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EFFECTS OF AMMONIA, SULFUR, AND ISOACIDS ON IN VITRO RUMINAL FERMENTATION OF TROPICAL FORAGES

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EFFECTS OF AMMONIA, SULFUR AND ISOACIDS ON *IN VITRO* RUMINAL FERMENTATION OF TROPICAL FORAGES

By

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ABSTRACT

EFFECTS OF AMMONIA, SULFUR AND ISOACIDS ON *IN VITRO* RUMINAL FERMENTATION OF TROPICAL FORAGES

By

Sol Anabel Rodríguez-Medina

The effects of two levels of NH_3, sulfur and isoacids on *in vitro* ruminal true digestibility (IVTD) of eight tropical forages was studied using a 2^3 factorial design. Trypticase was added to the final incubation media. This casein hydrolyzate should not have been added because it contributes NH_3, isoacids and sulfur. This raised the levels of NH_3 from the expected 5-10 to 17-26 mg/dl and isoacid levels about 3-fold. Sulfur levels were not increased. The results are discussed in light of trypticase addition. After a 48 h *in vitro* ruminal fermentation, NDF, ADF and lignin were measured to estimate IVTD. The results showed that IVTD increased with high levels of NH_3 and when isoacids were added to treatments low in NH_3. IVTD decreased when both NH_3 and isoacids were high. High levels of sulfur tended to decrease IVTD for all forages, except elephant grass. Addition of isoacids increased total VFA concentration.

I would like to dedicate this work to my beloved daughter, Aniella Sánchez, who will later understand the many sacrifices, patience, understanding, encouragement, endurance and love behind this two long years for both of us. I hope it will serve her as an example to achieve all her future goals in life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

N	Nitrogen
S	Sulfur
I	Isoacids
VFA	Volatile fatty acid
BCVFA	Branched-chain fatty acid
C2	Acetate
C3	Propionate
C4	Butyrate
IC4	Isobutyrate
IC5	Isovalerate
C5	Valerate
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
LIG	Lignin
ADFD	Acid detergent fiber digestibility
CWD	Cell wall digestibility
LIGD	Lignin digestibility
CELD	Cellulose digestibility
HEMD	Hemicellulose digestibility
IVTD	*In vitro* true digestibility

INTRODUCTION

Forages are an important source of feed for ruminants and other animals. In tropical regions, pastures become the main if not the only source of feed available for ruminants, horses and pigs. Tropical grasslands have the capacity to sustain animal production based upon extensive farming systems. Even though tropical forages may yield up to 22,000 $kg \cdot ha^{-1} \cdot yr^{-1}$ of dry matter (DM) (Tinnimit, 1974), animal production is seriously limited by the seasonal nature of pastures. In most tropical countries there are two seasons: wet and dry. During the wet season acceptable quality and quantity of forage is available. However, during the dry season not only the nutritional value decreases but also the amount of pasture to graze is drastically reduced.

Tropical forages do not supply sufficient energy for the production of meat and milk by ruminants. For dairy cows grazing tropical pastures, energy rather than protein was found the first limiting factor for milk production (Delgado and Randel, 1989). Most of the useful energy of tropical forages is obtained from carbohydrates. Their energetic value depends, to a great extent, on the digestibility of the carbohydrate fractions of cellulose and hemicellulose which are digested in the rumen by the action of microorganisms (Van Soest, 1982). For optimal digestion of fiber, rumen microorganisms need ammonia (NH_3), sulfur (S) and isoacids as well as other factors such as vitamins and minerals.

There are several grasses and legumes of major economic importance in tropical zones. A study of factors affecting ruminal fermentation of these forages is needed in order to find ways to enhance their economic value for ruminants.

REVIEW OF LITERATURE

Digestibility of tropical forages

The great potential of the tropics for animal production lies in the enormous yield of biomass that can be produced per unit of land area. However, productivity per animal in the tropics has remained low because pronounced wet seasons are almost always followed by long dry periods. Dry pastures are generally low in both protein and digestible energy (Preston and Leng, 1975). Of forages widely used in tropical regions, signal grass (*Brachiaria decumbens*), buffel grass (*Cenchrus ciliaris*), star grass (*Cynodon dactylon*), pangola grass (*Digitaria decumbens*), guinea grass (*Panicum maximum*), elephant grass (*Pennisetum purpureum*), leadtree (*Leucaena leucocephala*), and shad (*Gliricidia sepium*) are produced in the Dominican Republic.

It is common knowledge that digestibility decreases with increased maturity of forage (Van Soest, 1982). Low digestibility is associated with lower leaf to stem ratios and higher fiber contents (Panditharatne et al, 1987). The apparent DM, CP, NDF, and ADF digestibility of guinea silage fed to wethers was greater for 2 than for 3 weeks of plant growth. Chopping the grass before ensiling increased digestibility compared with unchopped forage.

Dry matter digestibility and intake of tropical grasses are considerably lower than those of their temperate climate counterparts (Minson and Bray, 1980). This is due to

the adverse effect of high temperatures in the tropics (McLeod and Minson, 1970). Furthermore, tropical grasses have a higher cell wall content and lower dry matter digestibility (DMD) than temperate grasses. Wilson and Hattersley (1989), worked with leaves of 12 *Panicum* with C_3 (temperate) and C_4 (tropical) photosynthetic pathways. They reported lower cell wall content (37-49%) and higher DMD (67-74%) for C_3 and higher cell wall content (49-67%) and lower DMD (53-67%) for C_4. Similar results were obtained by Hill et al. (1989) with C_3 and C_4 *Panicum* species.

The occurrence of high photosynthetic capacity in the leaves of C_4 plants confers some advantages which enable them to adapt to certain ecological conditions better than C_3 species (Spedding, 1971).

Fiber represents a significant fraction of the diet of herbivores. Consequently, the animals productivity is limited by their ability to consume and digest the fibrous portion of the diet. Allen and Mertens (1987) developed mathematical models to define the processes of fiber digestion and for evaluating factors affecting digestion of fiber in anaerobic systems. They found that the largest independent constraint on fiber digestion is the fraction of fiber that is indigestible, which represents up to one-third and one-half of the total fiber fraction of grasses and legumes, respectively. Van Soest (1973) stated that the extent to which cell walls are digested depends on the lignin fraction which determines the availability of cellulose and hemicellulose.

Tsai et al. (1967) in their study of the effect of dietary fiber on lactating cows in the tropics, observed an increased heat stress (measured as rectal temperature) as a result of an increase in fiber intake. They concluded that fiber level should be considered in

formulating rations for dairy cows.

McLeod and Smith (1989) studied the effect of fiber level of forages on eating and rumination behavior. It was concluded that when a ruminant is fed diets of high fiber content, voluntary intake is not always reduced because of restrictions in either rumen fill or rumination. Van Soest and Marcus (1964) examined 96 forages and found that there was no significant relationship between cell wall constituents and voluntary intake when forage cell wall content was less than 60% of the dry matter (including most legumes and a few immature grasses). However, when values were above 60% there was a marked decreased in voluntary intake with increasing content of cell walls.

Moir (1974) developed an equation to estimate the metabolizable energy from cell walls and digested cell walls which could be used not only with a wide range of grasses, but also appeared to apply to legumes. Dry matter digestibility (DMD), dry matter intake (DMI) and fiber fractions differed between forage classes and animal species (Reid et al., 1988). Also, C_4 grasses were consumed at levels higher than would be expected from their DMD and fiber concentrations. Ruminants appear to increase neutral detergent fiber intake (NDFI) in response to higher NDF concentrations in the forage. McLeod and Minson (1988) found no difference in breakdown between temperate and tropical forage when using a digestion-detrition simulator for 48 h digestion. The apparatus simulates digestion and detrition (rubbing) in the rumen. They reported that both digestion and detrition reduced forage particle size *in vitro*.

A study of the chemical composition and digestibility of 101 tropical grasses was conducted by Kayongo-Male et al. (1976). Grasses were harvested at 30 days of growth.

Wide ranges in the percentage of NDF (45.7 to 79.2%), ADF (30.9 to 45.3%), and hemicellulose (11.7 to 37.5%) were found. Digestibility estimates obtained by the Tilley Terry method and NDF digestibilities ranged from 42.6 to 66.0% and 22.0 to 62.0%, respectively. ADF seemed more important than lignin in determining digestibility values. It was pointed out that the significance of Lignin/ADF ratio in relation to digestibility estimates was much less for *in vitro* estimates than for estimates calculated from predictive equations. They concluded this indicated that lignification of cellulose had less influence on digestibility of tropical than of temperate forages.

Butterworth (1964) reported that digestible energy of twenty-four forages ranged from 2.39 kcal/g for pangola silage to 3.08 kcal/g for signal silage. In addition, no correlation was demonstrated between the content of crude fiber or crude protein and the digestible energy of the forages.

Ishizaki et al. (1976) found a positive correlation between the *in vivo* (65.4) and *in vitro* (68.2) digestibility of pangola grass by sheep.

Dry matter digestibility was 59.7 and 66.7% for leucaena and buffel, respectively, when lambs were fed a diet containing sisal pulp, sisal bagasse and urea (Yerena et al., 1978). Child et al. (1982) conducted a digestibility study with heifers using the nylon bag technique and found that the mean rumen digestion index (dry matter disappearance using the nylon bag technique) for ground leucaena samples varied from 13.6% for shattered pods to 90.6% for the small developing pods. The index for crude protein varied from 5.2 to 31.7. It was concluded that leucaena can serve as a high quality feed for livestock.

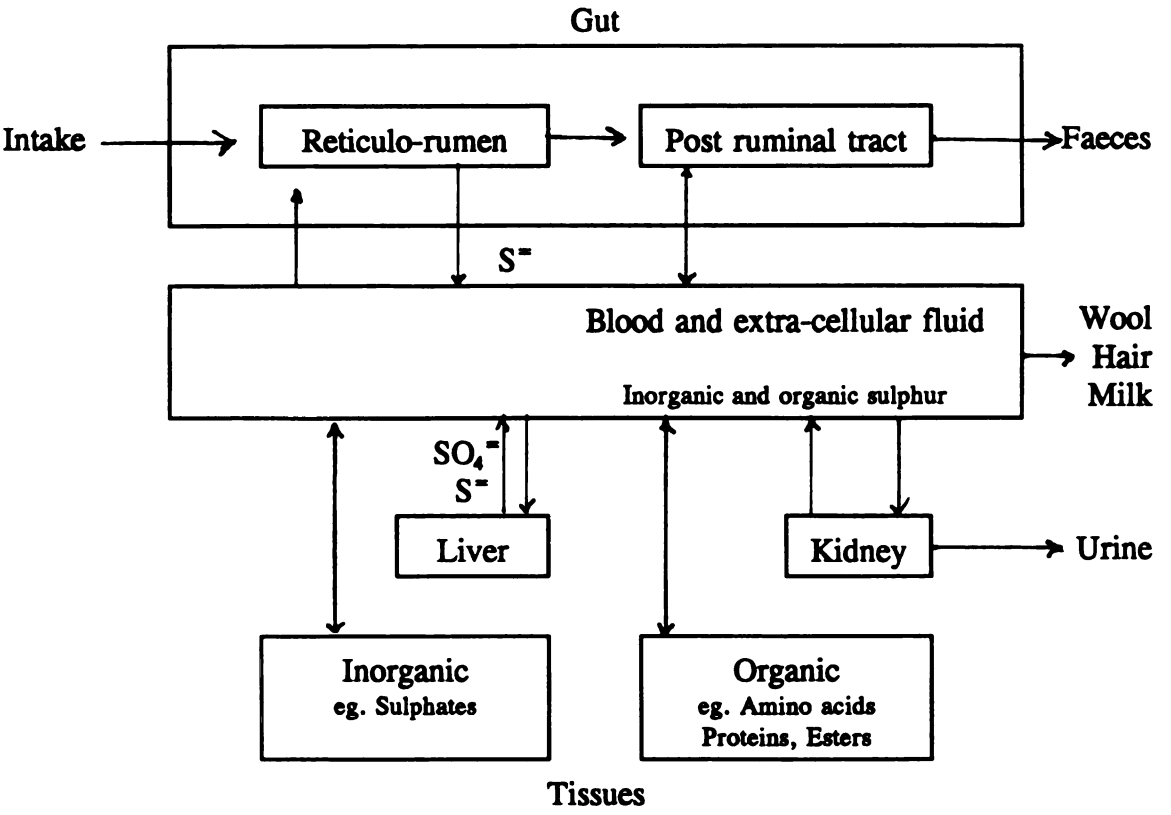

Figure 1. The fate of sulphur in ruminants. Adapted from Bray and Till (1975).

Sulfur nutrition in ruminants

Interest in S metabolism began when du Vigneaud demonstrated, by using radio-labeled cystathionine, that mammals convert methionine to cystine (Garrigus, 1970). The S-containing amino acids are important components of many proteins, enzymes, vitamins and several hormones. Sulfur plays a major role in protein structure (Johnson et al., 1970). It is a component of two essential amino acids, cysteine and methionine. Therefore, S is required by the ruminant to synthesize S-containing amino acids within the rumen. The fate of S in ruminants is illustrated in Figure 1.

The presence of rumen microorganisms permits the ruminant to utilize fibrous material and forage plants as sources of dietary nutrients. The microbes ferment forage to volatile fatty acids, and convert inorganic N and S to microbial protein.

