep than by cattle (Bray and Till, 1975) because more sulfur amino acids are deposited by animals depositing fiber.

Incorporating nutrients in drinking water also may increase the postruminal supply of sulfur amino acids. Fenn and Leng (1989) reported that adding 1.5 g/d of met to drinking water of sheep increased wool production by 12%. Bassett et al. (1981), feeding 2.6 g met per day for 112 days, increased grease fleece weight per goat by .50 kg. Under nutritional conditions in which the ruminant can respond to an increased protein supply, all amino acids appear to be absorbed from small intestine equally well (Wallace, 1994).

Plasma is the major vehicle for the transport of amino acids to and from various tissue of the body. While pools in tissue rather than plasma constitute the principal reservoir of amino acids for protein synthesis (Wolf and Bergman, 1972), amino acids exchange rapidly between plasma and tissue pools. Changes in concentrations of amino acids in plasma have been used to identify which specific amino acid limits production (Broderick et al., 1974). Conceptually, the concentration of an amino acid increases but only when the quantity supplied exceeds the quantity required. Until the requirement is exceeded by the supply, the concentration of the first limiting amino acid remains low and stable.

Due to its low cost, DL-met has been used traditionally to increase wool growth. However, levels are critical. An excess of met, by causing an imbalance, can depress wool growth. Hence, including an excess of met may depress wool or mohair production.

Work by Garza et al. (1990) indicated that 40 to 80% of water consumed by beef cattle bypassed the rumen. Based on this concept, we tested the impact of supplemental DL-met in drinking water for Angora goats. We provided several

levels of supplemental dietary DL-met and monitored responses in terms of performance, free amino acids in the plasma and mohair growth rates.

Materials and Methods

Experiment 1. For this study, supplemental DL-met was provided to increase sulfur intake from an equivalent of .15 (basal) to .18, .21 and .24%; these yielded nitrogen:sulfur ratios of 14.7, 12.3, 10.5, and 9.2, respectively. Methionine was provided in the drinking water to each of three or four goats per treatment maintained in metabolism crates

Three-year old male castrated goats (33 kg) were divided into four treatment groups in a completely randomized design. Each goat was provided with 1 liter of tap water containing either 0, 2.5, 5.0, and 7.5 g of DL-met per day with their meal. After it had consumed this quantity of water, each goat was given free access to tap water. Fresh water was available at all times except when water containing met was being provided. Each goat had free choice access to a pelleted diet (Table 1). This basal pelleted diet contained 13.8% crude protein, .15% sulfur, 4.4 kcal GE/g, 26% ADF and 42% NDF. Fresh feed was provided once daily, and feed residues were collected, weighed and discarded. This diet met the minimum nutrient requirements of Angora goats (NRC, 1981). The trial lasted 60 days. The goats were housed indoors in individual metabolism stalls (1.2 x 1.5 m). Ambient temperature was maintained at 20 $\pm$ 1^0C with 12 hr daily lighting during the winter season. Each diet was sampled once each week throughout the experiment and composite samples were analyzed for DM, CP (N x 6.25), ash (AOAC, 1990), ADF, NDF (Goering and Van Soest, 1970), and gross energy using an adiabatic oxygen bomb (Parr Instrument, Co. Inc., Moline, IL).). Sulfur analysis followed the procedure of Mottershead (1971).

Plasma Amino Acids. Jugular blood samples were collected 0, 1, 2 and 3 h postprandially into 10-mL tubes containing heparin (Becton Dickinson Vacutainer Systems, Rutherford, NJ). The tubes were chilled immediately in an ice bath, transported to the laboratory, and centrifuged at 3000 x g for 20 min to separate plasma. Aliquots of plasma were stored at -20 ^{o}C until analyzed. Prior to analysis, blood plasma samples (1 mL) were spiked with internal standards (.1 mL of .5 mM norvaline and .5 mM sarcosine) and deproteinized with .9 mL Seraprep (Pickering, Mountain View, CA). Amino acids analyses were performed with an AminoQuant (Hewlett Packard, San Fernando, CA) using UV detection and pre-column derivatization with o-phthaldialdehyde and 9-fluorenylmethylchloroformate.

Mohair Fiber Measurements. At the beginning (day 0) and end (day 60) of the experiment, each goat was totally sheared with an animal clipper (Stewart-Shearmaster Model EW310C head, Milwaukee, WI). Greasy (uncleaned) fleece was totally weighed; clean mohair weight, fiber diameter, and fiber length were determined in samples obtained from the left side of the animal at three specific positions: side, neck, and leg. Mohair was weighed and evaluated for greasy fleece weight, laboratory scoured yield (ASTM, 1990a), clean fleece weight, and staple length (ASTM, 1990b).

Statistical Analysis. Feed intake, body weight, mohair fiber measurements, blood, and ruminal traits were analyzed as a completely randomized design (Cochran and Cox, 1957). Calculations were performed using the GLM procedure of SAS (1985). Exact probability values are presented for all parameters analyzed.

Experiment 2. In this trial, 14 Angora wethers having a mean weight of 28 kg were assigned randomly within body weight groups to treatment. Three or four animals were assigned to each treatment. The same basal diet as in

Experiment 1 (13.8% protein, .15% S, 4.4 kcal GE/g, 26% ADF and 42% NDF) was used, but in this experiment the ration was not pelleted. For this study, supplemental DL-met (2.5 g/d) was added to water at a low level of dietary sulfur or to feed together with additional sodium sulfate. Three or four kids received either 1) the basal diet only, 2) the basal diet plus 2.5 g/d of DL-met in their drinking water, 3) the basal diet supplemented with sodium sulfate to provide .20% S, or 4) treatment 3 plus 2.5 g of met daily top-dressed on the feed. The basal diet without sodium sulfate contained .15% sulfur; sodium sulfate addition increased the dietary sulfur concentration to .20% of dry matter. Adding the sulfur provided by DL-met made total sulfur intake equivalent to dietary sulfur concentrations of .15, .18, .20 and .23% per day; these provided nitrogen:sulfur ratios of 14.7, 12.3, 11.0 and 9.6, respectively. The trial lasted 60 days.

All other methods were similar to those of experiment 1 except that in this trial, D and L-lactate concentrations in the ruminal and plasma samples were measured using Sigma kit 826 (Sigma Diagnostics, St. Louis, MO).

Experiment 3. In this trial, three 3-year old fistulated Angora wethers (45 kg) were used in a 3 x 3 Latin square design experiment. For this study, amounts of supplemental DL-met were provided to increase sulfur intake from an equivalent of .15 (unpelleted basal diet) to .18% (nitrogen:sulfur ratios of 14.7 and 12.3) by adding DL-Methionine (2.5 g/d) to either the drinking water or the feed. The third goat received control diet. This trial lasted 63 days with three period of 21 days (each with 14 days for diet and treatment adaptation and seven days for sampling and measurement).

Ruminal contents. Ruminal samples were obtained at 0, 30 min, 1 h and 2 h postprandially. Ruminal samples were collected via an esophageal tube equipped with a strainer. Vacuum was applied to the esophageal tube using a 50 mL syringe. Ruminal fluid samples were examined for evidence of salivary

contamination and discarded if contaminated. The ruminal fluid was placed in 10 mL test tubes and preserved with .2 g of metaphosphoric for later VFA analysis following the procedure of Erwin et al. (1968). Total ruminal ammonia was analyzed by the method of Broderick and Kang (1980). The 5 mL samples used for ammonia analysis were preserved with .2 mL of 72% of sulfuric acid and held sealed at -20 ^{o}C. For fixing, staining, and counting protozoa, two mL of fresh ruminal fluid from each animal were transferred to bottles (1 mL per bottle) containing 24 mL of methylgreen-formalin-saline solution (Ogimoto and Imai, 1981); protozoa were counted using an Olympus microscope (BHA model, Olympus, Lake Success, NY) and a Petroff-Hausser bacteria counter (Hausser Scientific, Blue Bell, PA).

Plasma Amino Acids. Jugular blood samples were collected 0, 30 min, 1 h, and 2 h postprandially into 10-mL tubes containing heparin (Becton Dickinson Vacutainer Systems, Rutherford, NJ) for analysis of amino acids concentration in the plasma as described in experiment 1.

Experiment 4. In this trial, two wethers 12-month old Angora goats weighing 29 and 32 kg were used. Goats were fed the basal diet (Table 1) for 60 days. The ration was available *ad libitum*; one goat received no supplemental sulfur whereas the other received DL-met (2.5 g/d) provided in the drinking water once each day. Both kids received only one kg of diet on the day they were sacrificed.

Blood collection. Blood samples were taken from the jugular vein of both animals before feeding and 1 h later (post-slaughter) from the mesenteric vein. Blood was collected into three 7 mL tubes containing sodium heparin (Beckton Dicksons, Vacutainer Systems Rutherford, NJ). The tubes immediately were chilled in an ice bath and transported to the laboratory where they were

centrifuged at 1,500 x g at 4°C for 20 minutes. Plasma aliquots were stored at -20°C until analyzed as described in experiment 1.

Statistical analysis. Because only one animal received each treatment, no statistical analysis was possible. Values were compared numerically only.

Results and Discussions

Experiment 1. Performance results are presented in Table 2. With the two lower levels of sulfur in drinking water, goats adapted and consumed their water allotment readily. With the highest level of sulfur in water (7.5 g/d), water intake was retarded and one goat refused to drink all of its water. This may have altered results for the highest met treatment. Feed intake tended to be increased ($P < .12$) by including met in the water. Treatment differences in weight gain (g/d) were not detected even though treatment means differed by nearly 30% (219 vs 282 g/d). Both feed intake and ADG numerically tended to be highest with 5 g met per day. Shorn body weight gains tended to be increased ($P < .13$) by including met in the drinking water.

Weights of greasy fleece tended to be greater ($P < .09$) with met supplementation with 2.5 g/day appearing to be a threshold. Compared with goats receiving no supplemental met, providing 2.5 g met in drinking water each day tended to increase ($P < .12$) clean mohair production (37.5% greater). Total dietary sulfur at this dose rate was equivalent to .18% sulfur for a nitrogen:sulfur ratio of 12.3. The dietary sulfur requirement for goats is reported to be .18 to .26% of sulfur (NRC, 1981) for young and mature goats, respectively. Hence, this response might be attributed simply to addition of sulfur alone. Prevalence of undesirable fiber types (kemp and med fibers), fiber diameter, and length were not significantly affected by DL-met supplementation. In the past, methionine supplementation has been associated with increases in wool fiber diameter (Reis, 1967; Sahlu and Fernandez, 1992).

The time by treatment interaction was not significant (P <.61) for plasma amino acid concentrations. Hence, means for treatments and time effects will be presented. Plasma amino acids concentrations averaged across time are presented in table 4. Except for met, plasma concentration of each essential amino acid was lower when supplemental met was provided. For most of amino acids and their sums, decreases were linear with quantity of met dosed: ser, gly, thr, ala, val, phe, ile, leu (P <.01) and lys (P <.02), total, essential and nonessential amino acids (P <.01). Plasma his responded quadratically (P <.01) to met supplementation in drinking water. Methionine as a percentage of essential amino acids or of total amino acids exhibited a quadratic increase (P <.01).

Effects of postprandial time on plasma amino acid concentrations are presented in Table 5. Most of amino acids in the plasma decreased numerically after feeding except for lys which decreasing cubically. Plasma free met increased linearly (P <.05) over time. Methionine as a percentage of essential amino acids and total amino acids exhibited linear increases (P <.01 and .02) over time. These changes would indicate that administering met in drinking water had increased the quantity of met absorbed and available for metabolism.

Experiment 2. Feed intake (Table 2) tended to be increased numerically when met was provided in drinking water (1077 vs 1020) or top-dressed on the feed (1066 vs 1012). DL-met in feed increased clean mohair production by 35% (.74, .76, .87 and 1.00 kg; P <.11). Mohair diameter, length,and percentages of undesirable (med and kemp) fibers were not altered by met.

L-lactate in the plasma increased when sulfur intake was increased (Table 3) being highest (P <.09) with the .20% sulfur equivalent. Concentrations of D-

lactate in plasma and of D and L lactate in the rumen were not altered by dietary sulfur level.

For plasma amino acids, the time by treatment interaction was not significant (P>. 68). Supplemental sulfur (Table 6) tended to linearly increase plasma concentration of some amino acids and totals: ser, gly, ala, arg, total amino acids, nonessential amino acids (P <.01), leu, lys (P <.02), and total essential amino acids (P <.04). Supplemental sulfur resulted in a cubic response in plasma free met concentration. With .18% sulfur (2.5 g/d met in water), met peaked at 24.7 mM. At higher sulfur intakes, plasma free met concentrations averaged 18.7 (added sodium sulfate only) and 21.3 mM (met added to the feed). Mohair production by these young goats was maximum with .23% sulfur in this experiment. This is less than the sulfur requirement for growth of young goats (.26%; Qi et al., 1992) but higher than the level recommended (.18%) for growing goats by NRC (1981).

Effects of postprandial time on plasma free amino acids for young goats are presented in table 7. Linear decreases over time were noted for ser, gly, arg, (P <.01), ile (P <.07), leu and lys (P <.02). However, probably due to administration of met, plasma concentrations of met, cys, phe, and met as a portion of total and of essential amino acids tended to be higher at 2 h than at 4 h post-feeding.

Experiment 3. Plasma free concentrations of adult fistulated wethers are presented in the table 8. Either adding met to either drinking water or feed increased plasma concentrations for glu (P <.02), arg (P <.01), met (P <.02), met as a percentage of essential amino acids (P <.06), and as a percentage of total amino acids (P <.03). Compared to providing met in drinking water, met in feed produced higher concentrations of plasma free val (P <.04), met (P <.05) and phe (P <.03). Methionine. either as a percentage of essential (P <.01) or of total

amino acids (P <.01) was higher when met were added to feed than when met was supplemented in the drinking water.

Effects of postprandial time on plasma amino acid concentrations are presented in table 9. Plasma amino acids concentrations for ser, thr, ala, val and leu increased quadratically over time whereas tyr responses were cubic. Methionine as a percentage of total amino acids increased linearly with time post-feeding. Total, essential, and nonessential amino acid concentrations in plasma tended to be highest at one hour post-feeding (Table 9).

Effects on ruminal measurements are presented in Table 10. Total VFA (mM) concentration in the ruminal fluid samples was not altered by met supplementation (control (C) vs added met in drinking water (W) or in top-dressed on feed (F) (P > .67), but response to supplementation tended to be highest when met was added to the drinking water (F vs W; P = .11). Ruminal ammonia decreased when met was supplied to goats; molar proportion of VFA, protozoal numbers, and pH were not altered by met supplementation.

Plasma amino acid responses differed when an equal amounts of met (2.5 g/d) were provided in the drinking water vs added to the feed. Although total met concentration in the plasma was increased with either method of administration, the increase from supplementation in drinking water was 26% versus 72% when met was added to the feed. This is a surprising because most previous researchers have suggested that met is rapidly destroyed by ruminal bacteria and have attributed little or no wool growth to dietary met to such destruction. Although Liebholtz (1971 a, b) reported that free amino acids can be absorbed through the ruminal wall, she concluded that absorption was nil under practical conditions because ruminal concentrations of free amino acids normally are extremely low. However, with administration of a large quantity of met, perhaps

some may be absorbed through the rumen wall. A second possible mechanism is retarded degradation of met which permits rapid flushing of met through the rumen when it is fed. Cottle and Velle (1989) administered lysine, thr and met into the rumen and reported that apparent degradation during the first four hours was highest for lysine and lowest for met They concluded that some of the thr and met, even when not protected from ruminal degradation, flowed from the rumen intact. Bird and Moir (1972) previously had reported that met is not degraded as rapidly as other amino acids in the rumen.

Reis and Schinckel (1963) reported that dietary supplementation with DL-met (2.46 g/d) or L-cysteine (2 g/d) increased wool growth by 35 to 130%. Their diet supplied the equivalent of 2-3 g of cysteine per day. Our results indicate that DL-met (in water) increased mohair production by 37.5% in adult Angora goats in the first experiment and by 35% in young Angora (via feed) goats in the second experiment. The mechanism by which an increased dietary supply of met might increase wool or mohair growth was suggested by Roberts et al. (1955) cited by Reis, (1979) to be an effect of met on ruminal microbes rather than on an increase in the postruminal supply. They contended that the rate of trans-sulfuration limited bacterial growth. Enhanced protozoal numbers with supplemental met (Gil, 1973) also might be expected. Pittman and Bryant (1964) cited by Reis, (1979) found that exogenous met was stimulatory or essential for growth of certain strains of ruminal bacteria although cross-feeding would be expected to meet any methionine needed by microbes within the rumen. These results do not negate the potential for increasing postruminal met supply by including met in drinking water. In all three of these studies, supplemental met increased plasma free met concentrations. If feed is being provided to animals, including met in the feed may be a simpler but still effective and convenient

alternative for administering water-borne nutrients, especially under grazing situation when supplementation via feed may be neither feasible nor practical.

Experiment 4. Free amino acid concentrations in jugular and mesenteric plasma from goats are presented in table 11. These goats were allowed only one hour to eat their feed prior to slaughter. During this time, the control goat (29 kg) consumed 230 g of feed and drank 500 mL of water while the goat receiving met in water (32 kg) ate 410 g of feed and drank 580 mL of water which contained 1.45 g DL-met.

Compared with the jugular vein plasma cys concentration prior to feeding, the mesenteric plasma cys one hour after feeding for the control goat was 64% lower. For the goat receiving met in water, plasma cys had increased by 7%. During this time period, plasma met had increased by 24% in the control animal versus 39% for the supplemented animal. Plasma free val concentration decreased in both animals while leu increased in both. Lys decreased in the control animal but increased in the supplemented animal.

When compared with plasma data from sheep (Tagari and Bergman, 1977), our goats had higher values for val and leu while their sheep had higher values for phe and lys. However, their diets were very rich in protein (15.6 to 19.8%) compared with ours (13.8%).

Implications

Supplementation of diets or of water with DL-met increased mohair production without increasing mohair diameter. Based on plasma methionine concentrations, even an unprotected form of methionine when included in feed increased the quantity of met absorbed. Whether all the responses can be attributed to an increased supply of met or merely to an increased supply of sulfur remains to be determined though supplementing sulfur-rich diets with met.

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Table 1. Composition of experimental diets (% of DM).

Ingredient	
Cottonseed hulls	38.00
Ground corn	33.10
Soybean meal 44%CP	18.00
Alfalfa hay good	7.60
Limestone 38% Ca	0 - 0.3
Salt	1.50
Sodium sulfate	0.30
Dicalcium phosphate [a]	0.50
Ammonium cloride	0.50
Vit. A, D, E [b]	0.20
Chemical composition [c]	
CP %	13.80
DM %	92.81
ADF %	25.25
NDF %	43.40
S %	0.15 - 0.29
GE, Mcal / Kg of DM	4.40

[a] Dicalcium phosphate containing 22 % Ca, 19.5 % P.

[b] Contained 2,200 IU of vitamin A, 1,B10100 IU of vitamin D, and 2.2 IU of vitamin E per gram.

[c] Chemical composition was measured.

Table 2. Responses to DL-Methionine in drinking water (experiment 1) in drinking water plus in the the top of the feed (experiment 2)

Treatment				Mohair	Feed	Clean		Grease	Fiber	Fiber	Kemp	Med
Methionine		Diet		Production	Intake	Mohair	ADG	Mohair	diameter	length	Fiber	Fiber
g	%S	S, %	N:S	(g/d)	(g/d)	(kg)	(g/d)	(kg)	(um)	(cm)	(%)	(%)
Experiment 1												
0		0.15	14.7	12	1536	.72	219	.85	37.1	2.2	.15	.29
2.5 or 0.03		0.18	12.3	17	1839	.99	267	1.29	37.0	2.3	.13	.30
5.0 or 0.06		0.21	10.5	13	1860	.76	282	.94	34.4	2.1	.18	.35
7.5 or 0.09		0.24	9.2	12	1739	.73	280	.86	37.8	2.3	.18	.32
Met. addition (Interaction)				.12	.12	.12	.13	.09	.47	.84	.71	.82
Experiment 2												
0		0.15	14.7	12	1020	.74	158	.77	32.2	1.9	.17	.35
2.5 or 0.03		0.18	12.3	13	1077	.76	200	.84	32.9	2.1	.22	.43
0		0.20	11.0	15	1012	.87	133	.88	32.2	2.1	.17	.31
2.5 or 0.03		0.23	9.6	17	1066	1.00	172	1.10	31.5	1.9	.24	.42
Met. addition (Interaction)				.11	.15	.11	.20	.12	.88	.70	.41	.77

Table 3. Responses to DL-Methionine in drinking water.

Sulfur %	.15	.18	.20	.23	SE	Probability
L-lactate, mg/dL, Rumen	2.08	2.83	2.45	2.67	.26	.33
D-lactate, mg/dL, Rumen	5.42	5.83	5.56	5.89	.56	.94
L-lactate, mg/dL, Plasma	14.17 [ab]	15.83 [ab]	21.33 [a]	13.56 [b]	2.06	.09
D-lactate, mg/dL, Plasma	.42	.33	.22	.11	.26	.89

Table 4. Plasma free amino acids response to supplemental DL-Methionine in the drinking water for adults goats (mM).

	Dietary sulfur, %					Probability <		
Item	.15	.18	.21	.24	SE[a]	L	Q	C
Ser	113.62 [a]	103.69 [a]	65.38 [b]	74.50 [b]	6.61	.01	.17	.02
Gly	437.65 [a]	367.11 [ab]	296.79 [b]	310.94 [b]	29.42	.01	.17	.03
Thr	73.88 [a]	60.62 [b]	65.46 [ab]	45.56 [c]	4.13	.01	.44	.01
Ala	256.84 [a]	197.01 [b]	184.29 [b]	195.63 [b]	10.89	.01	.01	.01
Arg	166.63	157.69	157.08	161.88	10.58	.77	.53	.79
Tyr	71.24	72.46	71.24	65.64	4.76	.44	.50	.51
Val	220.81 [a]	178.00 [bc]	189.62 [ab]	146.72 [c]	13.18	.01	.99	.01
Phe	64.58 [a]	46.19 [bc]	54.01 [b]	42.91 [c]	3.08	.01	.27	.01
Ile	86.24 [a]	65.83 [b]	74.36 [ab]	58.49 [b]	5.25	.01	.68	.01
Leu	152.84 [a]	116.64 [bc]	132.38 [ab]	97.48 [c]	9.42	.01	.95	.01
Lys	63.58 [a]	55.04 [ab]	62.63 [a]	48.74 [b]	3.15	.02	.94	.01
His	63.57 [a]	49.10 [b]	52.33 [b]	55.69 [ab]	3.13	.18	.01	.08
Met	19.72 [b]	22.43 [b]	29.72 [a]	16.89 [b]	1.99	.90	.01	.11
TAA[b]	1791.19 [a]	1491.82 [bc]	1435.28 [b]	1321.07 [c]	95.03	.01	.43	.01
TEAA[c]	758.70 [a]	594.33 [bc]	694.50 [ab]	512.48 [c]	39.14	.01	.80	.01
TNEAA[d]	1032.49 [a]	897.49 [b]	740.78 [b]	808.59 [b]	45.01	.01	.16	.01
MPEE[e]	2.60 [c]	3.77 [b]	4.28 [a]	3.30 [bc]	.31	.02	.01	.36
MPAA[f]	1.10 [c]	1.50 [b]	2.07 [a]	1.28 [bc]	.14	.05	.01	.08
TEAA/TAA	.42 [ab]	.40 [b]	.48 [a]	.39 [b]	.01	.99	.19	.18

Means with the same letter are not significantly different ($p < .05$). [a]SE = standard error. [b]TAA = total of amino acids.
[c]TEAA = total essential amino acids. [d]TNEAA = total nonessential amino acids.
[e]MPEE = methionine/TEAA x 100. [f]MPAA = methionine/TAA x 100.

Table 5. Plasma free amino acids response to supplemental DL-Methionine in the drinking water for adults goats (mM) .

	Time					Probability <		
Item	0	1	2	3	SE[a]	L	Q	C
Ser	96.69	89.00	84.20	84.95	8.26	.24	.56	.37
Gly	349.95	350.30	344.64	358.62	33.41	.89	.83	.84
Thr	67.89	61.71	57.23	59.28	4.88	.16	.36	.32
Ala	200.66	208.22	209.21	205.99	13.05	.76	.65	.79
Arg	171.13	161.17	157.45	151.27	11.20	.25	.87	.31
Tyr	72.09	71.73	69.07	67.05	5.03	.47	.87	.61
Val	194.14	179.04	182.84	183.86	15.03	.69	.58	.61
Phe	54.01	50.41	51.57	52.18	6.63	.79	.53	.67
Ile	78.62	70.33	68.93	67.30	3.71	.19	.56	.23
Leu	135.73	122.82	122.82	119.81	5.98	.32	.63	.34
Lys	62.46 [a]	57.55 [ba]	59.468 [ba]	50.22 [b]	10.88	.36	.48	.01
His	57.40	54.54	53.67	53.67	3.73	.50	.71	.57
Met	19.78	21.46	25.21	25.27	2.36	.05	.71	.22
TAA[b]	1560.55	1498.28	1486.31	1479.47	88.70	.39	.85	.29
TEAA[c]	685.82	626.78	638.80	611.60	43.24	.31	.83	.22
TNEAA[d]	874.73	871.50	847.51	867.87	37.25	.65	.63	.55
MPEE[e]	2.88 [b]	3.42 [ab]	3.95 [a]	4.13 [a]	.34	.01	.30	.04
MPAA[f]	1.26 [b]	1.43 [ab]	1.69 [a]	1.71 [a]	.18	.02	.47	.10
TEAA/TAA	.44	.42	.43	.41	.01	.61	.49	.61

Means with the same letter are not significantly different ($p < .05$). [a]SE = standard error. [b]TAA = total amino acids.

[c]TEAA = total essential amino acids. [d]TNEAA = total nonessential amino acids.

[e]MPEE = methionine/TEAA x 100. [f]MPAA = methionine/TAA x 100.

Table 6. Plasma free amino acids response to supplemental DL-Methionine in the drinking water for young goats (mM).

	Dietary sulfur, %					Probability <		
Item	.15	.18	.20	.23	SE[a]	L	Q	C
Glu	140.26	139.08	171.37	154.74	11.51	.22	.59	.85
Ser	200.25 [b]	193.28 [b]	248.67 [ab]	264.48 [a]	16.85	.01	.60	.12
Gly	788.77 [b]	777.50 [b]	1061.34 [a]	933.00 [a]	40.14	.01	.22	.45
Thr	87.93 [b]	95.56 [b]	116.67 [a]	92.58 [b]	4.68	.14	.01	.84
Ala	270.05 [b]	272.16 [b]	323.12 [a]	324.22 [a]	12.16	.01	.99	.10
Arg	163.04 [b]	166.12 [b]	186.11 [ab]	201.46 [a]	8.57	.01	.61	.03
Tyr	69.74	82.05	71.53	83.84	4.05	.15	.99	.02
Cys	12.34	13.60	13.35	11.85	.68	.54	.13	.63
Val	233.66	277.63	278.99	258.83	12.07	.23	.23	.25
Phe	55.60	64.34	60.53	61.22	2.32	.34	.19	.12
Ile	78.12 [b]	92.04 [ab]	95.63 [a]	91.16 [ab]	4.49	.07	.10	.13
Leu	133.33 [b]	163.67 [a]	168.04 [a]	170.07 [a]	8.56	.02	.21	.03
Lys	105.02 [b]	119.84 [ab]	131.86 [a]	127.67 [ab]	6.44	.02	.21	.09
Met	14.90 [c]	24.66 [a]	18.68 [bc]	21.25 [ab]	1.52	.14	.07	.01
TAA[b]	2353.01 [b]	2481.53 [b]	2945.89 [a]	2796.37 [a]	82.41	.01	.17	.05
TEAA[c]	705.56 [b]	837.78 [a]	870.39 [a]	822.78 [ab]	33.73	.04	.04	.09
TNEAA[d]	1647.45 [b]	1643.75 [b]	2075.50 [a]	1973.59 [a]	65.90	.01	.51	.11
MPEE[e]	2.06 [b]	2.98 [a]	2.16 [b]	2.56 [ab]	.17	.53	.26	.02
MPAA[f]	.63 [b]	.99 [a]	.63 [b]	.78 [b]	.06	.89	.17	.02
TEAA/TAA	.30 [b]	.34 [a]	.30 [b]	.29 [b]	.01	.31	.21	.54

Means with the same letter are not significantly different ($p < .05$). [a]SE = standard error. [b]TAA = total of amino acids.

[c]TEAA = total of essential amino acids. [d]TNEAA = total nonessential amino acids

[e]MPEE = methionine/TEAA x 100. [f]MPAA = methionine/TAA x 100.