Orskov (1982) found that microbial biomass may contain as much as 8 g of S/kg DM, found mainly in the protein fraction. Sulfur in feed is reduced to sulfide (H_2S) in the rumen and incorporated into microbial protein or absorbed directly as H_2S. Sulfide is the key intermediate between the breakdown of ingested and recycled S and its utilization or loss from the ruminant system (Bray and Till, 1975).

The general pathway of S amino acid biosynthesis from sulfate and other inorganic S compounds is presented in Figure 2 (Roy and Trudinger, 1970).

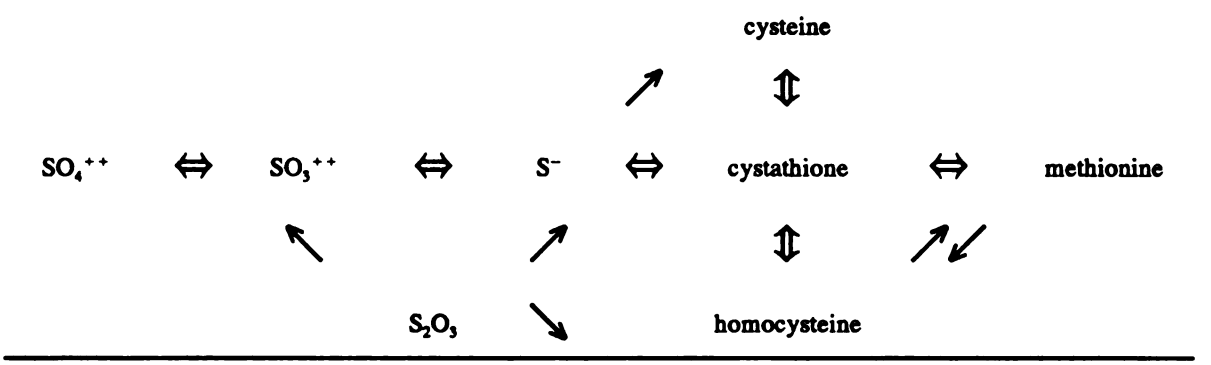

Figure 2. Pathway of sulfur amino acid biosynthesis from sulfate and other inorganic sulfur compounds.

Sulfur metabolism by rumen microorganisms

Rumen bacteria rapidly reduce inorganic S and incorporate it into organic compounds. The optimum pH for the reduction of sulphate is 6.5 (Kandylis, 1984). However, the capacity of the reticulo-rumen system for sulphate reduction is partially

dependent on a period of adaptation to dietary S (Bray and Till, 1975).

The microbes can utilized both inorganic and organic S to synthesize S-containing compounds available for absorption (Kandylis, 1984). If dietary S is inadequate, microbial activity is slowed. Sulfur losses may occur because of the formation of volatile H_2S (Goodrich and Garret, 1986).

Emery et al. (1957a and b) conducted an *in vitro* study with labelled sulfate and substrates representative of concentrate and forage rations. They observed that cysteine formation was twice as rapid as methionine formation and sulfate incorporation into amino acid was more rapid with forage as the substrate.

Pittman and Bryant (1964) observed that some strains of *Bacteroides ruminicola* required cysteine and methionine. Sulfate reduction in the rumen is executed by both assimilatory and dissimilatory microorganisms. Assimilatory reduction involves sulfate reduction to sulfide with accompanying incorporation into cellular materials. Dissimilatory microorganisms reduce sulfate without using it, producing free hydrogen sulfide (Peck, 1970).

Moir (1970) reported that *in vitro* incubations fermenting cellulose with ^{35}S, methionine and cysteine accounted for 28 and 34% of the S-protein produced, while sulfide accounted for 88%. Hume and Bird (1970) also reported accumulation of sulfide in the rumen following sulfate administration.

Sulfide is probably the central metabolite in ruminal S metabolism. Therefore, dietary S utilization by rumen microorganisms depends on the quantity and source of available S, as well as on the loss of sulfide from the rumen (Moir, 1970). The relative

rate of sulfide formation from S compounds is as follows: cysteine > inorganic S > methionine (Bray and Till, 1975). Bray and Hemsley (1969) reported ruminal sulfide levels of 0, 2, and 6 μg sulfide/ml of rumen fluid in sheep fed diets containing .06, .14, and .32% S, respectively. However, Hume and Bird (1970) found values for rumen sulfide of .5, 1.9, 4.3, and 3.5 μg sulfide/ml when consuming a diet containing either 0.08, 0.2% sulfate S or 0.2% cysteine S and 0.4% sulfate+cysteine S, respectively.

Sulfur requirements

To obtain a well functioning rumen, rumen microorganisms need to be supplied with adequate amounts of S. Different species may have different requirements depending on a variety of factors such as age and condition of the animals, natural diet vs. purified diets, source of S and dietary N, or *in vitro* vs. *in vivo* experiments.

Bird (1972a) showed that less dietary S is required by cattle than sheep, and cattle may tolerate a wider N:S ratio in the feed than sheep. Apparently, this is because S is recycled more effectively in cattle. In another study, Bird (1972b) found that for sheep a small increase in S intake (0.36 g/d) improved the nutritive value of a S deficient, low protein roughage (oat hull) diet in which urea was supplied. Earlier work performed by Thomas et al. (1951) showed that growing lambs fed semi-purified rations with less than 0.1% S and supplemented with urea had improved rates of gain and N retention after Na_2SO_4 was added to the diet.

Purified diets with and without S and containing urea as the source of N fed to

sheep resulted in decreased intake and weight loss in the S deficient animal (Whanger and Matrone, 1970). Moreover, it was found that gram positive organisms were predominant in the rumen of sheep fed S adequate diets while gram negative bacteria predominated in the rumen of S deficient sheep (Whanger and Matrone, 1970). Hence, there was an accumulation of lactate in the rumen of sheep fed the low S diet, and just traces in the rumen of the S fed sheep. In addition, there was more butyric and higher fatty acids when S was adequate while more acetate and propionate was found when S was deficient.

Several studies were conducted by Bouchard and Conrad (1973a, b and c) concerning the requirements of S by lactating dairy cows. They found that dietary S of .12% and .18% produced a zero and a positive S balance, respectively. Therefore, they concluded that those levels should approximate the limits of S requirements in lactating dairy cows. They also compared the availability of different sources of dietary S. Sodium and calcium sulfate provided an availability of S of about 77 to 87%. However, S from lignin sulfonate was poorly digested (42 to 53%). Dietary S requirements and variation within species and physiological stage of the animal are presented in Table 1.

Table 1. Sulfur requirements of ruminants.

Ruminant	Sulfur, % DM	Reference
Growing-finishing cattle	0.10	Goodrich & Garret, 1986
Growing-finishing sheep	0.14-0.26	Goodrich & Garret, 1986
Calf starter concentrate	0.20	NRC, 1989
Growing heifers	0.16	NRC, 1989
Dry pregnant cow	0.16	NRC, 1989
Lactating cow	0.20	NRC, 1989
Mature bulls	0.16	NRC, 1989

Generally, rations composed of natural plant and animal components usually contain adequate S to meet requirements of ruminants. However, some grasses are low in available S (Johnson et al., 1970). Animals fed low quality forages may respond to S supplementation (Goodrich and Garret, 1986). Rees et al. (1974), reported an increased digestibility in sheep fed pangola grass supplemented with S. However, an increased voluntary intake of 44% was found when the same grass was fertilized with S. Bray and Hemsley (1969) found that sulphate supplementation increased crude fiber digestion and N and S retention when added to a poor quality forage diet fed to sheep. In another study, Guardiola et al. (1983) observed increased total fiber digestibility in lambs fed low or high quality forages supplemented with sulfate or methionine. Kennedy and Siebert (1972) and Kennedy (1974) studied the effect of sulfate additions to rations composed of tropical spear grass and urea in sheep. Sulfur supplementation resulted in improved dry matter digestibility, N retention and feed intake. Inorganic S added to the

rumen can be absorbed directly into the blood (Kennedy and Milligan, 1978). Serum inorganic sulfate-S were increased to a maximum of 35-46 mg/L by infusion of sulfate into the rumen or abomasum of sheep given bromegrass.

Nitrogen to Sulfur ratio

There is a close association between S and N in both plant and animal cells. Most diets that contain required levels of protein will also provide adequate levels of S (National Research Council, 1989). Poor quality diets supplemented with Non-Protein Nitrogen (NPN) will require additional S supplementation (Goodrich and Garret, 1986). The relative proportion of S to N in these diets is important.

The N:S ratio of body tissue is about 15:1 (Garrigus, 1970). According to Bray and Till (1975), it could be argued that the ratio of N to S retention should be of the same order as the ratio in body tissue. This is true for cattle. However, sheep have a larger S requirement for wool production, requiring an overall N:S ratio of 13.5:1 (Bray and Till, 1975). Hume and Bird (1970) found a microbial protein N:S ratio of 18:1. For ruminal bacteria, the ratio of total N:S has been reported to range from 11:1 (Moir, 1970) to 22:1 (Bird, 1972a). Nitrogen to S ratios of feedstuffs range from 10:1 for most cereal grains, 18.2:1 for legumes (e.g. peanut meal) to 26.9:1 for zein (Moir, 1970).

A close relationship has been observed by several investigators between dietary N and S content in ruminant diets. The ratio of the two elements is used as a guide to recommend proper levels of supplemental S. Bray and Hemsley (1969), using a simple oat hull, urea, and mineral diet with sheep, found dry matter digestion increased from

46.6 to 51.9% when sulfate-S intake was increased to narrow the N:S ratio from 24 to 9.7:1. Moir (1970) described the relationship between N and S intake and N balance. They found that the maximum N balance was achieved with a dietary N:S ratio of 10:1.

The National Research Council (1989) estimated the S requirement for lactating cows at 0.20% of the total diet. This implies a N:S ratio of 12:1. Slyter and Weaber (1971) reported that calves fed adequate S were efficient in retaining N. Saliva appears to be the major source of recycled S (Kandylis, 1983). The N:S ratio in the residual-S (protein) fraction of the saliva remains relatively constant at 11-12:1.

Adequate S supplementation can be achieved with methionine, elemental S or sodium sulfate (Johnson et al., 1970). However, supplemental S may be less available than the S source found in the natural diet (National Academy of Sciences, 1976). Organic sources are preferred such as D-L-methionine. Inorganic sources like sodium sulfate and elemental S are the least available (Goodrich and Garret, 1986). However, Onwuca and Akinsoyinu (1989), reported that elemental S supplementation to small ruminants improved dry matter intake, live weight gain and N utilization.

The S for the microbes is generally derived from degradation of dietary protein, and therefore a deficiency in S is likely to occur only if there is a deficiency of N. Such a deficiency will lower the number of lactic acid fermenting microorganisms in the rumen (Johnson et al., 1970). Sulfur deficiency may result in reduced milk production and weight gain, anorexia, low dry matter digestibility, profuse tearing and salivation, dullness, emaciation and in extreme cases, death (Goodrich and Garret, 1986). Kandylis (1984), reported that S toxicity may occur if dietary S exceeds .3 to .4% of the D.M.

Ammonia requirements

Despite the amino acid needs of the ruminant animal, there are N requirements for the rumen microbiota especially when the animal is expected to use forage and other cellulose containing energy sources (National Academy of Sciences, 1976). Because of the limitations in quantity of protein synthesized by the rumen microbiota, it becomes necessary to provide adequate dietary protein or provide all other intermediates essential for microbial protein synthesis (Chalupa, 1973).

Studies on the nutrition of ruminal cellulolytic bacteria emphasize the importance of their interaction with other microbial species to synthesize chemicals such as B-vitamins, ammonia and certain VFAs often essential for bacterial growth. The major microbial nutrients include minerals, S, and N, which leads to ammonia formation (Bryant, 1973).

Ammonia is a vital ingredient in microbial synthesis (National Academy of Sciences,1976) and is produced by ruminal microbes from both protein and NPN substances (Allison, 1969). It is not the only nitrogenous nutrient required for ruminal microbial growth (McDonald, 1948), but is the main one (Allison, 1969; Hungate, 1966). Bryant and Robinson (1961) studied the N requirements of some cellulolytic bacteria and found that ammonia was utilized as the sole source of N by different strains of *Ruminococci sp.*

Other sources of ammonia in the rumen include urea from blood, salivary proteins, epithelial cells sloughed from the mouth, esophagus and ruminal epithelium (Nolan et al., 1973). Supplementation of ammonia to meet optimal ammonia

concentrations in the rumen depends on the amount of ammonia which can be degraded from dietary components, the amount of recycled endogenous urea and levels of other components like energy and minerals. The availability of energy from different sources is a key factor in evaluating effects of supplemental ammonia in the ruminant. Pidgen (1971) as cited by National Academy of Sciences (1976) report that the lignocellulose complex accounts for most of the energy in mature forages. Nitrogen composition of roughages will affect their individual rate of digestion. Of the N in fresh forage 70-90% is in protein (Waldo, 1968) in the sense that it can be made insoluble by denaturation. The 10-30% of the N that is soluble is often considered NPN and contains nucleic acids, peptides, amino acids, amines and purines and occasionally nitrate (Spedding and Diekmahns, 1972 as cited by Tamminga, 1986). In fresh grass, total N content is usually high and a large proportion of it is rapidly degraded in the rumen (Tamminga, 1986). Diets based on fresh grass contain low amounts of energy and a surplus of rumen degradable protein. In order to utilize NPN efficiently with such a diet, supplementation with an adequate energy source to favor microbial growth is required (Tamminga, 1986). Russel and Hespell (1981) indicated that insufficiency of peptides, amino acids, and branched chain fatty acids at certain times after feeding may be a major factor causing energetic uncoupling, resulting in continued production of fermentation products without concomitant bacterial growth in the rumen. A scheme for ruminal degradation of proteins is presented in Figure 3.