Table 7. Plasma free amino acids response to supplemental DL-Methionine in the drinking water for young goats. (mM)

	Time				Probability <	
Item	0	2	4	SE[a]	L	Q
Glu	152.03	152.03	147.13	11.65	.82	.59
Ser	231.78	221.97	214.24	17.51	.01	.60
Gly	911.80	862.15	849.51	40.64	.01	.22
Thr	104.27	95.08	90.16	4.74	.14	.01
Ala	272.97 [b]	296.19 [ab]	312.94 [a]	12.31	.01	.99
Arg	194.25 [a]	175.45 [ab]	159.60 [b]	8.68	.01	.61
Cys	12.38 [b]	14.55 [a]	11.42 [b]	.69	.54	.13
Tyr	75.96	79.37	72.26	4.10	.15	.99
Val	263.54	268.49	242.90	12.22	.23	.04
Phe	56.68 [b]	64.25 [a]	58.85 [ab]	2.35	.34	.19
Ile	90.30	88.62	83.91	4.54	.07	.10
Leu	144.73	168.38	154.00	8.67	.02	.21
Lys	132.09 [a]	117.10 [ab]	106.15 [b]	6.52	.02	.21
Met	16.21 [b]	22.30 [a]	19.41 [b]	1.53	.14	.07
TAA[b]	2658.97	2625.92	2522.48	83.45	.01	.17
TEAA[c]	807.80	824.21	755.38	34.15	.04	.04
TNEAA[d]	1851.17	1801.71	1767.10	66.73	.01	.51
MPAA[e]	.61 [b]	.85 [a]	.78 [ab]	.06	.89	.17
MPEE[f]	1.99 [b]	2.66 [a]	2.55 [a]	.17	.53	.26
TEAA/TAA	.30	.32	.30	.01	.31	.21

Means with the same letter are not significantly different ($p<.05$). [a]SE = standard error.

[b]TAA = total of amino acids. [c]TEAA = total of essential amino acids. [d]TNEAA = total nonessential amino acids.

[e]MPEE = methionine/TEAA x 100. [f]MPAA = methionine/TAA x 100.

Table 8. Plasma free amino acids response to supplemental DL-Methionine fed on the top of the feed and in drinking water for adults fistulated goats. (mM).

	Treatments				contrast	
Item	C	F	W	SE[a]	C vs W & F	F vs W
Glu	138.19 [b]	160.81 [a]	173.03 [a]	10.00	.02	.39
Ser	257.94	237.85	260.91	12.60	.58	.21
Gly	983.09	942.06	943.50	54.60	.55	.98
Thr	85.99	94.99	101.67	5.90	.09	.43
Ala	262.62	272.05	293.63	15.20	.28	.32
Arg	233.14 [ab]	270.27 [a]	264.68 [ab]	11.17	.01	.72
Tyr	93.61	97.64	105.16	6.14	.31	.39
Val	179.87 [ab]	201.77 [a]	171.08 [b]	10.42	.61	.04
Phe	53.39	44.20	53.69	3.07	.24	.03
Ile	62.21	69.66	67.95	3.71	.15	.74
Leu	103.50	108.48	104.19	4.78	.63	.53
Lys	85.28	91.07	97.14	7.75	.36	.58
His	60.95 [b]	67.04 [ba]	73.29	4.73	.11	.38
Met	20.88 [b]	35.91 [a]	26.26 [b]	3.41	.02	.05
TAA[b]	2620.66	2693.80	2736.18	117.00	.44	.71
TEAA[c]	651.79	704.00	690.52	31.70	.24	.72
TNEAA[d]	1968.87	1989.80	2045.66	98.07	.42	.60
MPEE[e]	3.26 [c]	4.97 [a]	3.42 [b]	.41	.06	.01
MPAA[f]	.73 [c]	1.23 [a]	.84 [b]	.11	.03	.01

[a]SE = standard error. Means with the same letter are not significantly different (P < .05)

[b]TAA = total of amino acids. [c]TEAA = total of essential amino acids.

[d]TNEAA = total nonessential amino acids. [e]MPEE = methionine/TEAA x 100.

[f]MPAA = methionine/TAA x 100.

Table 9. Plasma free amino acids response to suplemental DL-Methionine fed on the top of feed and in drinking water. for adults fistulated goats.(mM)

	Time					contrast		
Item	0	30 min	1h	2h	SE[a]	L	Q	C
Glu	146.93	147.47	182.01	152.86	11.22	.31	.20	.06
Ser	244.80 [c]	264.86 [b]	270.88 [a]	228.41 [d]	14.20	.50	.03	.59
Gly	984.77	992.15	992.22	855.71	61.60	.16	.25	.64
Thr	89.01 [c]	97.68 [b]	104.68 [a]	85.51 [c]	6.67	.90	.04	.41
Ala	257.42 [c]	279.66 [b]	312.15 [a]	255.18 [c]	17.20	.73	.02	.21
Arg	253.98	251.62	279.53	239.00	12.60	.76	.14	.08
Tyr	95.24 [b]	90.49 [c]	112.72 [a]	96.75 [b]	6.93	.39	.42	.04
Val	174.62 [c]	185.89 [b]	206.57 [a]	169.88 [d]	11.78	.90	.04	.21
Phe	52.05	53.19	50.98	45.48	3.47	.16	.34	.99
Ile	64.63	65.60	74.82	61.38	4.19	.97	.09	.11
Leu	106.45 [c]	108.49 [b]	113.72 [a]	92.89 [d]	5.40	.15	.04	.23
Lys	89.68	91.04	91.04	91.97	8.71	.84	.94	.99
His	63.31	70.73	70.29	64.06	5.15	.93	.17	.92
Met	23.21	25.19	33.04	28.67	3.91	.18	.38	.37
TAA[b]	2646.10 [b]	2724.06 [b]	2894.65 [a]	2467.75 [c]	133.00	.53	.04	.45
TEAA[c]	659.66 [c]	700.86 [ab]	735.79 [a]	635.79 [c]	36.60	.82	.05	.43
TNEAA[d]	1986.44 [c]	2023.20 [ab]	2158.86 [a]	1831.96 [d]	131.07	.52	.04	.38
MPEE[e]	3.52	3.59	4.49	4.51	.46	.10	.94	.52
MPAA[f]	.88 [b]	.92 [b]	1.14 [a]	1.16 [a]	.12	.05	.96	.54

[a]SE = standard error. Means with the same letter are not significantly different (P < .05). [b]TAA = total of amino acids.

[c]TEAA = total of essential amino acids. [d]TNEAA = total nonessential amino acids.

[e]MPEE = methionine/TEAA x 100. [f]MPAA = methionine/TAA x 100.

Table 10. Means of ruminal fluid, volatile fatty contents (mol/100mol), ammonia, protozoa and pH.

	Treatments				Probability <	
Item	C	F	W	SE	C vs W & F	F vs W
VFA, molar percentage						
Acetate, %	67.18	68.28	68.17	.48	.38	.75
Propionate, %	20.15	20.08	19.86	.48	.93	.89
Isobutyrate, %	.92	.93	.81	.05	.51	.12
Butyrate, %	9.39 [a]	8.06 [b]	8.61 [ab]	.32	.19	.97
Isovalerate, %	1.21	1.39	1.33	.11	.64	.43
Valerate, %	1.15	1.26	1.22	.04	.49	.26
Total, m*M*	79.13 [ab]	76.78 [b]	84.51 [a]	1.69	.67	.11
Protozoa, thousand/mL	40	28	52	.07	.85	.25
pH	6.15	6.17	6.08	.04	.61	.56
Ammonia, mg/dL	15.45 [a]	12.05 [b]	12.45 [b]	1.61	.01	.59

Means with the same letter are not significantly different (P <.05)

Table 11. Relative amounts of free essential amino acids and cysteine appearance in the plasma of jugular and mesenteric vein compared with previously published data from sheep in the portal vein.

Diet	Control-0	Control-1	Met-0	Met-1	Tagari & Bergman(1978)	
Vein	Jugular	Mesenteric	Jugular	Mesenteric	HP	MP
		(CP = 13.8)			CP=19.8	CP=15.6
	*Relative (%) of amino acid in the plasma					
Amino acid						
Thr	9.3	9.3	11.5	10.5	11.8	8.6
Cys	1.4	0.9	1.4	1.5	3.4	1.9
Val	36.7	26.3	35.7	25.1	14.4	8.5
Ile	11.5	10.4	10.7	11.0	15.1	13.7
Leu	20.0	33.1	18.9	27.0	10.3	10.8
Phe	6.0	6.4	6.0	7.9	19.6	22.7
Lys	13.0	10.9	13.9	14.5	22.2	26.9
Met	2.1	2.6	1.8	2.5	3.3	6.9
Total	100.0	100.0	100.0	100.0	100.0	100.0

Control-0 = Control at 0 time in the jugular vein.

Control-1 = Control 1 hour after feed in the mesenteric vein.

Met-0 = Methionine at 0 time at the jugular vein .

Met-1 = Methionine one hour after feed in the mesenteric vein.

* Relative appearance = Total amino acid appearance divided by the total eight essential amino acid plus cystein X 100.

HP = high protein and MP = medium protein

CHAPTER IV

THE INFLUENCE OF ADDING METHIONINE TO DRINKING WATER OR TO FEED ON THE GROWTH RATE OF MOHAIR BY YOUNG ANGORA GOATS

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ABSTRACT. Effects of supplementing sulfur as DL-methionine in drinking water or top-dressing sodium sulfate on feed on mohair production were examined in two experiments with Angora kids. In the first trial, no added sulfur was compared with supplements of 2.5 g DL-methionine in the drinking water or an equivalent amount of sulfur from sodium sulfate (2.4 g) top-dressed on feed. Each treatment was provided to four 12 mo old wethers goats (37 kg) for 60 days. The basal diet, containing 40% roughage, 13.8% protein and .26% sulfur, was available free choice. In the second trial, the same 12 animals were used in a crossover design (30 d periods) to compare supplemental methionine in water with sodium sulfate added to the feed. Rates and efficiencies of gain and mohair production were not altered either by sulfur supplementation or by sulfur source. However, plasma amino acid concentrations indicated that methionine in

drinking water increased the post-ruminal methionine supply. Failure of supplemental DL-methionine to increase mohair production suggests that post-ruminal supply of methionine already was adequate to produce the maximum growth rate of mohair.

KEY WORDS: Methionine, Goats, Sulfur.

Introduction

Qi et al. (1991) estimated that the sulfur requirement for mohair production is 0.267% of diet dry matter. When dietary sulfur is at or above this level, responses to methionine supplementation theoretically could be attributed either to a direct contribution of this essential amino acid to the post-ruminal methionine supply or to some indirect effect of sulfur-containing amino acids on ruminal metabolism as noted with in vitro ruminal fermentation studies (Gil et al., 1973). The magnitude of response to supplemental dietary methionine is interrelated strongly with other nutritional factors including total dietary protein, sulfur, and energy (Chandler et al., 1976).

Although many nutritional factors can influence wool and mohair production by sheep and goats, mohair production generally has been increased when the post-ruminal supply of protein has been increased. Such responses have been attributed largely to methionine and cysteine, the sulfur-containing amino acids (SAA). This is because these SAA are considered to be the first limiting amino acids in bacterial protein for wool production. Using cattle and sheep maintained completely on infused purified nutrients, several workers (Storm and Orskov, 1983; Asplund 1987) have estimated requirements for amino acids.

Cystine or its reduced dimer, cysteine, is not an essential amino acid for animals; it can be synthesized from methionine plus serine. During de novo synthesis of cystine, sulfur is derived from methionine and the carbon skeleton

originates from serine (Murray et al., 1993). Although serine can be synthesized by the liver, methionine is not synthesized by animal tissues. Hence, to meet an animal's requirement for cystine, either cystine or methionine must be available. Approximately, one-third of the methionine requirement of sheep can be satisfied by cysteine. Wool growth has been increased dramatically by post-ruminal administration of either intact protein or methionine (Reis et al., 1990, Sahlu et al., 1992). Infusing methionine abomasally into sheep at a rate of 2 g/day increased wool growth by 67%. In contrast, simply adding these amino acids to the diet generally has not increased wool growth; this has been attributed to extensive ruminal destruction of SAA by bacteria. Unprotected amino acids are generally believed to be partially or completely destroyed during passage through the rumen and never reach the small intestine for absorption. Nevertheless, Cottle and Velle (1989) found substantial amounts of these amino acids flowing out of the rumen intact when they were supplemented in the diet. How to increase ruminal escape of SAA has been the topic of research by Ferguson et al., (1967) and Ferguson (1975). One alternative method to increase ruminal "escape" is to supply a nutrient in the ruminant's water supply (Garza et al., 1990). The objective of this research was to examine relative mohair growth responses to sulfur provided either as sulfate added to the feed or as DL-methionine added to the drinking water.

Materials and Methods

Experiment 1. In this study we examined the effect of supplemental sulfur. Levels tested included the basal level of sulfur recommended by Qi et al. (1991) of .26% (H), or this level supplemented with .03% added sulfur from either sodium sulfate in feed or DL-methionine added to drinking water.

One-year old wethers Angora goat were allocated randomly to the three treatments. Each treatment was provided to four wethers 12 mo old (37 kg). The supplemental sulfur was either not provided or added to drinking water or feed. The added sulfur was provided either as sodium sulfate (treatment S) added to the feed (2.4 g top-dressed on feed) or as DL-methionine (2.5 g) added to the drinking water (treatment M). Total sulfur intakes provided the equivalent of .26% S, .29% S and .29% S. Diet compositions are presented in the Table 1.

The trial lasted 60 days. The methionine (2.5 g/d) was mixed with 1 liter of water, which was provided when the daily meal was fed. After each kid consumed this quantity of water, it was given free access to tap water. All goats had free choice access to a feed; refused feed was collected once daily, weighed, and discarded. The goats were housed indoors in individual metabolism stalls (1.2 x 1.5 m). Ambient temperature was maintained at 20 $\pm$ 1^{0}C. Feed was available ad libitum with fresh feed added once daily. All diets met the minimum nutrient requirement for Angora goats (NRC, 1981). Each diet was sampled once each week throughout the experiment. Composited samples were analyzed for DM, CP (N x 6.25), ash (AOAC, 1990), ADF, NDF (Goering and Van Soest, 1970), and for GE using an adiabatic oxygen bomb (Parr Instrument Co., Inc., Moline, IL). Shorn body weights were determined on the first and last day of the experiment; total fleece was collected and weighed (ASTM, 1990ab). Sulfur was analyzed by the method described by Mottershead (1971).

Ruminal contents. Ruminal samples for ammonia and VFA measurement and plasma samples for amino acid quantification were obtained at 0, 2 and 4 hours postprandially. Ruminal samples were collected via an esophageal tube equipped with a strainer. Vacuum was applied to the esophageal tube using a 50 ml syringe. Ruminal fluid samples were examined for evidence of salivary

contamination and discarded if contaminated. The ruminal fluid was placed in 10 mL test tubes and preserved with .2 g of metaphosphoric for later VFA analysis following the procedure of Erwin et al. (1968). Total ruminal ammonia was analyzed by using the method of Broderick and Kang (1980). The 5 mL samples for ammonia analysis were preserved with .2 mL of 72% of sulfuric acid and held sealed at -20 °C.

Plasma Amino Acids. Jugular blood samples were collected 0, 2, and 4 h postprandially into 10-mL tubes containing heparin (Becton Dickinson Vacutainer Systems, Rutherford, NJ). These tubes were chilled immediately in an ice bath and transported to the laboratory. There, tubes were centrifuged at 3000 x g for 20 min to separate plasma. Aliquots of plasma were stored at -20 °C until analyzed. Prior to analysis, blood plasma samples (1 mL) were spiked with internal standards (.1 mL of .5 mM norvaline and .5 mM sarcosine) and deproteinized with .9 ml Seraprep (Pickering, Mountain View, CA). Amino acids analyses were performed using AminoQuant (Hewlett Packard, San Fernando, CA) using UV detection and pre-column derivatization with o-phthaldialdehyde and 9-fluorenylmethylchloroformate.

Mohair Fiber Measurements. At the beginning (day 0) and end (day 60) of the experiment, each goat was totally sheared. Weight of total (greasy) fleece, and clean mohair weight were measured. Fiber diameter and fiber length were determined in samples obtained from the left side of the animal at three specific positions: side, neck, and leg.

Statistical Analysis. Feed intake, body weight, mohair fiber measurements, blood, and ruminal traits were analyzed as a completely randomized design. (Cochran and Cox, 1957). Calculations were performed using the GLM procedure of SAS (1985). Significance was declared at levels of $P < .05$; $P < .10$

was interpreted to indicate a trend. Exact probability values are presented for all parameters analyzed.

Experiment 2. Methods were quite similar to those of Experiment 1 except that only two treatments were imposed in a crossover design; trial duration and sampling times were different. Each animal received each treatment with half being on each treatment during each period. The two treatments were identical to treatments M and S used in Experiment 1. Twelve male Angora wethers (40 kg) were assigned randomly to two groups after they had been weighed and shorn. Both groups were fed the diet containing .26% sulfur (Table 1). One group received 2.5 g met daily in its drinking water (for a total intake equivalent to .29% sulfur); the other received 2.4 g sodium sulfate placed on top of its daily feed (for a total intake equivalent to .29 % sulfur).

In this experiment, each period lasted 30 days. Times of ruminal and blood sampling were immediately before feeding and one hour after the feed was first offered. Other measurements and analyses were similar to those of Experiment 1 with animals being shorn on the final day of each period. Statistical analysis was for a crossover design so that animal effects could be removed.

Results and Discussions

Experiment 1. No treatment differences were significant for animal weight, feed intake, ADG, or ratios of feed to gain, gain to feed, or mohair to feed (Table 2). Most animals lost weight so that the mean gain and feed efficiency both were negative. No differences in mohair growth (greasy or clean) in response to methionine supplementation were detected although mohair production per day tended ($P<.08$) to be greater (29%) with dietary sodium sulfate in the feed than with methionine added to drinking water. Urinary nitrogen tended to be lowest with methionine in drinking water.

For ruminal measurements, no treatment by time interactions were significant (P>.56); therefore, means for treatment and time will be presented. Means for ruminal measurements are presented in Table 3. Although the sum of VFA in ruminal fluid was not significantly different (P = .33 to .86) among treatments, acetate percentage was greatest and propionate percentage tended to be least with sodium sulfate added to the diet than with the other two treatments. Sulfur supplementation averaged across sulfur sources decreased isovalerate (P<.04) and valerate (P<.06) proportions. Compared with sulfur added as methionine, sulfur added as sodium sulfate had a greater effect increasing acetate (P<.01) and decreasing (P<.05) propionate. Although ruminal ammonia was slightly lower with methionine in water than with the other treatments, differences were not significant.

Effects of postprandial time on ruminal measurements are presented in Table 4. Total mM concentrations in the rumen samples obtained 2 and 4 h postfeeding were almost double those measured in samples obtained before feeding for these kids fed one meal each day. These increases to a plateau are reflected as linear and quadratic effects of time (P<.02). Ruminal ammonia concentration also was higher 2 hour postprandially than before feeding although only the linear effect proved to be significant (P<.02).

The time by treatment interaction was not significant (P >.86) for plasma amino acid concentrations. So again, treatment and time means will be presented. Plasma concentrations averaged across time are presented in Table 5.

Except for methionine and cystine, plasma concentration of each amino acid was mathematically lower with methionine provided in drinking water than with the other two treatments. In contrast, plasma methionine concentration was greater (P<.02) when methionine was provided in drinking water than with the

other two treatments. Presumably, this reflects an increased post-ruminal supply of methionine and increased utilization of other essential amino acids when methionine was provided in the drinking water. The trend for a decreased urinary N concentration noted earlier also would reflect increased retention of protein. Subdivided by sulfur level and sulfur source, the higher level of sulfur tended to decrease plasma threonine (P<.09), valine (P<.07), tyrosine (P<.10) and leucine (P<.06). Plasma serine was greater mathematically (P<.31) with sulfate than methionine in drinking water, but plasma methionine was greater (P<.02) with methionine in drinking water than with sulfate added to the feed. Sulfur amino acids as a percentage of total amino acids (P <.01) was lower when sulfur was added as sodium sulfate than methionine; total essential amino acids as a percentage of total amino acids (P <.06) decreased when sulfur intake was increased (Table 5).

Effects of postprandial time on plasma amino acid concentrations are presented in Table 6. Concentrations of individual amino acids tended to be greater 2 h after feeding than before feeding, but they had declined at 4 h post-feeding to concentrations below pre-feeding concentrations for all of the non-essential amino acids. In contrast, plasma concentrations at 4 h postfeeding of all essential amino acids remained above pre-feeding levels. Presumably, this reflects preferential catabolism of the nonessential amino acids as a source of energy. Plasma methionine and cystine were increased at 2 h postfeeding and remained elevated at 4 h (linear P<.02 and quadratic effects P<.05). Total sulfur amino acids and sulfur amino acids as a percentage of total amino acids exhibited linear increases (P <.01 and .01) over time.

Experiment 2. Animal weights, feed intake, ADG, and ratios of feed to gain, gain to feed, and mohair to feed were not significantly different whether added sulfur was provided as methionine in drinking water or as sodium sulfate added

to the feed (Table 7). Clean mohair production tended to be greater (7%) with methionine in the drinking water, but this difference was not significant (P<.39). This increase could simply reflect the slightly (7%) greater feed intake of animals receiving methionine.

No treatment by time interactions were detected for ruminal measurements; hence, treatment and time means will be presented. Ruminal VFA concentrations were not different (P > .23) between treatments (Table 8) except for valerate which was greater (P < .03) with sodium sulfate added to the diet. Ruminal pH was not different, but ruminal ammonia concentration tended to be greater (P<.07) with the sulfate than the methionine treatment; this matches a trend noted in Experiment 1.

Concentration of each VFA was greater 1 h after than before feeding (Table 9). Similar effects were noted in Experiment 1 although changes were less dramatic there, presumably because energy intakes were higher in Experiment 2. VFA concentrations had increased by 68% at 2 hour in the first experiment and at 1 hour postprandially in the second experiment.

Reflecting the higher VFA concentrations, ruminal pH was lower after than before feeding. Ruminal ammonia concentration was greater after than before feeding, again matching results of Experiment 1.

No time by treatment interactions were noted for plasma amino acid concentrations; hence, amino acid means for individual treatments and individual times will be discussed. Concentrations of total nonessential amino acids tended to be higher with the sulfate than the methionine treatment while the essential amino acids tended to be higher with the methionine than the sulfate (Table 10). However, no differences in methionine or cystine or in concentrations of essential amino acids were significant. The proportion of total amino acids that was essential was greater (P<.04) with the met treatment. Plasma amino acid

concentrations for all amino acids except methionine and cystine tended to be lower 1 h after than before feeding (Table 10). However, the only significant decrease (P<.02) was for glutamic acid. Sulfur amino acids as a portion of total amino acids tended to be greater (P<.07) after feeding.

DL-methionine given in drinking water had no effect on performance or efficiency in these trials. No increases in mohair growth (clean) in response to methionine supplementation in drinking water was detected. Clean mohair production tended to be higher with sodium sulfate in Experiment 1 but with methionine in Experiment 2. Failure of methionine in drinking water to increase mohair production could be attributed to 1) failure of methionine to escape ruminal fermentation or 2) failure of an increased post-ruminal supply of methionine to increase mohair growth.

Garza et al. (1990) indicated that 40 to 80 percent of a water soluble marker in drinking water provided to steers bypassed the rumen. Carneiro et al. (1994) reported that clean mohair production was increased by 37% (P < .12) when 2.5 g of methionine added in the drinking water for adult goats. However, in that study, the control diet (.15% S) was not isosulfurous with other diets and when methionine was added dietary sulfur became .18 %. With diets that are very low in sulfur, a mohair growth response might be expected from any source of added sulfur. In experiment 1, increases in relative and absolute plasma concentrations of methionine and cystine suggest that the post-ruminal methionine supply was increased when methionine was provided in drinking water. These results support the concept that methionine added to water can increase blood concentrations of sulfur amino acids. However, failure of this increased supply of methionine to increase mohair production suggests that the

basal supply of methionine already may have been adequate to provide for this rate of mohair production.

Precise amounts of post-ruminal methionine available to the animals in these trials were not determined. Amounts (moles of SAA) might be estimated from feed intakes and compared with S deposition in mohair. Such values are presented in Table 10. Results suggest that 30 to 40 moles of SAA in the plasma supply of met and cys should be adequate for maximum rates of mohair production.

One hour after feeding, the concentration of almost all the amino acids in the plasma was reduced except for SAA (Cys and Met) that were increased. That an increased supply of methionine will decrease plasma glycine concentration is not particularly surprising. Glycine is involved in several reactions (formation of cysteine, creatinine, and glutathione) in which methionine also participates. Since arginine is involved in urea formation, reduction in the arginine concentration following methionine administration also might be expected. Decreases in the plasma levels of glycine, valine and arginine concentration following methionine administration have been reported previously by Cottle and Velle (1989); declines in glycine were found by Strath and Shelford (1978) and Tao *et al.* (1974).

Generally, our results support those of Reis et al. (1978), and Doyle and Moir (1979). They concluded that in most studies where wool production responses to supplemental methionine have been detected, diets were sulfur-deficient. Only occasionally when dietary sulfur supply was adequate has wool growth increased when the supply of protected methionine or protein has been increased (Doyle and Moir, 1979)

Implications

Mohair growth was not increased by methionine supplementation in drinking water. However, plasma responses indicate that methionine in drinking water partially escaped ruminal fermentation. Failure of an increased methionine supply to increase mohair production suggests that when the dietary sulfur supply is adequate, the post-ruminal methionine supply is adequate so that an increased supply of bypassed methionine will not increase mohair production.

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Table 1. Composition of experimental diets (% of DM).

Ingredient	
Cottonseed hulls	38.00
Ground corn	33.10
Soybean meal 44%	18.00
Alfalfa hay good	7.60
Limestone 38%	0 - 0.3
Salt	1.50
Sodium sulfate	0.30
Dicalcium phosphate [a]	0.50
Ammonium cloride	0.50
Vit. A, D, E [b]	0.20
Chemical composition [c]	
CP %	13.80
DM %	92.81
ADF %	25.25
NDF %	43.40
S %	0.15 - 0.29
GE, Mcal / Kg of DM	4.40

[a] Dicalcium phosphate containing 22 % Ca, 19.3 % P.

[b] Contained 2,200 IU of vitamin A, 1,100 IU of vitamin D_3, and 2.2 IU of vitamin E per gram.

[c] Chemical composition was measured.

Table 2. Means of intake, body weight, mohair production and urinary nitrogen.

Items	Diet			SE	Probability <	
	Basal	Methionine	Sulfate		1 vs 2 & 3	2 vs 3
Initial weight	37	38	37	1.52	.86	.68
Final weight	36	37	36	2.44	.80	.90
Intake, g/d	1,056	961	1,042	76.34	.58	.48
ADG, g/d	-.02	-.02	-.01	.02	.80	.83
Feed:Gain	-.03	-.03	-.01	.01	.80	.72
Gain:Feedx1000	-28	-24	-116	.01	.34	.09
Hair:Feedx1000	1.16	1.06	1.21	.12	.87	.39
Mohair						
Clean, g total	1,197	1,002	1,260	93.28	.58	.08
Clean, g /day	20	17	21	1.56	.58	.08
Urinary nitrogen, %	1.29	.78	1.34	.19	.44	.11

ADG = Average daily gain [1000*(final weight - initial weight) / days].

Table 3. Means of ruminal fluid volatile fatty acid contents, and ammonia.

	Diet				Probability <	
Item	Basal	Methionine	Sulfate	SE	1 vs 2 & 3	2 vs 3
Total, m*M*	56.13	54.44	53.29	2.32	.33	.86
VFA, moles/100 moles.						
Acetate	66.36 [b]	66.67 [b]	69.42 [a]	.55	.01	.01
Propionate	17.34 [a]	17.01 [a]	15.16 [b]	.74	.12	.05
Isobutyrate	.81	.84	.72	.09	.87	.47
Butyrate	12.90	13.51	12.63	.48	.81	.19
Isovalerate	1.32	.93	.81	.17	.04	.59
Valerate	1.27	1.16	1.14	.05	.06	.75
Ammonia, mg/dL	14.48	11.94	14.81	1.67	.51	.33

Means with the same letter are not significantly different ($p < .05$).

Table 4. Means of ruminal, fluid volatile fatty contents and ammonia.

Item	Time (hr) 0	2	4	SE	Probability < Linear	Quadratic
Total, m*M*	38.35 [c]	64.54 [a]	59.73 [b]			
VFA, moles/100 moles						
Acetate	70.73 [a]	66.91 [b]	65.18 [c]	.55	.01	.13
Propionate	12.48 [b]	18.36 [a]	18.30 [a]	.74	.01	.95
Isobutyrate	1.20 [a]	.57 [b]	.65 [b]	.09	.01	.49
Butyrate	13.07 [ab]	12.16 [b]	13.76 [a]	.48	.86	.03
Isovalerate	1.42 [a]	.67 [b]	.98 [ab]	.17	.01	.19
Valerate	1.10 [b]	1.33 [a]	1.13 [b]	.05	.04	.01
Ammonia, mg/dl	10.39 [b]	16.63 [a]	13.84 [ab]	1.67	.02	.24

Means with the same letter are not significantly different ($p < .05$).

Table 5. Means of amino acids in the plasma (μM).