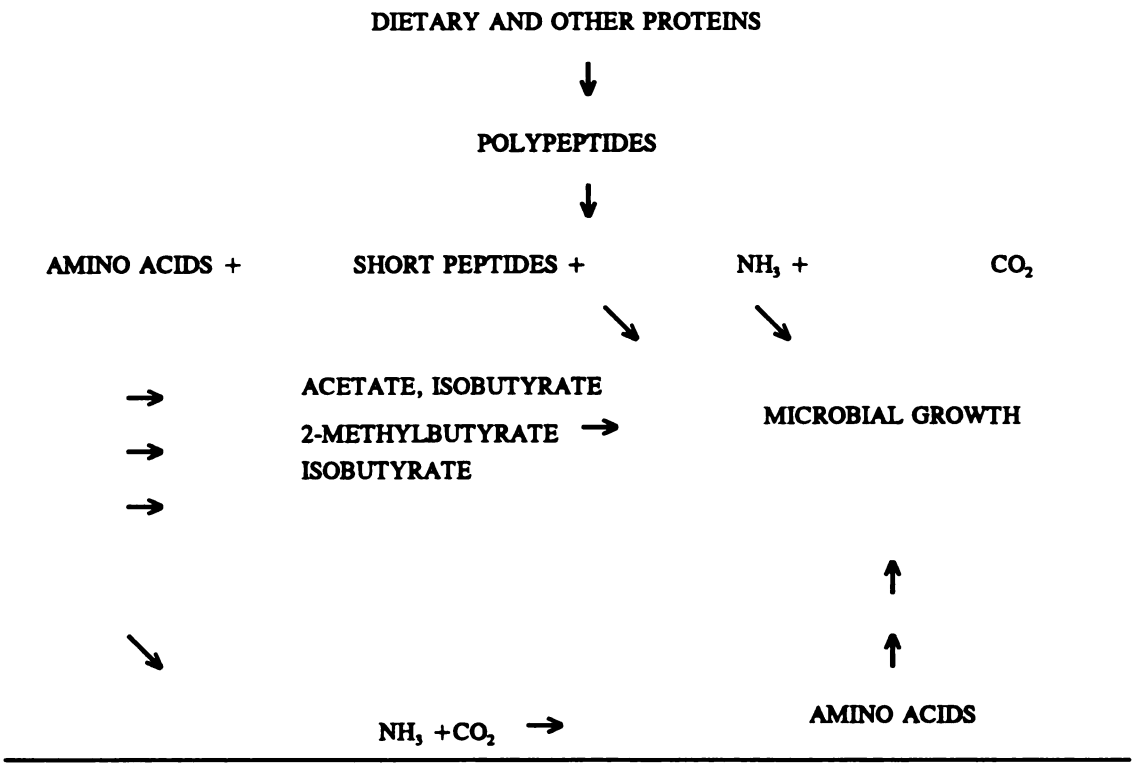

Figure 3. Ruminal degradation of proteins. Adapted from Russel and Hespell (1981).

Different sources and levels of ammonia precursors need to be considered. Urea is the main NPN source of N used for microbial protein synthesis. Other NPN products such as urea-carbohydrate, ammonium salts, ammoniated molasses, biuret etc. have been used as N sources for ruminants.

According to the National Research Council (1989), N is involved in the rumen in two ways. There is an efflux of N from the rumen which occurs through absorption and passage of ammonia, and an influx of N to the rumen through the diffusion of blood urea (Houpt and Houpt, 1968) and the secretion of salivary urea (National Research Council, 1989). Dietary NPN must be first transformed to ammonia in order to be

utilized for microbial growth. NPN is used most efficiently for rumen protein production when it produces an ammonia concentration that is optimal for bacterial protein synthesis (Chalupa, 1973). Some species of ruminal bacteria use exogenous amino acids (Allison, 1969). However, amino acids in peptides are more efficiently utilized than are free amino acids by other species (Pittman and Bryant, 1964). More recently, Cotta and Russel (1982) observed that high concentrations of peptides and amino acids resulted in high yields of bacterial protein but conversion of free amino acids to microbial cell protein was poor.

Hume et al. (1970) reported that the addition of NPN supplements to ruminant rations increased microbial protein synthesis while ammonia concentration remained low and constant up to a dietary N intake of 9 g/d. However, diets with a higher N intake (16 g/d) produced no further increase in rumen protein output and ammonia concentration increased. Satter and Slyter (1974) showed that ammonia in excess of 5 mg NH_3-N/dl of ruminal ingesta had no effect on fermentation rate. Rumen bacteria can scavenge ammonia from low concentration environments, but when ammonia starts to accumulate bacterial growth is not enhanced by providing additional NPN (Chalupa, 1973). Therefore, once ammonia concentration reaches 2 to 3 mg NH_3-N/dl, microbial needs are satisfied (Roffler and Satter, 1975a). However, because of its variation in the rumen, maintenance of an average concentration of 5 mg NH_3-N/dl is recommended (Roffler and Satter, 1975b). The same authors in a lactation study, observed that NPN supplementation did not improve milk production if the ration contained more than 12.5% CP or more than 4 mg NH_3-N/dl rumen fluid.

Huber and Kung (1981), in a review of the protein and NPN utilization in dairy cattle, explained that with increasing dietary N, rumen ammonia increases more rapidly with NPN than natural protein. Schaefer et al. (1980) determined that ammonia saturation constants for the predominant species of ruminal bacteria were less than 50 μM and that organisms growing in a medium of 1 mM (1.7 mg/dl) ammonia should achieve 95% of their maximum specific growth rate, but would not necessarily provide for maximum yields of bacterial cells. However, Bull et al. (1975) as cited by Huber and Kung (1981) showed an increased in microbial protein production until rumen ammonia reached about 20 mg/dl. In vivo studies suggested that synthesis of microbial protein is not maximized until rumen ammonia reached 10 (Hume et al., 1970) to 29 (Miller, 1973) mg/dl.

Mehrez and Orskov (1977) showed that NH_3 concentrations in the rumen needed to be much higher from what had been reported. At 95, 85, and 75% of maximal rate of substrate disappearance, NH_3 in the rumen digesta was 24, 19, and 15 mg/dl. When ruminal NH_3 was 7 mg/dl, only 50% of the maximal rate of breakdown was achieved. Ruminal NH_3 concentrations rise after consumption of a meal and net absorption is positively correlated with ruminal concentrations in goats, sheep, and cattle. Because of gut fermentation, a substantial portion (16-80%) of N is absorbed as ammonia N (NH_3-N). Net uptake of NH_3 is higher with forage diets than with high energy diets (Huntington, 1986).

Ammonia toxicity could be produced when urea is consumed in large quantities in a short time. Signs of toxicity include uneasiness, dullness, muscle and skin tremors,

excessive salivation, frequent urination and defecation, rapid respiration, incoordination, tetany and death (Bartley et al., 1976). Apparently, high rumen ammonia concentrations may exist without producing toxicity if the ration is readily fermentable and rumen pH is below 7.4 (Bartley et al., 1976).

Isoacids for microbial protein synthesis

It is well known that the microbial biomass consists of a multitude of microbes of different species (Hungate, 1966). Ruminal bacteria are by far the most frequently occurring group of organisms, although they do not always constitute the greatest biomass of the rumen microorganisms (Harrison and McAllan, 1980). Among them, the cellulolytic bacteria give the ruminant the ability to survive on poor quality fibrous forages (Orskov, 1982). Cellulolytic bacteria are strictly anaerobic and besides requiring ammonia and S, their growth rate is dependent on the presence of branched chain volatile fatty acids (Dehority et al., 1967; Bryant and Doestch, 1955; Allison, 1969) such as isobutyrate and isovalerate (Allison and Bryant, 1958). These branched chain acids appear to be formed mainly by degradation and deamination of branched chain amino acids from dietary protein by some of the non cellulolytic bacteria and ciliate protozoa (Slyter and Weaver, 1971). The branched chain volatile fatty acids cannot be synthesized by most rumen cellulolytic bacteria (Allison et al., 1974). Therefore, protein synthesis may be limited by the supply of these nutrients in diets containing low dietary protein (Hume et al., 1970). Slyter and Weaver (1971) in an *in vitro* study with several strains of cellulolytic bacteria, found branched chain fatty acids were formed by a mixed rumen

population fed a diet with no amino acids and rapidly fermented carbohydrates. However, branched chain fatty acids have been reported to increase total microbial synthesis (Hume, 1970a and b) and N retention in ruminants (Oltjen et al., 1971), although a beneficial effect has not always been attained (Cline et al., 1966).

Naga and Harmeyer (1975) studied the relationship between the production of VFA and synthesis of microbial protein *in vitro*. They observed a negative correlation between microbial growth and end products formed.

Some aspects of VFA production in the rumen

Large quantities of volatile fatty acids, particularly acetic, propionic and butyric acids, are produced in the rumen by microbial fermentation of dietary carbohydrates and protein and are absorbed into the bloodstream mainly through the rumen wall (Barcroft et al., 1944), constituting the major portion of absorbed energy (Bush et al., 1979). Early research (Masson and Phillipson, 1951; Kiddle et al., 1951) was conducted to study the concentration of these acids in blood leaving the rumen compare to its concentration in the rumen itself. Production of VFA is affected by the type and amount of plant material as well as by pH in the rumen (Van Soest, 1982).

Weller et al. (1967) measured by continuous infusion of ^{14}C labelled VFA, the total and individual VFA production in sheep fed lucerne hay. This study showed that the composition of the acids initially found in the rumen were 77-83% acetic , 15-18% propionic, and 1-7% butyric . Similar results were obtained with sheep fed different diets (Leng and Brett, 1966) and with grazing sheep (Leng et al., 1968). This last study

demonstrated a method to make comparisons between pastures on the basis of their potential yield of energy for sheep, by determining VFA production rates in grazing animals. They developed an equation to predict the amount of energy supplied by the acids.

In their studies of VFA metabolism in sheep, Krishna and Ekern (1974a and b) reported that the total amount of VFA produced when timothy hay was fed ranged from 3.80 to 3.93 mol/day or about 40-41% of the total metabolizable energy consumed. This agreed with previous findings reported by Marston (1948) as cited by Knox et al. (1967) that volatile short-chain organic acids may provide ruminants with 40-70% of their energy needs. Results of a short term study involving VFA intraruminal infusions indicated a higher efficiency of utilization for propionic acid than for acetic (Armstrong et al., 1958). Weller et al. (1969) measured the concentration of VFA by isotope dilution when sheep were grazing pastures. The VFA production in the rumen was found to increase during the period of growth of the pasture and to decline when it dried off. Gray et al. (1965, 1967), also conducted a series of experiments to study the production of VFA in the rumen of sheep. They observed the same rate of VFA production with different DMI of the same fodder ration. They found that the energy of the VFA produced in the rumen was equivalent to about 54% of the digestible energy of the diet.

The interconversion of acetic acid into butyric acid by sheep was reported to be between 50-80% when lucerne hay was fed (Weller et al., 1967) and from 51-66% if fed dried grass cubes (Bergman et al., 1965).

Isoacid requirements

Rumen bacteria are unique in their ability to synthesize amino acids by first carboxylating short chain acids to form alpha-keto acid analogues of amino acids and then, to utilize ammonia to form the complete corresponding amino acid. Isovalerate, isobutyrate and 2-methylbutyric acid are used for the biosynthesis of leucine, isoleucine and valine, respectively, as well as for synthesis of higher branched-chain fatty acids and aldehydes (Allison et al., 1962; Allison and Peel, 1971; Allison et al, 1974; Robinson and Allison, 1969). These branched-chain fatty acids and the straight-chain valeric acid stimulate growth and activity of cellulolytic and some noncellulolytic bacteria (Allison et al., 1962; Bryant, 1973; Dehority et al., 1967).

The addition of isoacids to ruminant rations have shown a positive effect on performance (Cline et al., 1966; Felix et al., 1980; Hemsley and Moir, 1963; Papas et al., 1984), dry matter digestibility (Soofi et al., 1982), microbial growth (Cline et al., 1958; Gorosito et al., 1985), insulin production (Horino et al., 1968), microbial protein synthesis (Hume, 1970a and b) and N retention (Umunna et al., 1975; Felix et al., 1976).

Low availability of isoacids limits ruminal fermentation, especially with high roughage diets, or high feed intake and high energy demand which is the case in lactation (Cook and Towns, 1987). Gorosito et al. (1985), observed an increase in cell wall digestion by ruminal bacteria supplemented with isoacids. Quispe (1982), conducted experiments where rumen acetate production was measured to determine the rate of fermentation. Her work showed that isoacids increased acetate production in sheep fed

pineapple tops. Later, Kone (1987) investigated the interaction of isoacids and Monensin on ruminal fermentation, observing that isoacids at 15 mg/dl increased acetate and VFA production while Monensin reduced acetate and VFA, but increased propionate production. The combination of both, increased acetate but did not eliminate the effect of the ionophore on propionate.