	Treatments				Probability <	
Items	Basal	Methionine	Sulfate	SE[a]	1 vs 2&3	2 vs 3
Ser	249.24 [ab]	174.12 [b]	299.72 [a]	39.31	.83	.31
Gly	893.08	680.67	897.82	103.29	.41	.15
Thr	114.73 [a]	81.84 [b]	102.66 [ab]	10.35	.09	.17
Ala	261.78	232.19	263.24	27.81	.68	.43
Arg	219.68	169.84	180.27	22.81	.12	.75
Val	294.44 [a]	209.13 [b]	256.70 [ab]	27.13	.07	.22
Met	24.37 [b]	34.05 [a]	21.38 [b]	3.48	.44	.02
Cys	15.82	16.18	17.17	1.67	.80	.68
Phe	63.99	53.81	55.77	5.90	.21	.82
Ile	107.56	80.53	93.94	10.61	.13	.38
Glu	121.96	101.02	114.54	14.48	.44	.51
Tyr	84.70	62.54	71.68	8.06	.10	.42
Leu	169.98	125.49	141.64	15.51	.06	.47
Lys	122.35	98.46	110.80	11.82	.23	.46
TAA[b]	2743.68	2119.87	2627.33	272.45	.23	.12
TEAA[c]	2010.18	1533.82	1860.98	184.41	.18	.22
TNEAA[d]	733.50	586.05	766.35	82.69	.54	.10
TSAA[e]	40.18	47.75	38.55	4.36	.58	.15
TSAA/TAA	.01	.02	.01	.01	.19	.01
TEAA/TAA	.73	.72	.71	.01	.06	.25

[a]SE = Means with the same letter are not significantly different ($p < 0.05$).

[b]TAA = Total amino acids.

[c]TEAA = Total essential amino acids.

[d]TNEAA = Total nonessential amino acids.

[e]TSAA = Total sulfur amino acids.

Table 6. Means of amino acids in the jugular plasma (μM) .

	Time (hr)				Probability <	
Item	0	2	4	SE[a]	Linear	Quadratic
Ser	230.27	274.00	218.46	41.15	.84	.33
Gly	808.00	896.50	767.10	104.66	.78	.40
Thr	94.09	107.42	97.72	10.84	.81	.39
Ala	254.83	267.87	234.51	27.30	.60	.49
Arg	178.82	194.52	196.46	23.04	.59	.81
Val	239.65	267.37	253.25	28.32	.74	.55
Met	16.80 [b]	32.73 [a]	30.27 [a]	3.75	.02	.05
Cys	9.30 [b]	19.61 [a]	19.92 [a]	1.59	.01	.01
Phe	49.06	62.57	61.94	5.88	.13	.33
Ile	77.09	102.32	102.61	10.80	.10	.35
Glu	106.48	127.35	103.69	13.62	.89	.19
Tyr	63.15	76.66	78.05	8.24	.18	.56
Leu	119.12	158.48	159.50	16.02	.08	.34
Lys	105.58	114.81	111.22	11.83	.74	.66
TAA[b]	2352.24	2702.21	2434.70	264.83	.83	.35
TEAA[c]	1688.23	1936.71	1780.04	188.54	.73	.39
TNEAA[d]	664.00	765.84	655.14	80.06	.94	.29
TSAA[e]	26.08	52.34	50.18	4.53	.01	.02
TEAA/TAAx100	71.90	72.13	72.66	.01	.44	.86
TSAA/TAAx100	1.14	2.08	2.27	.02	.01	.13

[a]Means with the same letter are not significantly different ($p < 0.05$).

[b]TAA = Total amino acids.

[c]TEAA = Total essential amino acids.

[d]TNEAA = Total nonessential amino acids.

[e]TSAA = Total sulfur amino acids.

Table 7. Means of intake, body weight, mohair production.

Items	Sulfur source		SE	Probability <
	Methionine	Sulfate		S Source
Initial weight	40	40	1.19	.87
Final weight	42	42	1.39	.98
Intake, g/d	1276	1194	71.97	.43
ADG, g/d	.07	.06	.01	.48
Feed:Gain	.01	.05	.01	.51
Gain:Feedx1000	288.4	225.8	.01	.70
Hair:Feedx1000	.63	.62	.04	.89
Mohair				
Clean, g total	777.52	724.71	42.70	.39
Clean, g/day.	25.92	24.16	1.42	.39

ADG = Average daily gain [1000*(final weight - initial weight) / days].

Table 8. Means of ruminal fluid volatile fatty acid contents, pH and ammonia.

	Sulfur source			Probability <
Item	Methionine	Sulfate	SE	S Source
Total,m*M*	52.92	57.76	2.78	.23
VFA, moles/100moles				
Acetate	68.08	67.33	.54	.29
Propionate	16.47	16.59	.43	.83
Isobutyrate	.82	.89	.03	.14
Butyrate	12.18	12.40	.38	.69
Isovalerate	1.27	1.48	.11	.18
Valerate	1.18 [b]	1.31 [a]	.04	.03
pH	6.72	6.65	.03	.14
Ammonia, mg/dL	12.23	14.88	1.00	.07

Means with the same letter are not significantly different ($p < 0.05$).

Table 9. Means of ruminal fluid volatile fatty acid contents, pH and ammonia.

	Time (hr)			Probability <
Item	0	1	SE	Time
Total,m*M*	41.31 [b]	69.36 [a]	2.78	.01
VFA,moles/100moles				
Acetate	68.55 [b]	66.86 [a]	.55	.03
Propionate	14.05 [b]	19.01 [a]	.43	.01
Isobutyrate	1.17 [a]	.55 [b]	.03	.01
Butyrate	12.94 [a]	11.64 [b]	.38	.02
Isovalerate	2.16 [a]	.81 [b]	.09	.01
Valerate	1.27	1.27	.03	.98
pH	6.91 [a]	6.46 [b]	.03	.01
Ammonia, mg/dL	11.63 [b]	15.48 [a]	1.00	.01

Means with the same letter are not significantly different ($p < .05$).

Table 10. Means of amino acids in the Jugular plasma (m*M*).

	Treatments			Probability <	Time			Probability <
Amino acids	M	S	SE[a]		0	1	SE[a]	
Ser	256.65	329.94	33.6	.13	306.42	280.18	33.6	.58
Gly	981.62	1093.85	82.2	.34	1109.26	966.21	82.3	.23
Thr	117.13	124.72	7.8	.49	127.04	114.80	7.8	.27
Ala	302.23	337.81	23.0	.28	320.20	319.83	23.0	.99
Arg	222.47	219.97	15.8	.91	232.11	210.34	15.8	.34
Val	274.17	247.57	17.2	.28	280.62	241.12	17.2	.11
Met	20.46	20.64	2.4	.95	18.71	22.39	2.4	.29
Cys	9.67	8.04	1.0	.26	8.40	9.28	1.0	.54
Phe	65.93	64.34	4.9	.80	67.46	62.81	4.3	.45
Ile	91.82	86.46	6.3	.55	94.48	83.79	6.3	.24
Glu	100.53	111.46	9.5	.42	122.02 [a]	89.97 [b]	9.5	.02
Tyr	92.15	91.25	7.3	.93	93.34	90.06	7.3	.75
Leu	148.91	139.28	11.2	.55	155.39	132.80	11.2	.16
Lys	139.45	133.61	9.5	.67	141.82	131.24	9.5	.43
TAA[b]	2823.19	3008.94	192.8	.55	3077.27	2754.82	192.8	.27
TEAA[c]	857.66	812.33	56.0	.57	881.03	788.96	56.2	.25
TNEAA[d]	1965.53	2196.61	155.6	.37	2149.44	1948.55	155.5	.37
TSAA[e]	31.32	29.55	3.5	.73	27.65	33.22	3.5	.28
TEAA/TAA x 10	31.00 [a]	28.00 [b]	.01	.04	28.63	28.64	.01	.80
TSAA/TAA x 10	1.07	.95	.001	.30	.90	1.21	.01	.07

[a]Means with the same letter are not significantly different ($p < .05$).

[b]TAA =Total amino acids.

[c]TAA = Total essential amino acids.

[d]TNEAA = Total nonessential amino acids.

[e]TSAA = total sulfur amino acids.

CHAPTER V

EFFECTS OF SUPPLEMENTAL SULFUR ON AMINO ACID COMPOSITION OF ISOLATED RUMINAL BACTERIA .

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ABSTRACT. The objective of this research was to determine whether a dietary deficiency of sulfur altered the amino acid composition of protein of isolated ruminal bacteria. Twelve Angora (BW ± SE = 18.1 ± .6 kg) and twenty Alpine (23.7 ± 1.0 kg) castrated male goats kids were individually fed isonitrogenous and isocaloric diets containing 2.28% N and either .11 (basal), .20, .28 or .38% sulfur for 10 weeks. Added sulfur came from supplemental $CaSO_4$. Goats were blocked by age and three Angora and five Alpine goats were assigned to each treatment. Non-attached ruminal bacteria were isolated by differential centrifugation and analyzed for amino acid (AA) composition. Differences between the two breeds were detected in AA composition of ruminal bacteria (mg/g DM) for lys ($P < .07$) and the phenolic AA ($P < .03$). Among the nonessential AA, differences were noted for asp ($P < .15$), pro ($P < .01$) and tyr ($P < .02$). Total nonessential AA also tended to be different ($P < .13$) between

breeds. In all cases, values were higher for bacteria from Angora than Alpine goats. When expressed per 100 g of AA, similar statistical differences were detected with lys and phenolic AA being greater ($P < .04$) for Angoras and, among the nonessential AA, differences being noted for ala ($P < .12$), pro ($P < .01$) and tyr ($P < .03$). In addition; however, Alpine goats had a higher proportion of total AA in the form of essential AA ($P < .01$), total sulfur AA ($P < .02$), more met ($P < .05$) and more cys ($P < .07$). As a percentage of essential AA, the differences were small but values were greater for lys ($P < .02$) and his ($P < .08$) for Angora and higher proportion of met ($P < .16$), and phe ($P < .11$) for Alpine. Sulfur supplementation of the diet produced a quadratic increase in cys concentration when expressed as mg/g bacterial DM ($P < .08$), and a linear increase ($P < .05$) when expressed as g/100g of total AA of bacteria. Sulfur supplementation also linearly increased ser expressed either mg/g bacterial DM ($P < .07$) or as g/100g of total AA ($P < .10$). Added sulfur tended to linearly decrease concentrations of phe, thr and ala ($P < .11$; $P < .16$; and $P < .07$) when expressed per 100 g of AA. Added sulfur linearly decreased ($P < .07$) total essential AA and but increased ($P < .15$) total nonessential AA. Adding $CaSO_4$ in the diet of growing kids altered amino acid composition of protein of isolated ruminal bacteria, increasing the proportional concentrations of cys and ser but not met.

KEY WORDS: Sulfur, Goats, Amino acid, Bacteria.

INTRODUCTION

The quantity of protein and thereby of sulfur amino acids (AA) available for digestion and absorption by ruminants equals the sum of dietary protein that escapes ruminal fermentation and microbial protein synthesized the rumen. The former depends on the quantity of protein fed as well as its potential to resist

microbial attack within the rumen. The latter, although usually limited by the quantity of energy fermented within the rumen, also can be limited by supply of other nutrients required for microbial growth (NRC, 1985). Low ruminal S concentrations can depress microbial growth and fiber digestibility (Kandylis, 1984). Weston et al. (1988) also proposed that the concentration of AA containing sulfur (met, cys, cys-cys) in ruminal microbes might be reduced by a deficiency of sulfur. The objective of this research was to determine whether a dietary deficiency of sulfur altered the amino acid composition of protein of isolated ruminal bacteria.

Materials and Methods

Animals and Diets. The experimental design was described previously (Qi et al., 1993). Briefly, 32 male, castrated goat kids were individually fed. Twelve were Angora goats kids (BW ± SE = 18.1 ± .6 kg) and twenty Alpine goat kids (23.7 ± 1.0 kg). Isonitrogenous and isocaloric diets were fed; diets contained, by analysis, 2.28% N and either .11 (basal), .20, .28 or .38% sulfur (with added sulfur coming from $CaSO_4$) during a 10 week growth trial (July to October, 1991). Each animal, housed in its own stainless steel cage (2.3 X 1.0 X 1.0) in an open barn with forced air ventilation, was given ad libitum access to its diet with 3 Angora and 5 Alpine kids being fed each diet. Diets (Table 1) were formulated to meet ME, CP, Ca and P requirements for growing goats according to NRC (1981). Calcium intake from $CaSO_4$ was balanced by adding $CaCO_3$ to the supplemented diets; $Si0_2$ was added to equalize the nutrient density among diets. Fresh feed was provided once daily and fresh water was available continuously.

Sample collection and Analysis. On day 70 of the trial, ruminal samples were taken by stomach tube at 0 and 4 h postprandially and composited across time but within each animal (280 mL). The first 20 to 30 mL of ruminal fluid from each stomach tube sample was discarded to reduce contamination from saliva. Immediately after collection, the ruminal fluid was centrifuged (Sorvall RC-5B, Du Pont, Wilmington, DE) at 500 x g for 10 min. to remove the feed particles and protozoa; then the supernatant fluid was centrifuged further (20,000 x g for 20 min) to sediment bacteria. The supernatant fluid was discarded, and the bacterial pellet was washed twice with physiological saline (.9% NaCl), and once with distilled water. Examined microscopically, these bacterial pellets were essentially free of contaminants. These pellets were lyophilized and contents of DM and amino acid were determined. Complete details for the protein hydrolysis and AA analysis have been given by Barkholt and Jensen (1989). Tryptophan was not measured.

Statistical Analysis. The goats kids, blocked by breed and age, were assigned randomly to one of the four rations in a complete randomized block design. For analysis, we considered that we had two 2 blocks or breeds (Cochran and Cox, 1957) with three Angora and five Alpine goats being fed each of the four sulfur levels. Data were subjected to ANOVA according to GLM procedure of SAS(1985). The analysis for Angora and Alpine kids was conducted in a factorial experiment with two factors: breed (2) and sulfur levels (4) as randomized complete block design. Mean amino acid compositions of isolated ruminal microbes were compared with literature values; the probability that determined values differed from literature values was estimated using literature standard errors and t-tests. The residual mean square was used to test for diet and breed effects Exact probability values are presented for all

parameters analyzed. Regressions were used to detect linear, quadratic and cubic effects of dietary sulfur concentration. Because the analyzed dietary S concentrations were not equally spaced, polynomial values were calculated (Verneque, 1994) to employ in these regressions.

Results and Discussion

Goats in this trial responded to sulfate supplementation with quadratic increases in ADG and DMI, each peaking with the .20% S diet (Qi et al., 1993). Apparent retention of S also reached a plateau with .20% S in the diet. Such differences suggest that .20% S was near the S requirement for these kids.

Amino Acid Concentrations of Isolated Ruminal Bacteria. Mean amino acid contents for ruminal bacteria (g/100 g AA) isolated from ruminal fluid of our goats are presented in Table 2 and Figure 1. That table also presents amino acid compositions of ruminal microbes that have been reported by Clark et al. (1992), by Ibrahim and Ingalls (1971) and by Hoeller and Harmeyer (1964) as cited by Purser and Buechler (1965). Compared with mean values from the summary of 18 studies (441 bacterial samples from animals fed 61 dietary treatments in 35 experiments) compiled by Clark et al. (1992), measured compositions for the bacteria that we isolated fell more than 2 SD away for pro (2.2 SD below), ile (2.0 SD below), phe (2.0 SD below) and tyr (4.5 SD above). Despite these statistical differences, our means all fell within the range of literature values presented by Clark et al. (1992). Values from Ibrahim and Ingalls (1971) for bacteria from dairy cattle generally were intermediate between our values and those of Clark et al. (1992). Values for cystine were not provided in the summary by Clark et al. (1992), but our values (1.3%) were slightly higher than those reported by Purser and Buechler (1966; 1.0%) and by Weller (1957; .7 to .8%) for sheep and Hoeller and Harmeyer(1964; 1.1%), for goats. But our

values were less than half those reported Ibrahim and Ingalls (1972) (3.0 to 3.2%) for lactating cows.

Perhaps species differences in the amount of sulfur available for recycling could alter cys content of bacteria. In an attempt to determine whether the animal species differences might be responsible for differences in methionine and cystine content of microbial protein, we compared values for microbial protein isolated the rumen of sheep versus cows from 21 different publications (Weller et al., 1957; Hoeller and Harmeyer, 1964; Purser and Buechler, 1966; Meyer et al., 1967; Bergen et al., 1968; Hoogenraad and Hird, 1970; Ibrahim and Ingalls, 1972; Williams and Dinusson, 1973; Burris et al., 1974; Ulyatt et al., 1975; Czerkawski, 1976; Ling and Buttery,1978; Storm and Ørskov, 1983; John, 1984; Hvelplund et al., 1986; Cecava et al., 1988; Titgemeyer et al., 1989; Cecava et al., 1990a; Cecava et al., 1990b; Cecava and Parker, 1993; Cunningham et al., 1993). Results are showed in Table 3 and Figure 1. The cys content per 100 g of AA of bacteria from cattle averaged 1.62 g (SE = .3); for sheep, this value was 1.0 g (SE = .5). For met, bacteria from cattle averaged 2.2 g (SE = .2) versus 2.4 g (SE = .2) for bacteria from sheep. These differences were not statistically significant. The mean cys concentration from our study was 1.3 g, which although being 1 SE below the mean for cattle, was slightly above the mean for sheep. Our value for methionine (3.4 g/100g of AA) was slightly above the mean for value for both cattle and sheep. Hoeller and Harmeyer (1964) as cited by Purser and Buechler (1965) found cys and met values from ruminal bacteria from goats that were very similar to ours (1.1 g/100g of AA for cys and 3.0 g/100g of AA for met).

Comparison of Breeds. Differences between breeds of goats in amino acid composition (mg) per g of dry rumen bacteria were detected for lys ($P < .07$), and phenolic AA ($P < .03$) as shown in Table 4. Among the nonessential

AA, differences were noted for pro ($P < .01$) and tyr ($P < .03$). Total nonessential AA also tended to be higher ($P < .13$) for bacteria from Angoras. When expressed per 100 g of AA (Table 5); statistical differences remained similar, with lys ($P < .04$) and phenolic AA ($P < .04$) being higher for Angora goats and, among the nonessential AA, differences being noted for pro, gly ($P < .01$), and tyr ($P < .03$). Total nonessential AA also tended to be different ($P < .01$) between breeds. Compared with bacteria from Angoras, bacteria from Alpine goats had a higher proportion of leu ($P < .08$) and total AA in the form of essential AA ($P < .01$), total sulfur AA ($P < .02$) more methionine ($P < .05$) and more cystine ($P < .07$). Among the nonessential AA Alpine goats also had higher gly ($P < .01$). As a percentage of essential AA (Table 6), the differences, although small, were significantly greater for lys ($P < .02$) in bacteria isolated from Angora than from Alpine kids.

Effects of Sulfur Supplementation. Sulfur supplementation of the diet produced a quadratic response in cys concentration when cys was expressed as mg/g bacterial DM ($P < .08$) and a linear increase ($P < .04$) when cys was expressed as g/100g of total AA of bacteria (Tables 7 and 8 and Figure 2). Serine responded to sulfur supplementation by increasing linearly when expressed either as mg/g bacterial DM ($P < .07$) or as g/100g of total AA ($P < .09$) as shown in figure 3. Regardless of the means of expression, met concentrations were not significantly altered by sulfate supplementation (Table 7, 8 and 9). Added sulfur tended to linearly decrease concentrations of ala ($P < .07$) when expressed per 100 g of AA. Glutamic acid, asp and tyr increased cubicly ($P < .09$; $P < .06$; and $P < .11$) with added sulfur when expressed per 100 g of amino acid. With added sulfur, total essential amino acid percentage decreased linearly ($P < .07$) being replaced by nonessential amino acids (linear increase; $P < .15$).

Regression of cystine concentration (mg per g bacterial DM) against dietary S (Figure 2) suggests that cys content of bacterial crude protein should peak when dietary S equals .263% of diet dry matter. This value is only slightly higher than the S required for maximum ADG (.22%) or DMI (.24%) according to Qi et al., (1993).

Cysteine is the primary sulfur amino acid incorporated into wool protein. The high concentration of cys in keratin relative to the cys concentration in the plant material suggests that wool growth may be limited by supply of cys or by sulfur AA. Reis (1967) reported that dietary methionine can replace cystine. Williams et al. (1972) reported that wool growth was stimulated more by DL-methionine than by L-cystine in merino sheep (41 vs 32%) suggesting that met has stimulatory effects on wool growth beyond its function as a cys precursor. Experiments with mixtures of AA infused into the abomasum have indicated a very specific role for met in the control of wool growth. Omission of methionine from a mixture of 10 essential L-AA reduced wool growth appreciably below pre-treatment rates, and the inclusion of cysteine in this mixture failed to increase wool growth.

Presumably, differences in amino acid composition of isolated bacteria from goats fed diets containing different levels of sulfur can be ascribed to differences in selection pressure for species or strains of bacteria that require less S. Differences between goat breeds are harder to explain With greater mohair production, perhaps less S is recycled by Alpine than Angora goats. Qi et al. (1993) found that Alpine kids in this study has lower ruminal pH and were fauna-free due to isolation from adults during growth. Perhaps differences in ruminal conditions may exist that in turn could cause differential degradation of specific bacteria or bacterial proteins. Schelling and Hatfield (1968) evaluated the adequacy of amino acids in microbial protein by feeding purified diet to

lambs. Protozoa were absent from the ruminal population of lambs fed their purified diet and the number of bacterial species in the rumen was limited. Absence of a thriving rumen protozoa population also might alter amino acid composition of ruminal microbes. In our study most of Alpine goats were defaunated. Alpine kids were fed pasterized milk, and kept in their isolated cages from birth to weaning at 75 days of age. Clarke (1978) suggested that the number of ruminal bacteria increases when protozoa are absent, so protozoa may markedly alter both the types of bacteria present and their chemical composition. Besides being proteolytic, ruminal protozoa possess a cysteine proteinase plus high aminopeptidase but limited deaminase activities (Forsberg et al., 1984). This may preferentially cleave cysteine from proteins. Cysteine and met both tended to be higher in Alpine (fauna free) than Angora goats.

Serine responses to added sulfur tended to be linear. In 1957, Schlossmann and Lynen as cited by Cooper (1983) showed that yeast contains an enzyme that catalyzes the reversible conversion of L-serine to L-cysteine. Combined with homocysteine, serine will produce cystathionine in the presence of one ketobutyrate. Cysteine is readily oxidized to cystine. Perhaps such linkage between these two AA can explain their parallel increases with sulfur supplementation.

Implications

Historically, amino acid composition of protein of ruminal bacteria was considered to be constant and fixed. Results of this study indicate that when sulfur is deficient, the cystine content of isolated ruminal bacteria was reduced. Breeds of goats also differed in composition of isolated bacteria, perhaps due to differences in protozoal numbers. Whether these differences can be attributed entirely to selection pressure for microbes having less need for sulfur or to alterations in the protozoal population is not certain. Performance benefits

heretofore attributed to an altered quantity of bacterial protein synthesis might instead be due to a slight alteration in concentration of the limiting amino acid in protein of ruminal bacteria.

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Table 1. Composition of experimental diets[a]

Item	Diet 1	2	3	4
Ingredient				
Ground peanut hulls	50.00	50.00	50.00	50.00
Corn starch[b]	37.40	37.40	37.40	37.40
Soybean meal	6.10	6.10	6.10	6.10
Urea	1.50	1.50	1.50	1.50
Na_2HPO_4	1.35	1.35	1.35	1.35
$CaCO_3$	.85	.53	.25	.00
$CaSO_4$	.00	.40	.80	1.15
Trace mineralized salt[c]	1.50	1.50	1.50	1.50
Vit A,D,E[d]	1.00	1.00	1.00	1.00
SiO_2	.30	.22	.10	.00
Chemical composition[e]				
GE, Mcal/kg	4.20	4.18	4.20	4.17
ME, Mcal /kg	1.80	1.67	1.72	1.70
N, %	2.28	2.28	2.27	2.29
S, %	.11	.20	.28	.38
N:S Ratio	21.27	11.69	8.20	5.99
ADF, %	29.77	29.62	31.98	30.41
ASH, %	6.65	6.66	6.69	6.93
Ca, %	.60	.62	.61	.62
P, %	.50	.49	.52	.51
Na, %	.97	.96	.98	.99
K, %	.57	.55	.58	.56
Cl, %	.75	.77	.76	.74
DCAB[f], meq/100 g	35.62	34.11	36.03	36.52
S, meq/100 g	3.35	6.08	8.68	11.91
DCAB:S[g], meq/100 g	32.27	28.03	27.35	24.61

[a]DM basis

[b]Dyets, Betlehem, PA.

[c]Containing (percentage): NaCl, 95.5 - 98.5;Mn, > .24; Fe, > .24; Mg, > .05; Cu, > .032; Co, > .011; I, > .007; Zn, > .005.

[d]Contained 2,200 IU of vitamin A, 1,200 IU of Vitamin D3, and 2.2 IU of vitamin E per gram.

[e]All values except ME were measured. Feed and fecal energies were measured; ME was calculated as DE x .82

[f]Dietary cation-anion balance was calculated as meq((Na +K) - Cl) / 100 g of diet DM.

[g]Dietary cation-anion balance was calculated as meq((Na +K) - (Cl +S)) / 100 g of diet DM.

Table 2. Amino acid composition of ruminal bacteria compared with previously published data.

Experiments compared	Present study		Clark et al. (1992)[a]		Ibrahim & Ingalls(1971)[b]		Hoeller & Harmeyer(1964)[c]
	Mean	SD	Mean	SD	Mean	SD	Mean
Essential AA							
Val	5.41	.22	6.20	.60	5.45	.17	4.20
Thr	5.02	.24	5.80	.50	5.40	.15	4.90
Ile	4.86 [b]	.30	5.70 [a]	.40	5.30 [ab]	.30	3.00
Leu	7.73	.23	8.10	.80	6.70	.30	3.60
Phe	4.82 [b]	.17	5.10 [a]	.30	4.70 [ab]	.20	4.90
Lys	6.77	.42	7.90	.90	7.00	.30	9.70
Met	3.45	.30	2.60	.70	4.40	.30	3.00
Arg	4.74	.27	5.10	.70	3.80	.10	6.10
His	1.82	.15	2.00	.40	3.20	.30	6.30
Nonessential AA							
Ala	8.29	.35	7.50	.60	7.10	.20	5.90
Asp	11.37	.25	12.20	.60	12.05	.40	11.60
Glu	13.71	.57	13.10	.70	12.15	.40	14.20
GLy	5.86	.20	5.80	.50	5.10	.14	5.10
Pro	2.57 [b]	.37	3.70 [a]	.50	3.30 [a]	.13	5.30
Ser	4.75	.48	4.60	.40	4.15	.10	4.90
Tyr	7.55 [a]	.96	4.90 [b]	.60	6.60 [a]	.84	4.40
Cys	1.27 [b]	.13			3.13 [a]	.10	1.10

[a] Value for bacteria from sheep and cattle.

[b] Value for bacteria from dairy cattle.

[c] Value for bacteria from goats as cited by Purser and Buechler (1965).

Mean = g/100 g of AA

Table 3. Published values for AA from rumen bacteria[f].

	Cys		Met	
	Mean	SE	Mean	SE
Goats (present study)	1.30	.10	3.40	.30
Sheep	1.03	.50	2.43	.20
Cow	1.62	.30	2.17	.18

[f]Average of amino acid composition of microbial protein isolated rumen of sheep and cattle from published paper (Ling and Buttery, 1978; Czerkawski,1976; Bergen et al.1968; Cecava et al., 1990; John, 1984; Storm and qroskov, 1976; Weller, 1957; Purser & Buechler, 1966; Cecava et al., 1988; Hvelplund, 1986; Cecava & Parker, 1993; Ulyatt et al., 1975; Hoogenraad and Hird, 1970; Ibrahim and Inghall, 1971; Titgemeyer et al., 1989; Burris et al., 1974; Meyer et al.,1967; Williams and Dinusson, 1973; Cunningham et al., 1993;

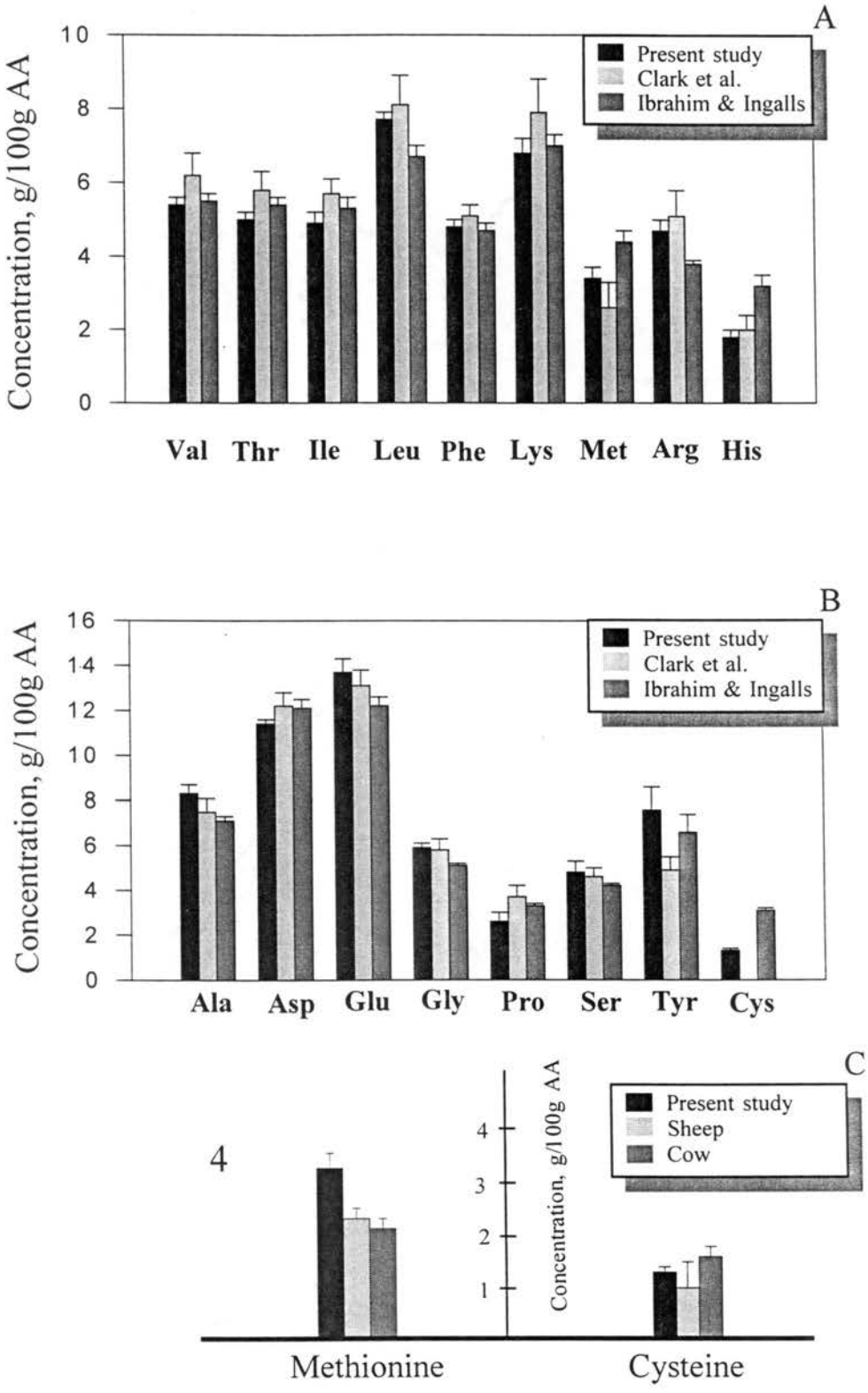


Figure.1a Amino acid composition of ruminal bacteria for essential AA (A) and (B) nonessential AA. and (C) published mean values for methionine and cysteine from 21 publications.