Cline et al. (1958), attempted to determine the nutrients needed for rumen microbial growth *in vitro*. By changing the carbohydrate source (starch, glucose and cellulose) and levels of urea, they found that as urea increased, the level of valeric acid decreased and cellulose digestion increased. This suggested that as microbial growth increased, the rate of utilization of valeric acid increased.

Previous work has shown that the addition of a mixture of four- and five-carbon branched chain and straight chain VFA to low protein diets may lead to an increase in the utilization of such a diet by ruminants. The addition of a mixture of branched chain VFA to sheep fed a protein free purified diet adequately supplied with NPN, increased protein production from 71 to 81 g/day. In another study , Hemsley and Moir (1963) found that the intake of a milled oaten hay diet was increased by the addition of 0.56% of a mixture of isobutyric, isovaleric, and n-valeric acids. Later, Cline et al. (1966), reported a significant increase in N retention by lambs supplemented with 4.18 g isobutyrate, 1.18 g n-valerate, and 5.9 g isovalerate per day. Studies conducted by Umunna et al. (1975), showed that feeding or rumen infusion of isobutyrate and/or isovalerate to lambs on high roughage, urea supplemented rations improved N retention and decreased urinary N loss. Rumen ammonia and blood urea were not affected by the

acids.

Low concentrations of isovalerate, 2-methylbutyrate and peptides in the diet can improve efficiency of synthesis of rumen bacterial protein from soluble carbohydrates up to 11.2% and 16.4%, respectively (Russell and Sniffen, 1984). Cummins and Papas (1985), reported that the addition of isoacids (C_4 and C_5) to a corn silage based diet, increased dry matter digestion and microbial growth *in vitro* regardless of the dietary crude protein content (13 to 16%). Soofi et al. (1982), measured the *in vitro* effects of branched-chain VFA , urea, starch and trypticase, and their interactions on dry matter disappearance of soybean stover finding a positive effect of branched-chain VFA on DM disappearance. The interaction of branched-chain VFA and trypticase appeared to provided a balance medium for bacterial growth.

Hefner et al. (1985) studied the effect of branched-chain VFA supplementation to corn crop residue diets. Dry matter and fiber digestibility tended to be higher when lambs were fed natural protein supplements, while urea and branched-chain VFA tended to decrease the extent of digestion. Gorosito et al. (1985), incubated mixed ruminal bacteria in an artificial medium with isolated plant cell walls and intact forages. They added an equimolar mix (.30 mM) of C_4 and C_5 acids and observed that cell wall digestion was increased 26.4%. The individual isoacids were equally as effective as the mixture, increasing cell wall digestion 25.4 to 26.6%. Valeric acid alone did not affect cell wall digestion.

Earlier work conducted by Falen et al. (1968) attempted to determine the effects of soybean meal (SBM), urea and phenylacetic, 2-methylbutyric, isobutyric and isovaleric

acids on the intake and digestion of low quality roughages. All treatments substantially increased intake, with isobutyrate giving the best response and isovalerate giving the lowest increase in VFA production. More recently, Varga et al. (1988) conducted an *in vitro* fermentation of diets containing SBM treated with 0.3% formaldehyde, or untreated. Formaldehyde treated SBM depressed fiber and protein digestion as well as VFA and microbial production. However, N provided as urea and carbon skeletons as isoacids restored fiber digestion.

An *in vivo* study conducted by Lassiter et al. (1958) indicated that the combination of isovaleric and valeric acids exerted a beneficial effect upon the growth rate of dairy heifers fed a low quality roughage. Research by Felix et al. (1976) suggested that mixtures of C_4 and C_5 branched-chain acids plus valeric acid improved N retention, milk production, and persistency of milk yield in dairy cows fed diets containing urea, corn silage, and corn grain. Isoacid fed cows could achieve an increase in milk production of 10% without any increase in feed intake (Papas et al., 1984).

The addition of salts of VFA to ruminant diets have been extensively studied. Rogers and Davis (1982) found that intraruminal infusions of mineral salts of VFA to steers reduced the molar percentage of ruminal propionate and increased that of acetate when high grain diet was fed. However, no effect was obtained when salts of VFA were added to a high roughage diet. Supplementation of diets for Holstein cows with ammonium salts of branched-chain VFA increased milk production by 8 to 10% (Peirce-Sandner et al., 1985) as cited by Otterby et al. (1990). Rogers et al. (1989), reported the milk production response of cows fed calcium or ammonium salts of

branched-chain VFA early in lactation was higher (7.9%) than the response of cows in mid- or late lactation. Dose response studies of dairy cows to 0, .4, .18, 1.2, or 1.6% ammonium salts of branched-chain VFA added to the concentrate portion of the diet with a forage:concentrate ratio of 50:50, 60:40, and 70:30 for first, middle, and late lactation, respectively. It was found that supplementation of ammonium salts of branched-chain VFA increased milk and milk protein yield during mid and late lactation. A tendency to gain less body weight was also observed (Otterby et al., 1990).

The addition of sodium and calcium salts of branched-chain VFA to high grain diets of small ruminants resulted in higher weight gain than to high roughage diets (Orskov and Allen, 1970).

With respect to the utilization of sodium and calcium salts of VFA by small ruminants, Orskov and Allen (1970) observed that addition of these salts to a high roughage diet promoted lower weight gains than to a high concentrate diet. Later research conducted by Poole and Allen (1970), showed that a mixture of sodium and calcium salts of acetic acid added to low (40%) and high (85%) concentrate diets exhibited a greater live-weight, empty body-weight and carcass-weight gain than lambs given unsupplemented diets. Response of weight gain to increasing levels of acetate salts was linear.

Among the factors affecting ruminal volatile fatty acid production, it was found by Peters et al. (1989) in an *in vitro* mixed bacterial population that total production of microbial products was greater at high than at low pH. Elliot et al. (1987) recently studied the influence of anaerobic fungi on rumen VFA concentration *in vivo*, indicating

an increased concentration of rumen propionic acid as a result of the removal of the rumen anaerobic fungi (RAF). This suggests that RAF may play an important role in the fermentation of high fiber diets.

As stated by Bergen (1979), the ruminal fermentation is a coupled process between carbohydrate degradation and microbial cell synthesis. Therefore, several factors such as ammonia, branched-chain VFA, S and carbon chains are required to be available at the same time in order to allow the optimal fermentation (Bergen and Yokoyama, 1977). The advantages of supplementing isoacids, S and a NPN source to ruminants have already been discussed. However, little information has been reported concerning the interaction among those three factors. Recent reports (Quispe et al., 1991; Brondani et al., 1991) indicated that in high fiber, low protein rations, S and N requirements have to be met before isoacids can elicit increases in microbial growth and cellulose digestion.

In the present study, an *in vitro* ruminal fermentation trial was carried out in order to evaluate the effect of the interactions of S, N and isoacids on high fibrous forages which are of common usage in Latin American countries.

MATERIALS AND METHODS

Materials

An *in vitro* experiment was conducted to investigate the effect of NH_3, isoacids and S on rate of fermentation of eight tropical forages. The forage samples collected in the Dominican Republic were *Brachiaria decumbens, Cenchrus ciliaris, Cynodon dactylon, Digitaria decumbens, Gliricidia sepium, Leucaena leucocephala, Panicum maximum and Pennisetum purpureum.* The stage of maturity of the forages was 35 days, with the exception of Leucaena (80 days), and Panicum (112 days). Samples were dried overnight at 60°C in an oven and ground in a Wiley mill to pass through a 1 mm screen.

Treatments and experimental design

Eight forages species in a 2^3 factorial design were studied. The factors were at two levels each of NH_3, S and isoacids. (Table 2).

The combinations of the three factors at two different levels resulted in 8 treatments (LLL, LLH, LHL, LHH, HLL, HHL, HLH and HHH) where NH_3, S and isoacids are the first, second and third factor, respectively, and L and H represent low and high levels . All treatments were tested using each one of the 8 forages.

Table 2. Calculated levels of NH_3, H_2S and isoacids in the fermentation flasks.

Levels	$(NH_3)^1$	$(H_2S)^1$	Isoacids
Low	5 mg/dl	2 mg/dl	0
High	10 mg/dl	6 mg/dl	15 mg/dl

[1] The levels of NH_3 and S achieved in the incubation mixture was 4.04 and 8.08 mg NH_3/dl for low and high NH_3, and 1.36 and 4.1 mg H_2S/dl for low and high S, respectively.

The levels chosen for the three factors were based on reports from previous experiments (Kone et al., 1989; Felix et al., 1980; Quispe et al., 1991). An equimolar mixture of isoacids 6 g/dl (isobutyric, 2-m-butyric, isovaleric and valeric) was prepared and neutralized with KOH, to pH 7.00. To obtain the final concentration required in the fermentation (15 mg/dl) a dilution of 400 fold was made.

Different levels of S were provided in the reducing solution by varying the amount of S ($Na_2S.9H_2O$). Ammonia was provided in the buffer solution as ammonium carbonate (NH_4HCO_3). The levels are shown in Table 3.

Table 3. Composition of the solutions used in the basal media for the different combinations of nitrogen and sulfur (LL, HL, LH, HH). The last column represents the media used at MSU forage laboratory[a].

TREATMENT	NH_3 5 mg/dl H_2S 2 mg/dl	NH_3 10 mg/dl H_2S 2 mg/dl	NH_3 5 mg/dl H_2S 6 mg/dl	NH_3 10 mg/dl H_2S 6 mg/dl	NH_3 86 mg/dl H_2S 70 mg/dl
			BUFFER SOLUTION		
H_2O ml	1000	1000	1000	1000	1000
NH_4HCO_3 mg	975	1950	975	1950	4000
$NaHCO_3$ g	630	630	630	630	630
			MACROMINERAL SOLUTION		
H_2O ml	1000	1000	1000	1000	1000
Na_2HPO_4 g	5.7	5.7	5.7	5.7	5.7
KH_2PO_4 g	6.2	6.2	6.2	6.2	6.2
$MgCl_2$ g	0.23	0.23	0.23	0.23	
$MgSO_4.7H_2O$ g					0.6
			MICROMINERAL SOLUTION		
$CaCl_2.2H_2O$ g	13.2	13.2	13.2	13.2	13.2
$MnCl_2.4H_2O$ g	10	10	10	10	10
$CoCl_2.6H_2O$ g	1	1	1	1	1
$FeCl_3.6H_2O$ g	8	8	8	8	8
			REDUCING SOLUTION		
H_2O ml	48	48	48	48	48
L(+)CYS.HCl.H_2O mg					313
1 N NaOH ml	2	2	2	2	2
$Na_2S.9H_2O$ mg	125	125	376	376	313

[a] According to Goering and VanSoest (1970).

In vitro rumen digestibility

Rumen fluid was collected from a Holstein cow that had been fed a wheat straw diet for several weeks, blended for 30 seconds in a steel blender at low speed, passed through cheesecloth and then glass wool under CO_2. Fermentations were conducted in 125 ml Erlenmeyer flasks containing 0.5 g substrate, 40 ml incubation media and 10 ml rumen fluid as described by Goering and Van Soest (1970). The incubation media (Table 4) was added to the flasks the night before the fermentation in order to hydrate the substrates. The next morning, all flasks were placed in a water bath at 40°C, covered with rubber stoppers and gassed continuously with CO_2. All flasks were inoculated with rumen fluid using an automatic syringe. Control rumen fluid samples were taken at the beginning, midpoint and end of the inoculating process and stored at -10°C until analyzed. Finally, two ml of a reducing solution (Table 3) were injected through the inlet tube and 30 min later the isoacid mixture was added.

Table 4. Composition of the basal media.

Distilled water	500 ml
Trypticase™	2.5 g
Micromineral solution	0.125 ml
Rumen buffer solution[1]	250 ml
Macromineral solution	250 ml
Resarzurin	1.25 ml

[1] This solution was prepared to provide different concentrations of NH_3 as required for the low and high levels.

After 48 h of fermentation, 2 ml of the incubation mixture were taken for analysis, and then 1 ml of toluene was added to stop the fermentation and the flasks were stored at 5°C. Zero time samples were taken.

Forage fiber analysis

A sequential analysis of NDF, ADF, and ADL was conducted with the omission of decahydronapthalene and $NaSO_3$ as modified by Robertson and Van Soest (1977). For NDF, all of the incubation mixture was transferred to a 600 ml Berzelius beaker using 100 ml of neutral detergent solution. The mixture was refluxed for 1 hour and filtered through a Gooch crucible, coarse porosity of 40 to 60 μm. The crucibles were washed two times with hot water and two times with acetone. Crucibles were air dried and then dried overnight at 100°C, weight was determined at 100°C. For ADF, the sample in the crucible was boiled in acid-detergent for 5 minutes and then transferred to a beaker and refluxed for 55 minutes, filtered and dried as described for NDF. Crucibles with ADF residue were treated with 72% sulfuric acid for 3 h, washed and filtered. Ashing the residue at 500°C permitted the determination of lignin.

True dry matter digestibility was calculated as 100 minus the percent of neutral detergent residue. Hemicellulose was calculated as NDF% - ADF%, and cellulose as ADF% - lignin %.

Analysis of ammonia concentration

Determination of ruminal ammonia concentration was performed as described by Chaney and Marbach (1962). A 5.00 μl aliquot of the incubation mixture was taken with a Hamilton syringe and a Cheney adaptor and stored in a 4 ml plastic tube. One ml of the phenol-nitroprusside and 1 ml of the sodium hydroxide-sodium hypochlorite solution was added using a Micromedic systems automatic pipetting station, model 24004 (Micromedic Systems, Inc., Horsham, Pa.). The mixture was covered with aluminum foil and incubated for 30 minutes at room temperature for color development. Absorbances were read at 625 nm in a Stasar II spectrophotometer (Gilford Ins. Lab., Burlington, Mass.). Ammonia standards were prepared from NH_4Cl and ranged from 5-20 mg of ammonia/dl of solution.

Hydrogen sulfide analysis

Ruminal hydrogen sulfide was determined according to manufacturers instructions using a sulfide micro ion sensing electrode, Lazar model ISM-146 connected to a Lazar model DPH digital pH meter (Lazar Res. Lab., Los Angeles, Ca.). A 250 μl aliquot of the incubation mixture was combined with 250 μl antioxidant buffer (a mixture of 125 g sodium salicylate, 42.5 g NaOH, 32.5 g ascorbic acid and distilled water up to 500 ml). The standard H_2S ranged from 0.01 to 100 ppm. The electrode was allowed to stabilize in a 10 ppm standard sulfide solution for 30 minutes before being used, and for 10 minutes between recording sample readings. Rinsing of the electrode with distilled water between samples was critical to obtain accurate readings. Potential readings

(millivolts) were plotted vs. sulfide concentrations on a logarithmic scale. Hydrogen sulfide was expressed as mg/dl of incubation mixture.

Determination of volatile fatty acid concentration

VFA analysis was performed using a Hewlet Packard 5730 A gas liquid chromatograph model 5730 A with flame ionization detector, a 7671 A automatic sampler and a 3380 integrator. A glass column (30" x 1/4" x 4 mm ID) was packed with 60/80 Carbopack C/0.3% Carbowax 20 M/0.1% H_3PO_4, lot # I46655 (Supelco cat. # 1-1825). Nitrogen was the carrier gas at 50 ml/min. The temperature program was 125°C for 4 minutes with a temperature increase of 8°C/min for 5.63 min. The final temperature was 170°C. Both, the injector and detector temperatures were 200°C.

One ml of each sample was centrifuged at 1000 RPM for 15 minutes, and a 10 μl aliquot was used for analysis. Prior to the injection, the 1 ml samples were acidified with 50 μl of 88% formic acid (Baker 0128-01). The VFA standards contained 60, 20, 20, 2, 2, 2, 2, mM of C2, C3, C4, IC4, 2MB, IC5, and C5, respectively. Data output was expressed as mM of the incubation mixture.

Statistical analysis

Overall significance of treatment effects was determined by ANOVA (Gill, 1978, Vol. 1, 2 and 3) according to the model in equation 1. Differences among grasses and legumes were determined by Bonferroni t-test and differences within the same specie were determined by Scheffe (Gill, 1978a).

(1) $$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta_l + (\alpha\delta)_{il} + (\beta\delta)_{jl} + (\gamma\delta)_{kl} + (\alpha\beta\delta)_{ijl} + (\alpha\gamma\delta)_{ikl} + (\beta\gamma\delta)_{jkl} + (\alpha\beta\gamma\delta)_{ijkl} + E_{(ijkl)m}$$

where, μ = the population mean

α_i = species effect (fixed)

β_j = isoacid effect (fixed)

γ_k = nitrogen effect (fixed)

δ_l = sulfur effect (fixed)

$(\alpha\beta)_{ij}$, $(\alpha\gamma)_{ik}$, $(\beta\gamma)_{jk}$, $(\alpha\beta\gamma)_{ijk}$, $(\alpha\delta)_{il}$, $(\beta\delta)_{jl}$, $(\gamma\delta)_{kl}$, $(\alpha\beta\delta)_{ijl}$, $(\alpha\gamma\delta)_{ikl}$, $(\beta\gamma\delta)_{jkl}$ and $(\alpha\beta\gamma\delta)_{ijkl}$ = interaction of the main effects (fixed)

$E_{(ijkl)m}$ = error term (random).

RESULTS

The effects of ammonia, S and isoacids on the volatile fatty acid concentrations and true digestibility of tropical forages using an *in vitro* rumen fermentation procedure are summarized in Table 5. Analysis of variance for *in vitro* true digestibility (IVTD) showed a significant variation ($P < .001$) between all species and among grasses and legumes (Table 6). Several interactions were significant. For species x NH_3, digestibility increased ($P < .003$) for all grasses as a result of increasing NH_3. For legumes, *Gliricidia* IVTD was not affected while *Leucaena* IVTD decreased when NH_3 level was increased (Figure 4). Sulfur at high levels tended to decreased ($P < .001$) IVTD of most of the forages (Figure 5). The interaction of isoacids x NH_3 (Figure 6), showed that the addition of isoacids to treatments with high level of NH_3 decreased ($P < .032$) digestibility of forages. When isoacids where added to treatments with low level of NH_3, IVTD increased.

The highest IVTD digestibility was found for *Pennisetum* and *Panicum* (64.61 and 64.89%) followed by *Brachiaria* and *Digitaria* (58.99 and 57.18%). Digestibility values for *Cenchrus* and *Cynodon* were 53.40 and 53.85%, respectively. Similar values were obtained for these grasses by Kayongo-Male et al. (1976). Stems from legumes had the lowest digestibility, 44.52 and 29.18% for *Gliricidia* and *Leucaena*, respectively (Table 5).

Table 5. Volatile fatty acid concentrations and true digestibility of tropical forages after a 48 hour *in vitro* rumen fermentation [1].

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodom dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximun*	*Pennisetum purpureum*
Acetate (mM)	$39.83^{a,b}$	46.32^{a}	51.19^{a}	$33.47^{b,c}$	$26.85^{b,c}$	$29.41^{b,c}$	$30.11^{b,c}$	26.97^{c}
Propionate (mM)	$7.74^{b,c}$	$8.63^{a,b,c}$	$10.04^{a,b,c}$	$12.24^{a,b}$	$10.40^{a,b,c}$	7.91^{c}	12.38^{a}	12.37^{a}
Butyrate (mM)	4.64^{a}	4.78^{a}	5.16^{a}	4.86^{a}	5.01^{a}	3.27^{b}	4.77^{a}	4.75^{a}
Acetate/propionate ratio	$5.15^{a,b}$	5.37^{a}	5.10^{a}	$2.73^{a,b}$	2.58^{b}	$3.72^{a,b}$	2.43^{b}	2.18^{b}
Total volatile fatty acids (mM)	$56.92^{b,c}$	$63.97^{a,b}$	71.05^{a}	$54.64^{b,c}$	49.06^{c}	44.07^{c}	$51.83^{b,c}$	48.40^{c}
In vitro true digestibility (%)	58.99^{b}	53.40^{c}	53.85^{c}	57.18^{b}	44.52^{d}	29.18^{e}	64.89^{a}	64.61^{a}

[1] Data are the average from all treatments. ^{a,b,c,d} Means in the same row with different superscripts differ ($P < .05$).

Table 6. Analysis of variance of the effects of ammonia, sulfur and isoacids on fiber fraction digestibility after a 48 h *in vitro* rumen fermentation of eight tropical forages.

	IVTD[a]			CWD[b]			ADFD[c]		
Source	df	MS	P	df	MS	P	df	MS	P
Species (SP)	7	2214.64	.001	7	4305.89	.001	7	4375.78	.001
Isoacids (I)	1	0.31	.745	1	1.11	.667	1	2.09	.607
Nitrogen (N)	1	26.67	.004	1	59.80	.002	1	34.54	.040
Sulfur (S)	1	188.45	.001	1	443.54	.001	1	543.88	.001
SP x I	7	3.11	.389	7	6.53	.373	7	8.96	.347
SP x N	7	13.26	.003	7	28.51	.002	7	30.48	.001
SP x S	7	19.46	.001	7	36.02	.001	7	31.98	.009
I x N	1	13.91	.032	1	25.46	.042	1	24.65	.081
I x S	1	0.10	.855	1	0.29	.826	1	5.29	.414
N x S	1	5.15	.187	1	10.63	.185	1	6.64	.360
I x N x S	1	0.03	.916	1	0.75	.724	1	0.29	.847
SP x I x N	7	2.50	.540	7	5.90	.444	7	14.85	.084
SP x I x S	7	13.00	.004	7	25.40	.006	7	20.94	.017
SP x N x S	7	9.02	.007	7	16.71	.013	7	22.47	.011
SP x I x N x S	7	3.34	.342	7	7.33	.297	7	6.55	.561
Error	64	2.89		64	5.93		64	7.82	
Grasses vs legumes	1	11584.01	.001	1	18149.59	.001	1	17822.18	.001

[a] *In vitro* true digestibility. [b] Cell wall digestibility. [c] Acid detergent fiber digestibility.

"Table 6 (cont'd)."

	HEMD[d]			CELD[e]			LIGD[f]		
Source	df	MS	P	df	MS	P	df	MS	P
Species (SP)	7	3782.33	.001	7	5368.78	.001	7	2241.98	.001
Isoacids (I)	1	0.64	.749	1	1.99	.655	1	15.90	.275
Nitrogen (N)	1	147.28	.001	1	13.64	.244	1	267.44	.001
Sulfur (S)	1	262.32	.001	1	723.99	.001	1	136.78	.002
SP x I	7	6.26	.438	7	13.99	.215	7	26.65	.064
SP x N	7	44.01	.001	7	39.49	.011	7	31.73	.029
SP x S	7	48.33	.001	7	56.73	.001	7	10.92	.564
I x N	1	19.14	.085	1	42.71	.042	1	17.78	.249
I x S	1	15.71	.118	1	4.48	.503	1	15.32	.284
N x S	1	17.78	.096	1	15.71	.212	1	4.83	.546
I x N x S	1	3.17	.479	1	0.02	.964	1	12.89	.325
SP x I x N	7	7.19	.343	7	17.62	.106	7	15.92	.309
SP x I x S	7	55.86	.001	7	28.55	.011	7	37.52	.012
SP x N x S	7	10.79	.118	7	34.00	.004	7	18.48	.218
SP x I x N x S	7	12.85	.061	7	6.72	.688	7	24.80	.086
Error	64	6.24		64	9.88		64	13.12	
Grasses vs legumes	1	15759.38	.001	1	22735.34	.001	1	173.72	.005

[d] Hemicellulose digestibility. [e] Cellulose digestibility. [f] Lignin digestibility.

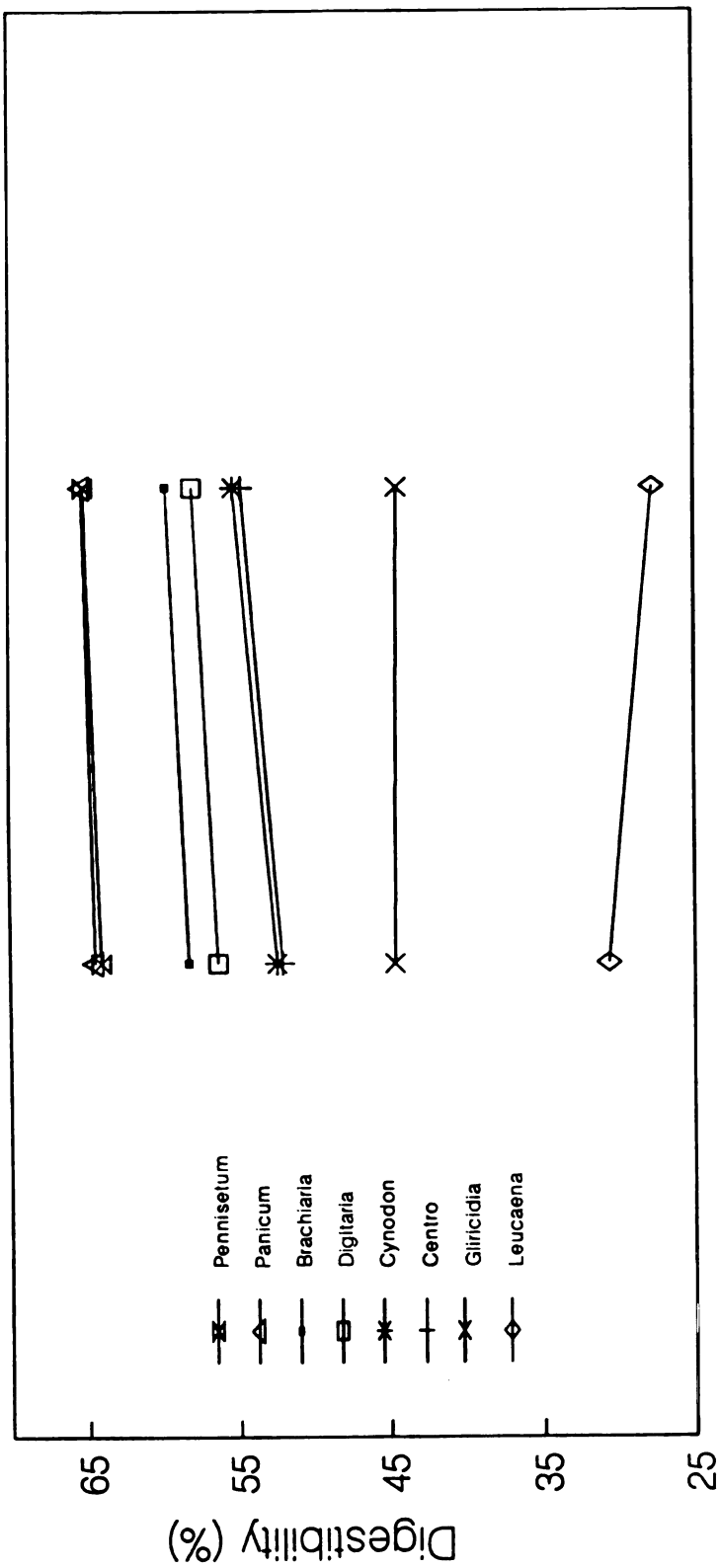

Figure 4. The effect of the interaction of forage species and NH3 on the in vitro true digestibility after a 48 h fermentation.