Table 4. Amino acid composition of ruminal bacteria from Angora and Alpine kids (mg /g of bacterial DM).

Item	Alpine	Angora	SE[e]	Probability
Essential AA				
Arg	13.36	13.88	.39	.44
His	5.12	5.34	.14	.39
Ile	13.74	13.93	.33	.74
Leu	21.82	22.38	.47	.48
Lys	18.69 [d]	20.56 [c]	.58	.07
Met	9.92	9.70	.34	.70
Phe	13.61	13.93	.29	.51
Thr	14.20	14.49	.32	.60
Val	15.32	15.63	.41	.67
TEAA[f]	125.78	129.84	2.99	.43
TSAA[g]	13.59	13.22	.44	.69
PhAA[h]	34.07 [b]	37.59 [a]	.92	.03
Nonessential AA				
Ala	23.41	23.92	.60	.62
Asp	31.78	33.55	.70	.15
Cys	3.66	3.59	.11	.72
Glu	38.59	39.91	.82	.35
Gly	16.59	16.82	.39	.72
Pro	6.63 [b]	8.67 [a]	.24	.01
Ser	13.55	13.45	.29	.85
Tyr	20.47 [b]	23.65 [a]	.74	.02
TNEAA[i]	154.68	163.56	3.34	.13
TAAj	280.46	293.40	6.27	.23

[abcd]Means with differant superscripts differ (a, b = P <.05; c, d =P < .10).

[e]Standard error for Alpine goats. SE for Angora goats was 1.35 times that for Alpine goats because the number of animal was less.

[f]Total essential amino acids.

[g]Total Sulfur amino acids (Cys + Met).

[h]Phenolic amino acids (Phe + Tyr).

[i]Total Nonessential amino acids.

[j]Total amino acids.

Table 5. Amino acid composition of ruminal bacteria from Angora and Alpine kids (g/100 g of amino acid).

Item	Alpine	Angora	SE[a]	Probability
Essential AA				
Arg	4.75	4.72	.06	.74
His	1.83	1.82	.04	.96
Ile	4.91	4.75	.07	.21
Leu	7.79 [c]	7.63 [d]	.05	.08
Lys	6.66 [b]	6.98 [a]	.09	.04
Met	3.52 [a]	3.29 [b]	.07	.05
Phe	4.86	4.76	.04	.12
Thr	5.06	4.94	.05	.19
Val	5.46	5.31	.05	.11
TEAA[b]	44.84 [a]	44.20 [b]	.17	.01
TSAA[c]	4.83 [a]	4.52 [b]	.07	.02
PhAA[d]	12.15 [b]	12.80 [a]	.17	.04
Nonessential AA				
Ala	8.34	8.14	.07	.12
Asp	11.33	11.45	.05	.21
Cys	1.30 [a]	1.22 [d]	.02	.07
Glu	13.77	13.62	.13	.49
Gly	5.92 [a]	5.73 [b]	.04	.01
Pro	2.36 [b]	2.96 [a]	.05	.01
Ser	4.85	4.59	.10	.16
Tyr	7.29 [b]	8.05 [a]	.20	.03
TNEAA[e]	55.16 [b]	55.76 [a]	.18	.01

[abcd]Means with different superscripts differ (a, b = P<.05; c, d = P,.10).

[a]Standard error for Alpine goats. SE for Angora goats was 1.35 times that for Alpine goats because number of animal was less.

[b]Total sulfur amino acids (Cys + Met).

[c]Total of essential amino acids.

[d]Phenolic of amino acids (Phe + Tyr).

[e]Total of nonessential amino acids.

Table 6. Amino acid composition (% of total essentials) of ruminal bacteria from Angora and Alpine kids.

Item	Alpine	Angora	SE[e]	Probability
Essential AA				
Arg	10.62	10.66	.23	.92
His	4.08	4.10	.18	.18
Ile	10.92	10.76	.26	.77
Leu	17.35	17.24	.30	.92
Lys	14.86 [b]	15.80 [a]	.32	.02
Met	7.89	7.48	.28	.16
Phe	10.82	10.74	.25	.11
Thr	11.28	11.17	.26	.37
Val	12.18	12.05	.29	.49

[e]Standard error for Alpine goats. SE for Angora goats was 1.35 times that for Alpine goats because number of animals was less.

[abcd]Means with different superscripts differ (a,b = P<.05; c, d = P<.10)

Table 7. Amino acid composition of ruminal bacteria from goats fed differents levels of sulfur (mg /g of bacterial DM).

Item	.11	.20	.28	.38	SE[a]	Linear	Quadratic	Cubic
Essential AA								
Arg	13.28	13.91	13.73	13.57	.63	.79	.53	.77
His	5.16	5.33	5.37	5.05	.23	.75	.30	.89
Ile	13.79	13.71	14.06	13.81	.54	.87	.87	.68
Leu	21.63	22.16	22.39	22.24	.76	.54	.64	.98
Lys	19.36	19.71	20.46	18.97	.94	.88	.33	.56
Met	9.84	9.84	10.02	9.56	.56	.77	.67	.77
Phe	13.67	14.06	13.74	13.61	.46	.80	.61	.65
Thr	14.28	14.68	14.29	14.11	.52	.67	.59	.66
Val	15.39	15.62	15.59	15.30	.67	.91	.71	.99
TEAA[d]	126.40	129.02	129.65	126.22	4.82	.99	.54	.94
TSAA[b]	13.12	13.61	13.82	13.21	1.42	.83	.42	.62
PhAA[c]	34.67	35.06	37.71	35.89	1.50	.36	.43	.33
Nonessential AA								
Ala	23.83	23.94	23.51	23.41	.98	.68	.92	.84
Asp	32.09	33.30	32.94	32.34	1.14	.94	.43	.78
Cys	3.28	3.77	3.80	3.65	.18	.15	.08	.74
Glu	38.16	40.59	39.55	38.72	1.35	.92	.24	.53
Gly	16.79	16.62	16.91	16.53	.64	.83	.87	.71
Pro	7.34	7.75	7.80	7.72	.39	.49	.52	.91
Ser	12.81	13.14	14.35	13.71	.46	.07	.27	.20
Tyr	21.01	20.99	23.97	22.27	1.21	.22	.44	.18
TNEAA[e]	155.31	160.10	162.83	158.35	5.40	.63	.39	.85
TAA[f]	281.71	289.12	292.78	284.57	10.27	.80	.45	.89

[a]Standard error; [b]Total Sulfur amino acids (Cys + Met); [c]Phenolic amino acids (Phe + Tyr); [d]Total essential amino acids; [e]Total nonessential amino acids; [f]Total amino acid.

Table 8. Amino acid in ruminal bacteria from goats fed differents levels of sulfur (g/100 g of amino acid).

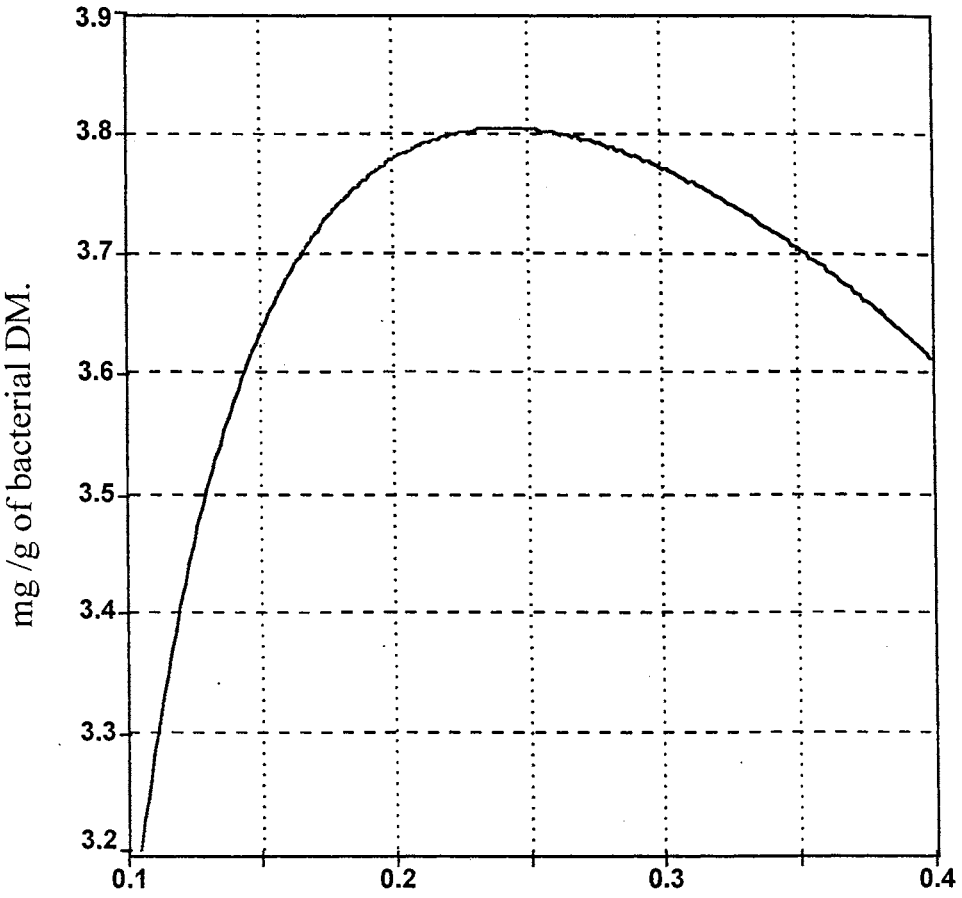
Item	.11	.20	.28	.38	SE[a]	Linear	Quadratic	Cubic
Essential AA								
Arg	4.71	4.81	4.69	4.75	.10	.85	.74	.50
His	1.83	1.85	1.84	1.78	.06	.68	.51	.70
Ile	4.90	4.75	4.80	4.87	.11	.96	.51	.43
Leu	7.69	7.67	7.67	7.81	.08	.33	.35	.81
Lys	6.84	6.80	6.98	6.66	.15	.46	.34	.25
Met	3.49	3.39	3.41	3.35	.10	.47	.81	.71
Phe	4.87	4.87	4.70	4.78	.06	.11	.41	.12
Thr	5.08	5.08	4.88	4.97	.08	.16	.59	.21
Val	5.46	5.40	5.31	5.38	.08	.39	.59	.58
TEAA[d]	44.87	44.60	44.29	44.34	.14	.07	.29	.89
TSAA[b]	4.65	4.69	4.71	4.63	.12	.93	.62	.89
PhAA[c]	12.26	12.14	12.92	12.60	.29	.17	.67	.13
Nonessential AA								
Ala	8.47	8.27	8.01	8.22	.11	.06	.07	.33
Asp	11.42	11.52	11.26	11.38	.09	.41	.97	.06
Cys	1.16	1.30	1.30	1.28	.04	.04	.06	.49
Glu	13.56	14.06	13.53	13.61	.20	.66	.38	.09
Gly	5.97	5.74	5.77	5.81	.55	.09	.04	.37
Pro	2.59	2.68	2.66	2.70	.09	.44	.74	.70
Ser	4.56	4.55	4.96	4.84	.16	.10	.68	.20
Tyr	7.39	7.27	8.22	7.82	.19	.13	.61	.11
TNEAA[e]	55.13	55.40	55.71	55.66	.29	.15	.48	.72

[a]Standard error; [b]Total Sulfur amino acids (Cys+ Met); [c]Phenolic amino acids (Phe + Tyr); [d]Total essential amino acids; [e]Total nonessential amino acid.

Table 9. Amino acid composition (% of total essential AA) of ruminal bacteria from goats with differents levels of sulfur.

Item	.11	.20	.28	.38	SE[a]	Linear	Quadratic	Cubic
Essential AA								
Arg	10.50	10.77	10.58	10.74	.21	.31	.56	.92
His	4.08	4.13	4.13	4.00	.10	.31	.66	.89
Ile	10.92	10.66	10.88	10.97	.24	.23	.46	.93
Leu	17.12	17.20	17.29	17.63	.27	.37	.40	.67
Lys	15.26	15.06	15.58	14.91	.36	.42	.38	.80
Met	7.81	7.72	7.82	7.62	.28	.28	.43	.74
Phe	10.82	10.92	10.61	10.80	.20	.27	.52	.68
Thr	11.31	11.41	11.05	11.19	.26	.28	.39	.88
Val	12.18	12.13	12.06	12.14	.37	.77	.67	.47

[a]Standard error.