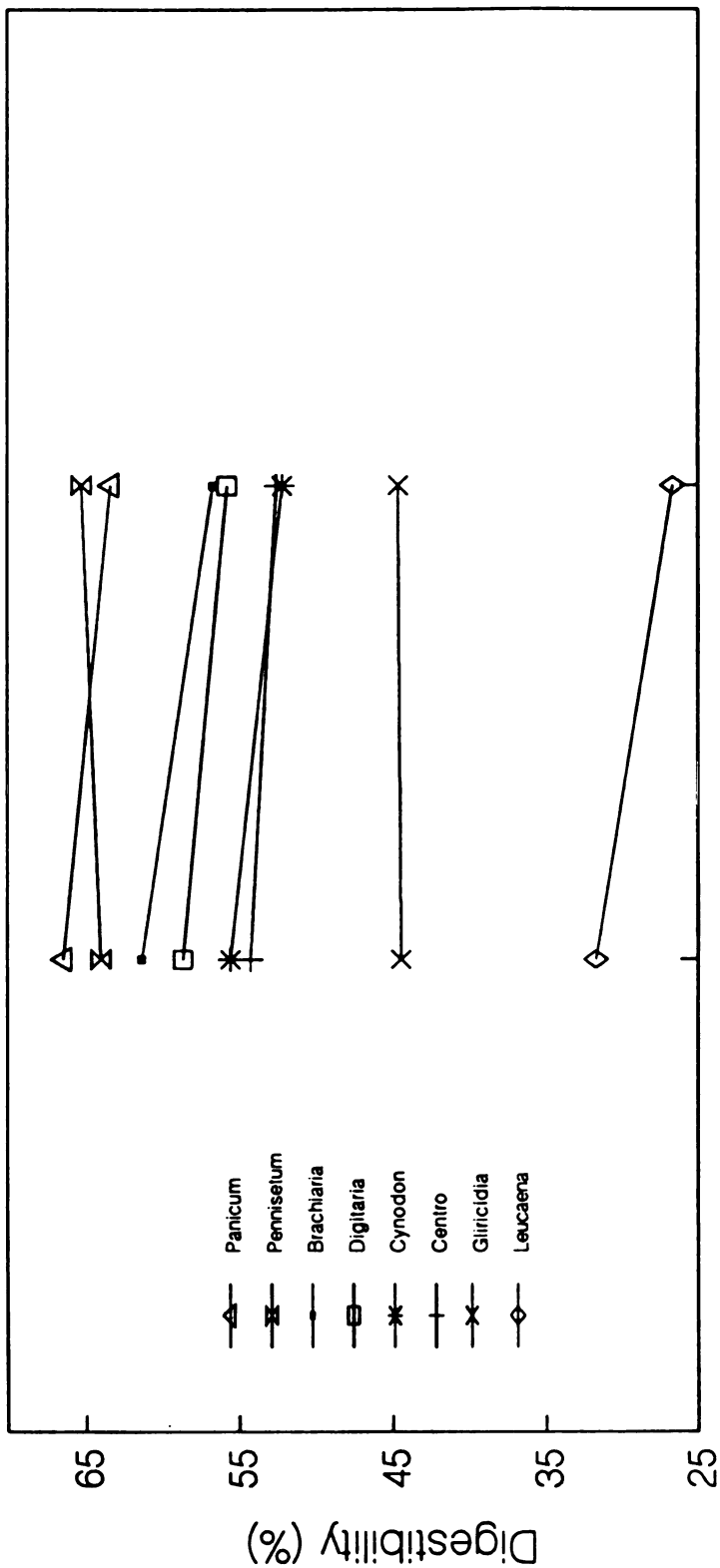

Figure 5. The effect of the interaction of forage species and sulfur on the in vitro ruminal true digestibility of forage species after a 48 h fermentation.

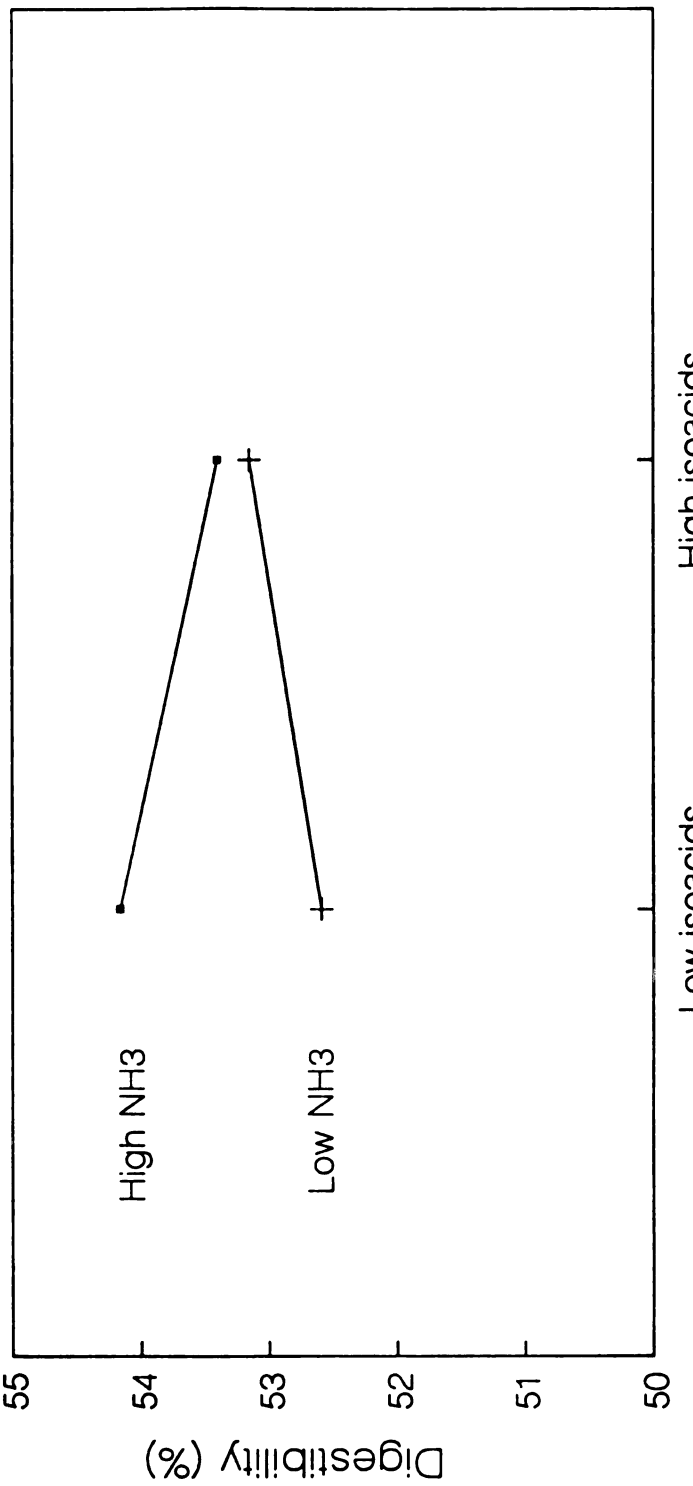

Figure 6. The effect of the interaction of isoacids and NH3 on the in vitro ruminal true digestibility of forage species after a 48 h fermentation.

Volatile fatty acid accumulation for the eight forage species studied is shown in Figures 7, 8, and 9. The variation among species was significant (Table 7). Total VFA production ranged from 44.07 to 71.05 mM (Table 5). Naga and Harmeyer (1975) reported *in vitro* rumen total VFA production of 11.89 $mMol \cdot g^{-1} \cdot h^{-1}$. Several *in vivo* studies have been conducted with sheep. Bergman (1965) obtained a total VFA value of 109 mM of rumen fluid with dried grass diet. However, Leng and Brett (1966) found a wide variation from 49.1 to 143.5 mM when sheep were fed four different diets containing combinations of lucerne, maize and wheaten straw. Weller et al. (1969) reported total VFA values of 100 to 136 mM in sheep grazing pastures. More recently, Elliot et al. (1987) found a total VFA concentration of 48 mM.

In this study, the molar proportion of acetate was 70% which is similar to values reported by Weller et al. (1969). However, the molar proportion of propionate (14%) was lower than values of 19 and 22% reported by Bergman (1965) and Weller et al. (1969), respectively. It was observed that when acetate concentration was lower, propionate was higher. Butyrate was fairly constant for all the species in the study (Table 5).

A positive effect of isoacids on total VFA concentration was observed (Figure 10). There was a variable effect of the interaction of species x NH_3 on acetate production as illustrated in Figure 11.

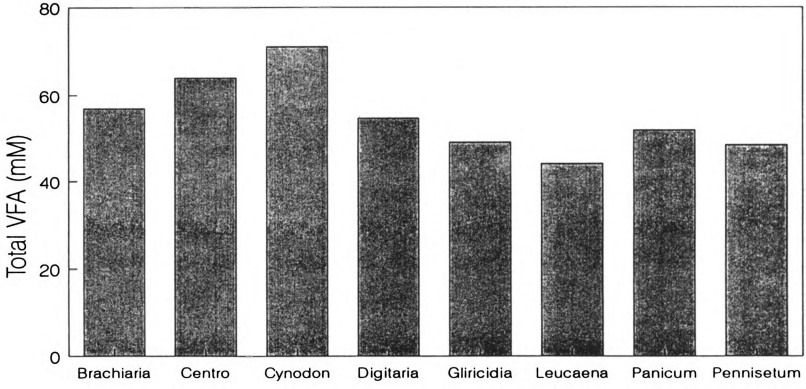

Figure 7. Total volatile fatty acid concentration after a 48 h in vitro ruminal fermentation of tropical forages.

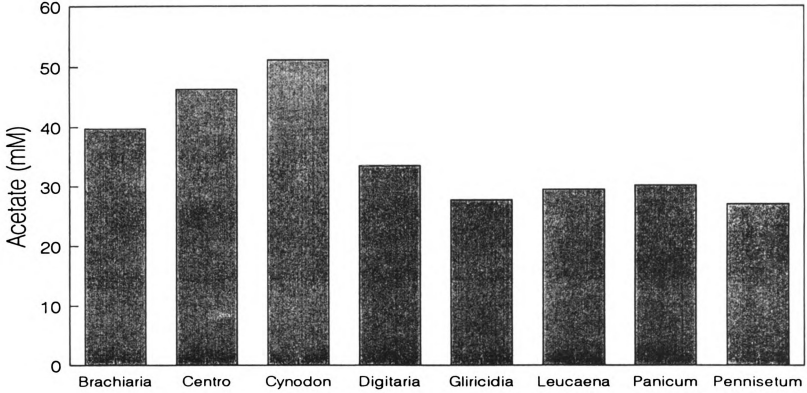

Figure 8. Acetate concentration after a 48 h in vitro ruminal fermentation of tropical forages.

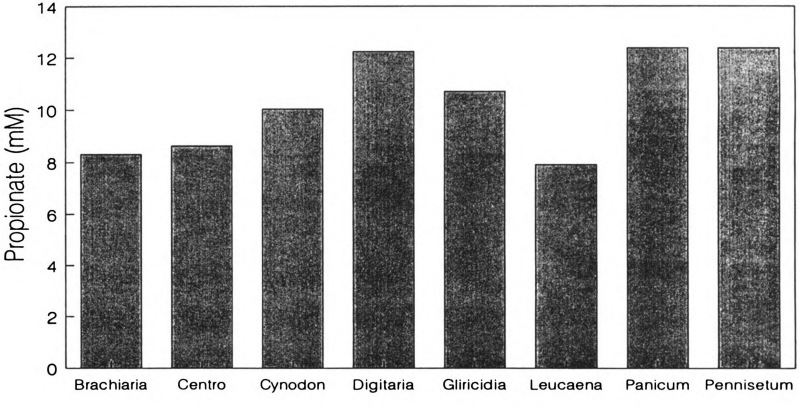

Figure 9. Propionate concentration after a 48 h in vitro ruminal fermentation of tropical forages

Table 7. Analysis of variance of the effect of ammonia, sulfur and isoacids on volatile fatty acid concentration after a 48 h *in vitro* rumen fermentation of eight tropical forages.

	Total VFA			Acetate			Propionate		
Source	df	MS	P	df	MS	P	df	MS	P
Species (SP)	7	1256.84	.001	7	1339.83	.001	7	57.30	.003
Isoacids (I)	1	530.61	.043	1	145.07	.275	1	13.49	.296
Nitrogen (N)	1	99.78	.375	1	154.64	.260	1	5.89	.489
Sulfur (S)	1	1.47	.914	1	3.72	.861	1	12.80	.309
SP x I	7	214.57	.972	7	46.57	.906	7	9.87	.581
SP x N	7	225.59	.102	7	244.76	.063	7	7.18	.751
SP x S	7	206.16	.138	7	169.31	.215	7	16.42	.242
I x N	1	0.98	.816	1	69.73	.448	1	44.23	.061
I x S	1	137.55	.298	1	118.71	.323	1	0.93	.783
N x S	1	1.75	.906	1	5.75	.827	1	0.11	.926
I x N x S	1	187.82	.225	1	152.06	.264	1	3.46	.595
SP x I x N	7	865.99	.447	7	101.64	.551	7	9.96	.575
SP x I x S	7	161.45	.269	7	241.38	.067	7	15.68	.270
SP x N x S	7	80.20	.720	7	148.52	.294	7	16.54	.238
SP x I x N x S	7	74.60	.756	7	86.82	.651	7	11.61	.472
Error	64	124.96		64	119.73		64	12.16	
Grasses vs legumes	1	3026.77	.001	1	2134.24	.001	1	44.02	.062

"Table 7 (cont'd)."

	Butyrate			C2/C3		
Source	df	MS	P	df	MS	P
Species (SP)	7	5.44	.001	7	28.64	.001
Isoacids (I)	1	0.77	.299	1	0.34	.799
Nitrogen (N)	1	0.14	.658	1	3.92	.386
Sulfur (S)	1	1.21	.146	1	2.68	.473
SP x I	7	0.52	.629	7	2.30	.869
SP x N	7	1.54	.045	7	4.74	.498
SP x S	7	1.10	.158	7	5.69	.371
I x N	1	0.51	.394	1	9.68	.175
I x S	1	2.08	.089	1	0.11	.886
N x S	1	1.12	.210	1	0.24	.829
I x N x S	1	0.08	.738	1	1.24	.626
SP x I x N	7	0.47	.688	7	1.91	.916
SP x I x S	7	0.14	.985	7	10.40	.066
SP x N x S	7	0.14	.985	7	5.79	.360
SP x I x N x S	7	0.47	.686	7	1.99	.908
Error	64	0.69		64	5.16	
Grasses vs legumes	1	11.27	.002	1	1.43	.601

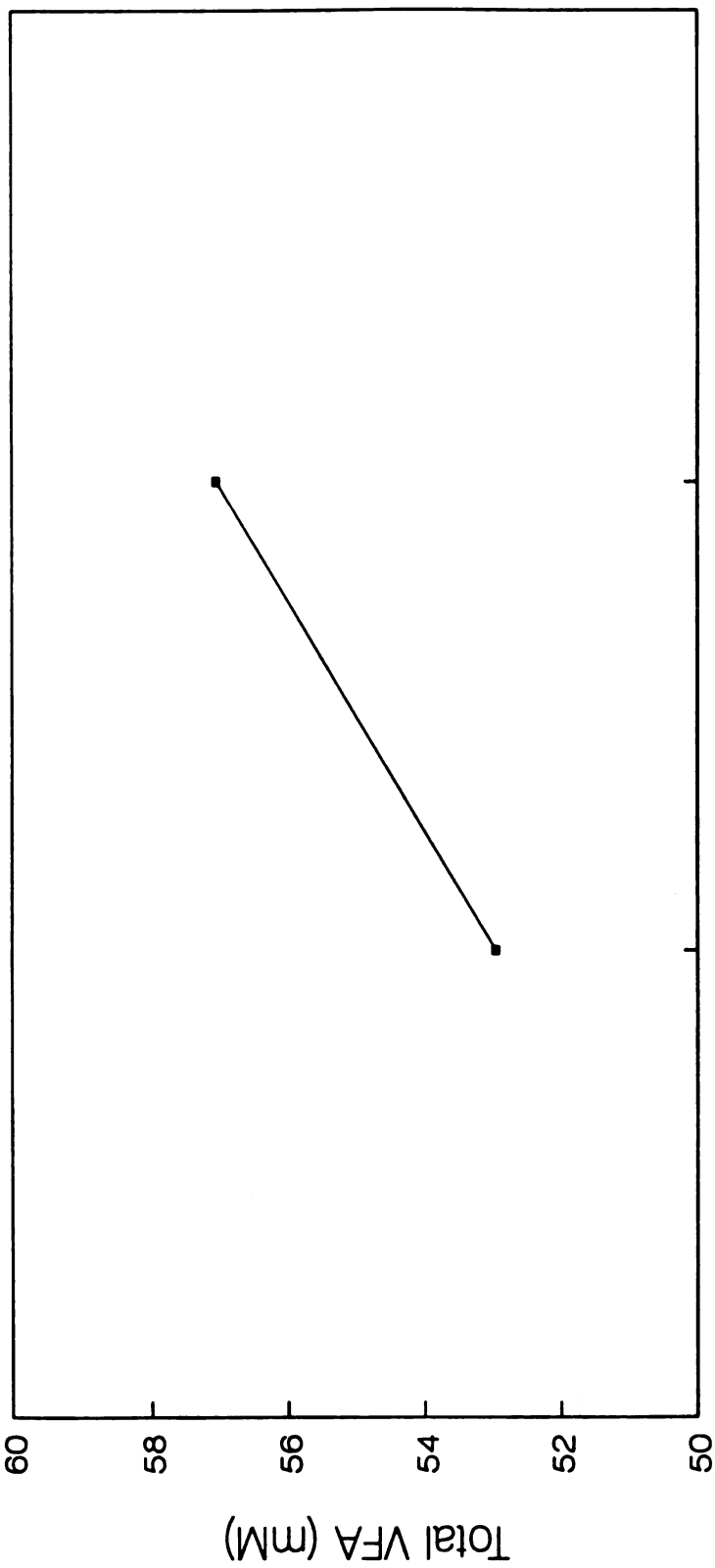

Figure 10. Effect of isoacids on total volatile fatty acid concentration after a 48 h in vitro ruminal fermentation of tropical forages.

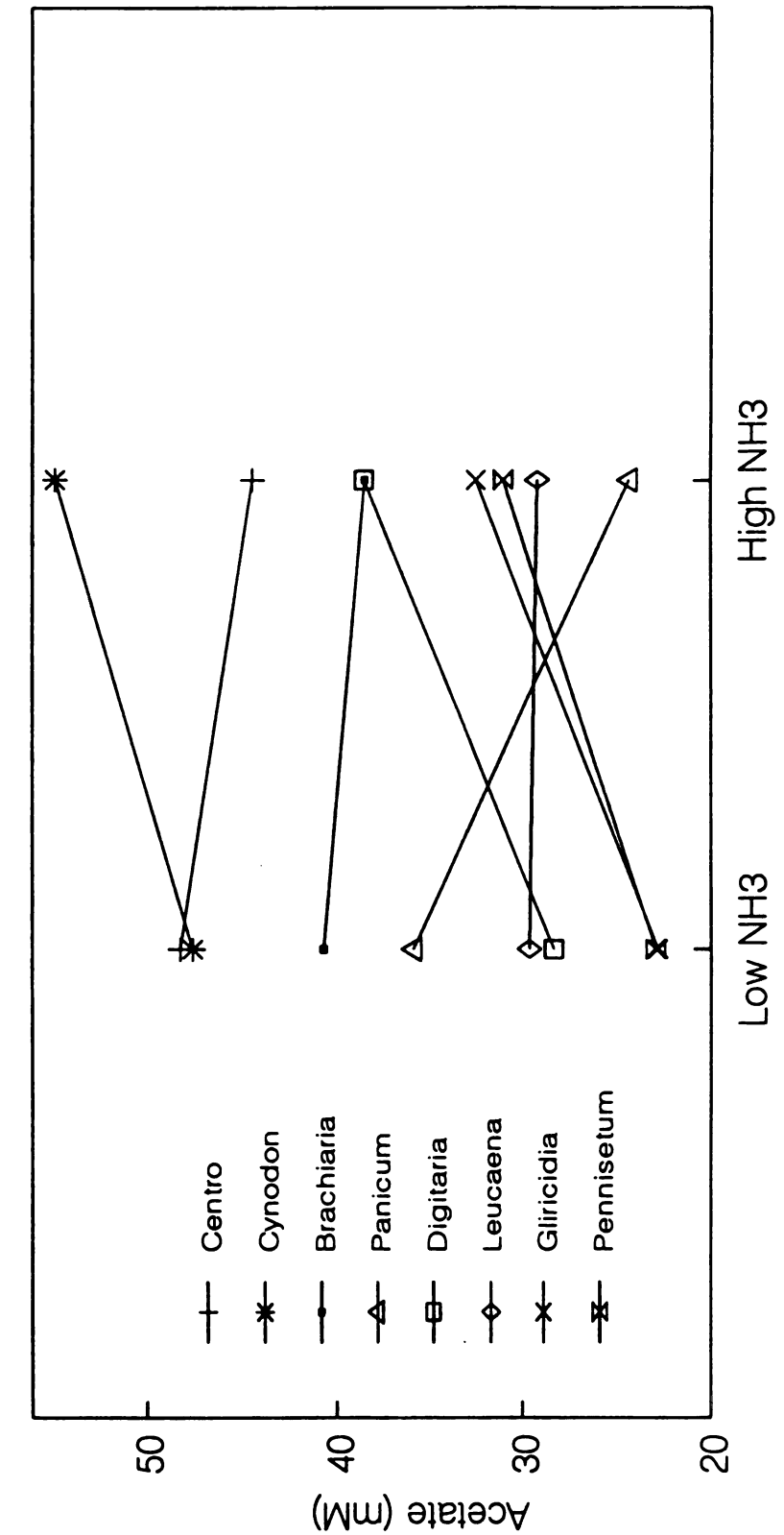

Figure 11. Effects of NH3 level on acetate concentration after a 48 h in vitro ruminal fermentation of tropical forages.

It was found that high NH_3 decreased ($P < .063$) acetate production from *Brachiaria, Cenchrus,* and *Panicum* while an increase in NH_3 increased acetate production from *Cynodom, Digitaria, Gliricidia,* and *Pennisetum.* On the other hand, there was a negative effect ($P < .061$) of isoacids on propionate concentration when NH_3 level was high. However, a positive effect of isoacids on propionate production was found with low levels of NH_3 (Figure 12). For the interaction of isoacids x S on total isoacids, the high level of isoacids increased ($P < .046$) the total concentration of isoacids for both low and high levels of S (Figure 13).

Isoacid concentrations after a 48 h *in vitro* rumen fermentation of tropical forages are presented in Table 8. Concentrations of C4 and C5 fatty acids were found to vary among species ($P < .061$). Isovalerate ranged from 0.75 to 1.05 mM. Valerate concentration ranged from 1.19 to 2.28 mM. The values for 2-methylbutyrate ranged from 0.61 to 0.92 mM accounting for differences between species. However, isobutyrate concentration was similar for all species studied (Table 8). Hefner (1985) reported ruminal molar proportions of isobutyrate and 2-metylbutyrate + isovalerate to be 6.3 and 1.5 molar % of total VFA when lambs were fed a corn diet supplemented with urea and isoacids. Umunna et al. (1975) found that addition of isobutyrate and isovalerate to high roughage diets fed to lambs did not affect dry matter digestibility. Similar results were obtained by Hemsley and Moir (1963), Cline et al. (1966) and Hume (1970a and b).

Table 8. Isoacids, ammonia and sulfur concentration after a 48 hour *in vitro* rumen fermentation of tropical forages [1].

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodom dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximun*	*Pennisetum purpureum*
Isobutyrate (mM)	0.99^{b}	1.00^{b}	0.96^{b}	0.96^{b}	1.48^{a}	0.96^{b}	1.08^{b}	1.02^{b}
2-M-butyrate (mM)	$0.67^{b,c}$	$0.70^{b,c}$	$0.79^{a,b}$	$0.61^{b,c}$	0.92^{a}	0.58^{c}	$0.78^{a,b}$	0.91^{a}
Isovalerate (mM)	$0.95^{a,b}$	0.98^{a}	$0.97^{a,b}$	0.75^{c}	1.02^{a}	$0.78^{b,c}$	1.05^{a}	$0.92^{a,b,c}$
Valerate (mM)	$1.65^{b,c}$	1.57^{c}	$1.97^{a,b}$	$1.82^{b,c}$	2.28^{a}	1.19^{d}	$1.68^{b,c}$	$1.48^{c,d}$
$IC4_2$+2MB+IC5+C5 (mM)	$4.22^{b,c}$	$4.24^{b,c}$	4.66^{b}	$4.13^{b,c}$	5.69^{a}	3.49^{c}	4.57^{b}	$4.32^{b,c}$
Ammonia (mg NH_3/l)	$24.81^{a,b}$	$24.27^{a,b,c}$	26.94^{a}	17.38^{d}	17.30^{d}	14.47^{d}	$22.34^{b,c}$	21.48^{c}
Sulfur (mg H_2S/l)	0.22^{a}	$0.20^{a,b}$	0.19^{b}	0.15^{c}	0.19^{b}	0.17^{c}	0.14^{c}	0.14^{c}

[1] Data is the average from all treatments. ^{a,b,c,d} Means in the same row with different superscripts differ ($P < .05$).