$y = a + bx^2 + c / x^2$

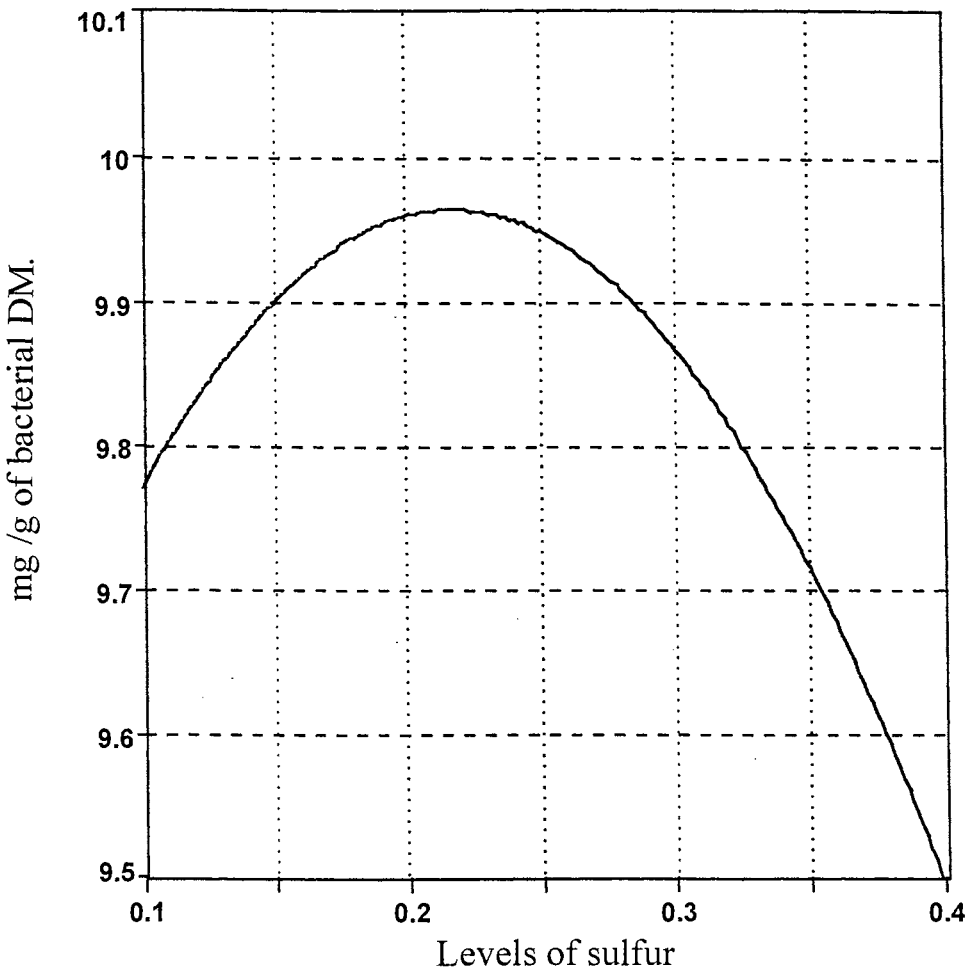
$r^2 = 0.99$

a = 4.1483133

b = - 2.9412111

c = - 0.010090043

Figure 2. Cystine best fit curve for the different levels of sulfur (mg /g of bacterial DM)



$y = a + bx^2 + cx$

$r^2 = 0.73$

a = 9.3055296

b = 6.0559865

c = -13.955713

Figure 3. Methionine best fit curve for the different levels of sulfur (mg /g of bacterial DM)

CHAPTER VI

SUMMARY

Scope of These Studies

A total of 87 goats were used in the studies included in this dissertation. Responses of goats to supplemental dietary sulfur as DL-methionine (met) or sodium sulfate placed in the drinking water or on top of the fed were analyzed. Sulfur adequacy was assessed in terms of mohair production, feed intake, plasma amino acids, bacterial sulfur amino acids, and ruminal parameters.

The effects of dietary sulfur were evaluated at two stages of growth (growing or adult Angora goats) and, with ruminal bacteria, for two goat breeds (Angora and Alpine). The methods used across all of the experiments were similar. All of the studies focused on bypass amino acid concentrations in the plasma and sulfur responses in terms of amino acid levels, mohair production and ruminal parameters.

When dietary sulfur concentrations were low, supplementation of sulfur in either the inorganic (sulfate) or the organic (met) form resulted in large increases in mohair production. Presumably, this reflects an inadequate supply of sulfur AA in protein of ruminal microbes relative to the amount needed for synthesis of mohair protein. When sulfur is deficient, in addition to reducing the total quantity of total bacterial protein supplied, the amino acid composition of ruminal bacteria also may be depressed. The relative importance of enhancing protein quality versus quality thereby remains uncertain. Our results suggest that microbial

protein levels increased when DL-methionine was supplemented. In addition, methionine in the diet partly escaped ruminal destruction even when this amino acid was not in some protected form. Although many researchers have enhanced performance by supplementing diets with rumen escape amino acids or protein, chemically or physically altering amino acids or protein to enhance bypass usually is costly and incompletely effective. Nutritionists must consider the cost of the diet and the supplement, not merely the enhanced production. For production of both wool and mohair, the metabolic requirement must focus on limiting amino acids whereas in the diet, sulfur adequacy is of primary interest. Only when sulfur supply is adequate should supplemental amino acids be considered . Supplementing amino acids in drinking water is a simple, cheap, reliable, and adaptable method to increase postruminal methionine supply which in turn may increase mohair production. Economic implications are the next topic that need attention.

Findings

In trial 1, 14 three-year old wethers weighing 33 kg were given ad libitum access to a 40% roughage diet (13.8% protein; .15% S) for 60 days. Three or four adult goats each received either 0 (basal), 2.5, 5.0, or 7.5 g DL-met daily in their drinking water. Feed intake, shorn body weight gain and greasy fleece weight tended to increase when met was included in the water. Providing 2.5 g met daily increased clean mohair production by 37.5%. Mohair diameter and length tended to be lowest for goats receiving 5 g met daily. The concentration of met in plasma exhibited a quadratic increase while ser, gly, thr, ala, val, phe, ile and leu exhibited linear decreases when met was added to drinking water; his decreased quadratically. Lys responded cubically ($P < .01$) to added DL-met in the drinking water. Total, essential, and nonessential amino acid concentrations

in plasma were higher with .15% of sulfur in the diet and decreased with met supplementation. Methionine as a fraction of total essential amino acids and of total amino acids were highest with .21% of dietary sulfur. Most of amino acids in the plasma were lower numerically at 4 hr than at 1 hr post-feeding. Maximum mohair production by these adult wethers was with .18% dietary sulfur (17 g/d) when the nitrogen: sulfur ratio was 12.3.

In the second trial, 14 one-year old wethers weighing about 29 kg were given ad libitum access to the same diet for 60 days as experiment one. Three or four kids received either treatment 1) the basal diet only, 2) the basal diet plus 2.5g/d of DL-met in their drinking water, 3) the same basal diet supplemented with sodium sulfate to equal .20% sulfur or 4) treatment 3 plus 2.5 g of met daily added to the feed. Sulfur intakes provided the dietary equivalents of .15, .18, .20, and .23% S. Feed intake tended to increase when met was provided in drinking water (1077 vs 1020) or on the feed (1066 vs 1012). Top-dressing the .20% S diet with DL-met increased clean mohair production by 35% (.74, .76, .87, and 1.00 kg per animal per treatment). Mohair diameter, length,and percentages of undesirable (med and kemp) fibers were not altered by met supplementation. L-lactate in the plasma was quadratically increased ($P < .09$) by sulfur supplementation, but D and L-lactate concentrations in ruminal fluid were not altered by sulfur level. The concentrations of met, thr, ala, arg, tyr, cys, ile, leu, lys in the plasma were increased by higher S levels; ser was lowest with .18% sulfur but increased at higher sulfur levels. Glu, gly, thr, val, ile and lys reached their peak values with .20% sulfur. Almost all amino acids increased linearly with postprandial time. For young wethers, mohair production (g/d) peaked at 17 g/d with .23% dietary sulfur and a nitrogen:sulfur ratio of 9.6.

In the third trial, we used three adult fistulated wethers. These goats received either 1) the basal diet containing .15% of sulfur, 2) the basal diet plus

2.5 g of DL-met daily in their drinking water for a sulfur intake equivalent to .18% dietary sulfur), or 3) the basal diet plus 2.5 g of DL-met daily top-dressed on the feed (.18% dietary sulfur). Methionine supplemented either in water or feed increased plasma concentrations of glu, arg, met, met as a percentage of essential or of total amino acids. Plasma met concentrations were highest when met was added to the feed; val, met, met as a percentage of essential or total amino acids were increased by met supplementation while phe was higher when it was placed in drinking water. Plasma concentrations of ser, thr, ala, tyr, val, leu, total, essential and nonessential amino acids exhibited a quadratic response over time peaking at 1 hr post-feeding. Of the amino acids, only met as a percentage of total amino acids was increased by met supplementation. Ruminal ammonia decreased when DL-met was added to the diet. VFA ratios, protozoal numbers, and pH were not altered by added met.

In the fourth trial, we used two yearling Angora wether goats weighting 29 and 32 kg. Goats were fed the .15% sulfur basal diet for 21 days. One goat received only the control diet while the other received this diet supplemented with DL-met (1.4 g/d) in its drinking water. Blood samples were taken from the jugular vein of both animals before feeding and from the mesenteric vein when the animals were slaughtered 1 hour post-feeding. These goats were allowed only one hour for eating their respective feed. During this time, the control goat (29 kg) consumed 230 g of feed and drank 500 mL of water while the goat receiving met in water (32 kg) ate 410 g of feed and drank 580 mL of water which contained 1.45 g DL-met. For the goat receiving supplemental met, plasma cys was 7% greater 1 h post-feeding than before feeding; in the goat not receiving met, plasma cys was 64% lower 1 h post-feeding. The increase in plasma met at 1 h post-feeding was 39% for the supplemented versus 24% for

unsupplemented animal. Compared with prefeeding concentrations, plasma val decreased and leu increased in both animals. Plasma free lys decreased in the control animal but increased in the supplemented animal over time. Plasma free val concentration decreased in both animals while leu increased in both. Lys decreased in the control animal but increased in the supplemented animal. When compared with plasma data from sheep (Tagari and Bergman, 1977), our goats had higher values for val and leu while their sheep had higher values for phe and lys. However, their diets were very rich in protein (15.6 to 19.8%) compared with ours (13.8%). Responses to met in water need to be tested with goats fed a higher dietary sulfur level to determine if the response can be attributed merely to an increased supply of sulfur.

In the fifth experiment, effects of supplementing sulfur as DL-methionine in drinking water or top-dressing sodium sulfate on feed on mohair production was examined. No added sulfur was compared with supplements of 2.5 g DL-methionine in the drinking water or an equivalent amount of sulfur from sodium sulfate (2.4 g) added to the feed. Each treatment was provided to four 12 mo old wethers goats (37 kg) for 60 days. The basal diet, containing 40% roughage, 13.8% protein and .26% sulfur, was available free choice. Failure of supplemental DL-methionine to increase mohair production suggests that post-ruminal supply of methionine already was adequate to produce the maximum growth rate of mohair.

In the sixth trial, the same 12 animals were used in a crossover design (30 d periods) to contrast supplemental methionine in water against sodium sulfate added to the feed. Rates and efficiencies of gain and mohair production were not altered by either sulfur supplementation or sulfur source. However, plasma

amino acid concentrations indicated that methionine in drinking water increased the post-ruminal methionine supply.

The objective of the seventh trial was to determine whether a dietary deficiency of sulfur altered the amino acid composition of protein of isolated ruminal bacteria. Twelve Angora and twenty Alpine castrated male goats kids were individually fed isonitrogenous and isocaloric diets containing 2.28% N and either .11 (basal), .20, .28 or .38% sulfur for 10 weeks. Added sulfur came from supplemental $CaSO_4$. Three and five goats of each breed, respectively, were blocked by age and assigned to each treatment. Non-attached ruminal bacteria were isolated by differential centrifugation and analyzed for amino acid (AA) composition. Differences between breeds were detected in AA composition of ruminal bacteria (mg/g DM) for lys ($P < .07$) and phenolic AA ($P < .03$). Among the nonessential AA, differences were noted for asp ($P < .15$), pro ($P < .01$) and tyr ($P < .02$). Total nonessential AA also tended to be different ($P < .13$) between breeds. In all cases, values were higher for bacteria from Angora than Alpine goats. When expressed per 100 g of AA, similar effects were detected with lys and the phenolic AA being greater ($P < .04$) for Angoras and, among the nonessential AA, differences being noted for ala ($P < .12$), pro ($P < .01$) and tyr ($P < .03$). In addition; however, Alpine goats had a higher proportion of total AA in the form of essential AA ($P < .01$), total sulfur AA ($P < .02$), more met ($P < .05$) and more cys ($P < .07$). As a percentage of essential AA, the differences were small but values were greater for lys ($P < .02$) and his ($P < .08$) for Angora and higher proportion of met ($P < .16$), and phe ($P < .11$) for Alpine. Sulfur supplementation of the diet produced a quadratic increase in cys concentration when expressed as mg /g bacterial DM ($P < .08$), and a linear increase ($P < .05$) when expressed

as g/100g of total AA of bacteria. Sulfur supplementation also linearly increased ser expressed either mg/g bacterial DM ($P < .07$) or as g/100g of total AA ($P < .10$). Added sulfur tended to linearly decrease concentrations of phe, thr and ala ($P < .11$; $P < .16$; and $P < .07$) when expressed per 100 g of AA. Added sulfur linearly decreased ($P < .07$) total essential AA, but to increase ($P < .15$) total nonessential AA. Adding $CaSO_4$ in the diet of growing kids altered amino acid composition of protein of isolated ruminal bacteria, increasing the proportional concentrations of cys and ser but not met.

Conclusions

- For mohair production, the optimum S was .18 for adults and at or above .26% of diet dry matter for young goats;
- For maximum bacterial cystine, the optimum S% was .26% of diet dry matter;
- No mohair response to methionine was found when the dietary sulfur level was above .26%;
- Based on plasma, methionine supplemented either in the drinking water or in feed partially escaped ruminal fermentation;
- Amino acid composition of bacteira is not constant. A sulfur deficiency reduces the cystine content of crude protein of isolated bacteria;.
- Breeds of goats may differ in composition of isolated bacteria;
- Ruminants can respond to additional sulfur-amino acids (in the drinking water or in feed) when sulfur-amino acids are deficient;
- Dietary amino acids may escape ruminal attack even when the amino acids being fed is not protected.

Limitations and Future Outlook

Due to the time and facility restrictions, several questions remain unanswered. We did not measure either duodenal flow or portal uptake of met

because of our surgical attempts were not successful. Such data could help quantify changes in ruminal escape and absorption amino acids. One also could infuse met or cys labeled with ^{35}S to quantify relative absorption, utilization, and interconversion of these sulfur amino acids as well as mohair synthesis rate.

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VITA

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