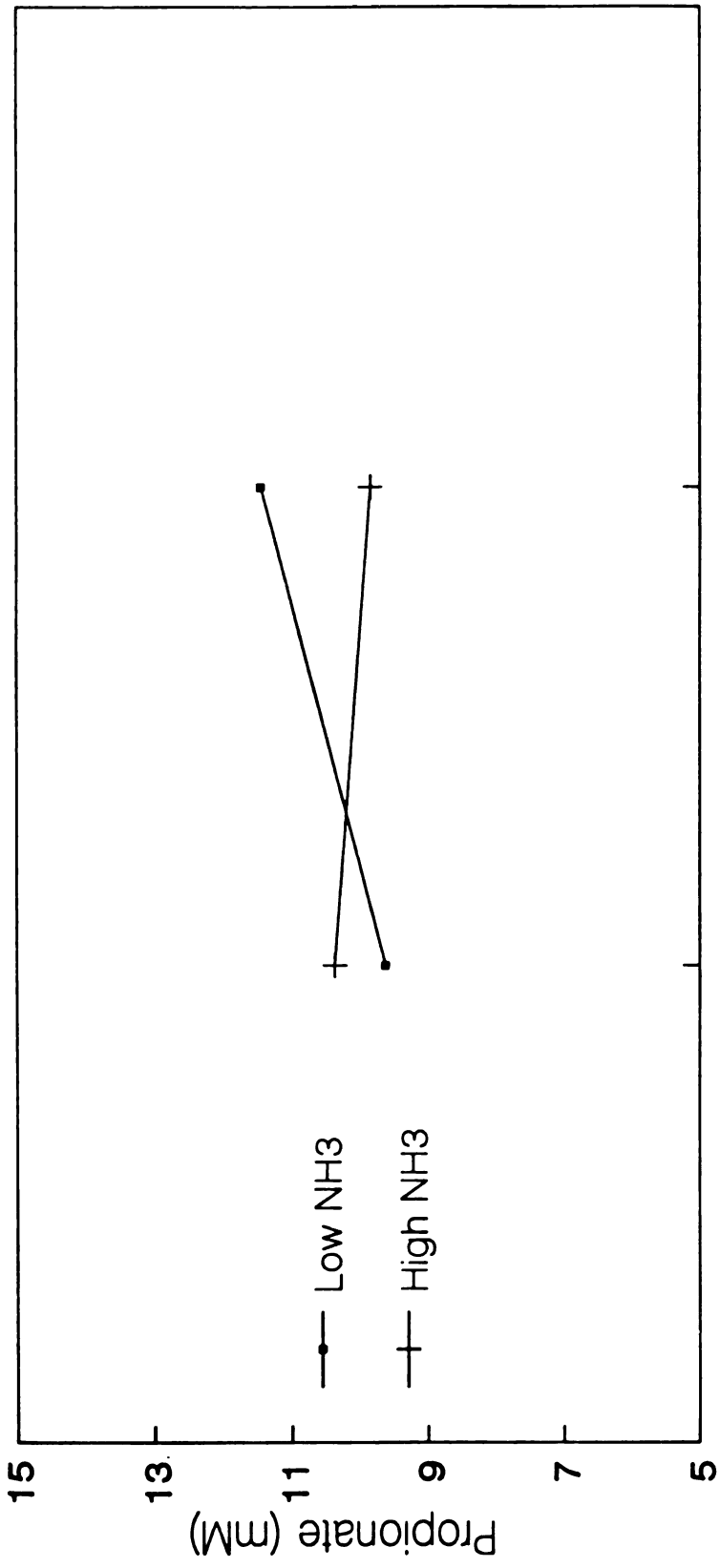

Figure 12. The effect of the interaction of isoacids and NH3 on propionate concentration after a 48 h in vitro ruminal fermentation of tropical forages.

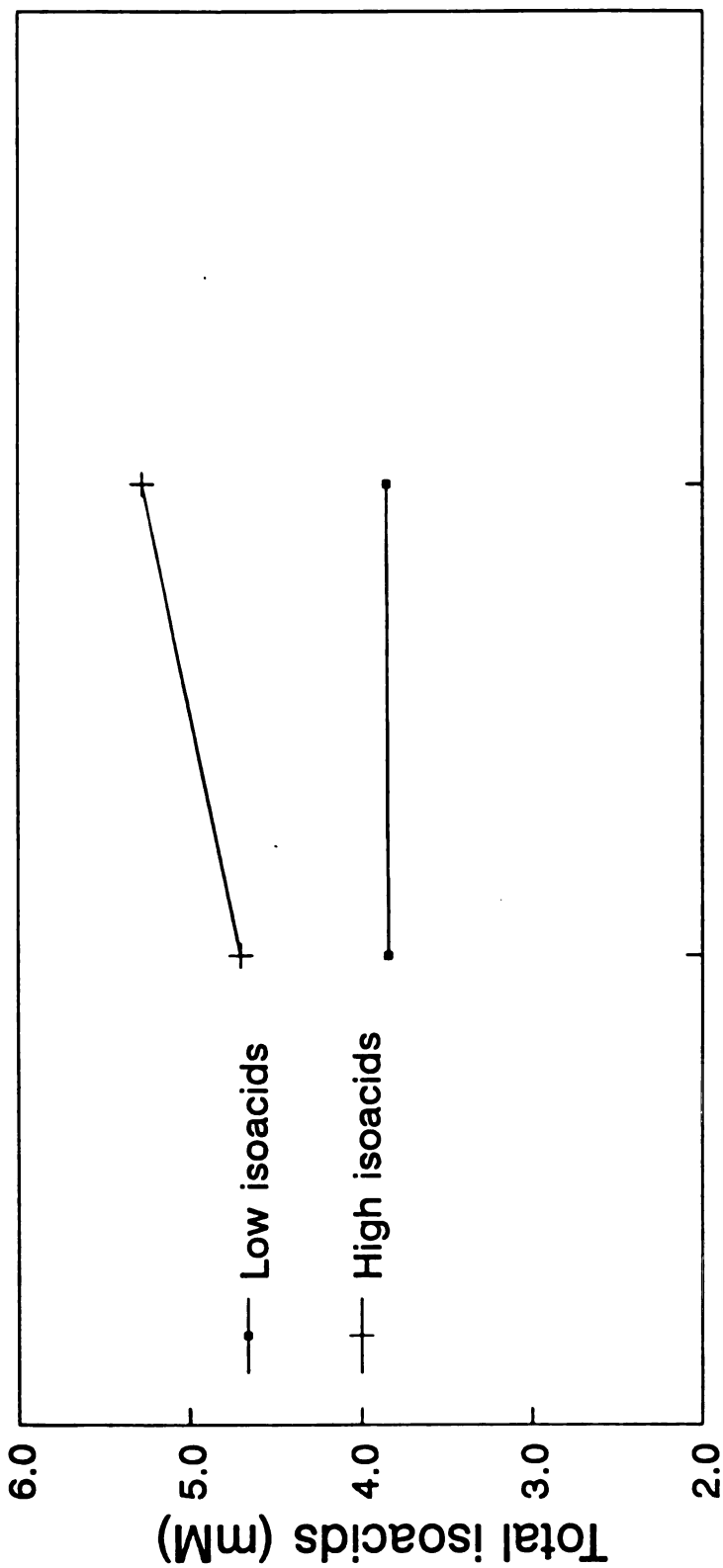

Figure 13. Effect of sulfur on the concentration of total isoacids after a 48 h in vitro ruminal fermentation of tropical forages.

Ammonia and sulfide concentrations after a 48 h *in vitro* rumen fermentation of tropical forages are presented in Table 8. Significant differences ($P < .05$) in NH_3 concentration were observed between species. Values ranged from 17.30 to 26.94 mg NH_3/dl for *Gliricidia* and *Cynodon*, respectively. The use of Trypticase in the incubation media explains the high levels of NH_3 found after the 48 h incubation. Sulfur concentrations ranged from 0.14 to 0.22 mg H_2S/dl for *Pennisetum* and *Panicum* and *Brachiaria*, respectively. However, these sulfide levels were at the limits of detection and are not considered significant.

The effects of NH_3, S and isoacids on the *in vitro* rumen fermentation of *Brachiaria decumbens* are summarized in Table 9. It was observed that IVTD (%) ranged from 53.50 (LHH) to 62.10 (LLH), although values were not statistically different. However, it was noticed that high levels of S (LHL) tended to decrease IVTD and the addition of isoacids (LHH) further decreased digestibility. The addition of high levels of NH_3 (HHH) tended to restore digestibility. A similar trend was observed for all other species. However, Quispe et al. (1991) found a positive effect of isoacids and S on acetate production when sheep were fed pineapple tops. The treatments for *Brachiaria* were not significantly different from each other for all the parameters studied. Lignin digestibility values were negative suggesting this was probably due to precipitation by phenolic compounds.

Table 9. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Brachiaria decumbens*.

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	37.05	38.93	30.49	26.99	37.80	37.64	36.46	31.69
LIGD (%)	-8.91	-7.05	-10.08	-19.10	-7.60	-4.28	-4.03	10.67
HEMD (%)	44.53	44.93	35.79	31.37	41.78	46.05	41.47	36.53
CELD (%)	43.36	45.23	36.04	33.31	44.03	43.39	42.01	37.50
CWD (%)	40.62	41.79	33.02	29.08	39.70	41.66	38.85	34.00
IVTD (%)	61.39	62.10	56.19	53.50	60.15	61.72	59.94	56.94
NH_3 (mg/dl)	14.86	22.02	26.99	29.85	24.44	25.14	27.98	27.21
H_2S (mg/dl)	0.25	0.23	0.22	0.22	0.21	0.19	0.19	0.23
Acetate (mM)	27.20	39.50	46.43	49.59	32.75	42.90	42.82	36.92
Propionate (mM)	6.16	8.90	8.48	8.01	7.67	7.37	10.31	9.57
Isobutyrate (mM)	0.62	1.31	0.94	1.39	0.67	0.72	0.98	1.36
Butyrate (mM)	3.24	4.75	5.25	5.16	3.63	4.22	5.45	5.43
2-methylbutyrate (mM)	0.39	0.82	0.58	0.95	0.47	0.55	0.62	0.95
Isovalerate (mM)	0.64	1.16	0.89	1.40	0.72	0.79	0.83	1.19
Valerate (mM)	1.00	1.67	1.73	1.98	1.15	1.34	1.88	2.14
Total volatile fatty acids (mM)	39.22	58.09	64.28	68.45	47.03	57.86	62.88	57.53
IC4+2MB+IC5+C5 (mM)	2.63	4.95	4.14	5.71	2.99	3.40	4.31	5.62
Acetate/propionate ratio	4.66	4.45	5.59	6.20	3.90	6.19	4.16	3.98

[1] LLL = low NH_3, low S, low (no) isoacid; H is high level of NH_3, S or isoacid.

Table 10. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Cenchrus ciliaris*.

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	28.52	31.86	28.18	31.58	37.76	35.29	32.10	27.85
LIGD (%)	9.35	1.27	5.84	10.08	20.28	10.46	14.25	11.36
HEMD (%)	24.90	30.98	26.29	28.02	35.07	34.32	31.87	32.35
CELD (%)	30.98	35.78	31.04	34.34	40.01	38.47	34.39	29.97
CWD (%)	26.97	31.48	27.37	30.06	36.61	34.87	32.01	29.77
IVTD (%)	50.53	53.83	51.15	52.78	56.83	55.84	53.76	52.50
NH_3 (mg/dl)	23.20^{b}	24.49^{a,b}	22.23^{b}	22.17^{b}	29.10^{a}	27.08^{a,b}	21.92^{b}	23.94^{a,b}
H_2S (mg/dl)	0.19^{a,b}	0.20^{a,b}	0.23^{a,b}	0.29^{a}	0.21^{a,b}	0.16^{a,b}	0.18^{a,b}	0.16^{b}
Acetate (mM)	48.86	47.72	48.44	47.85	49.23	49.85	37.62	41.00
Propionate (mM)	7.75	7.73	7.47	7.62	8.08	8.77	11.02	10.62
Isobutyrate (mM)	0.83^{b}	0.90^{b}	0.84^{b}	1.18^{a,b}	0.86^{b}	1.30^{a}	0.95^{a,b}	1.17^{a,b}
Butyrate (mM)	4.51	4.66	4.47	4.67	4.73	4.73	5.72	4.74
2-methylbutyrate (mM)	0.58^{c}	0.68^{b,c}	0.59^{c}	0.84^{a}	0.63^{c}	0.92^{a}	0.57^{c}	0.80^{a,b}
Isovalerate (mM)	0.83	0.99	0.73	1.12	1.31	1.07	0.75	1.00
Valerate (mM)	1.57^{b,c}	1.65^{b}	1.33^{c}	1.70^{b}	1.77^{a,b}	2.00^{a}	1.00^{d}	1.53^{b,c}
Total volatile fatty acids (mM)	64.91	64.31	63.86	64.97	66.61	68.63	57.61	60.84
IC4+2MB+IC5+C5 (mM)	3.80^{c,d,e}	4.20^{b,c,d,e}	3.48^{d,e}	4.84^{a,b}	4.57^{a,b,c}	5.28^{a}	3.26^{e}	4.49^{a,b,c,d}
Acetate/propionate ratio	6.31	6.18	6.49	6.28	6.10	5.69	3.83	4.44

[1] LLL = low NH_3, low S, low (no) isoacid; H is high level of NH_3, S, and isoacid. ^{a,b,c,d,e} Means in the same row with different superscripts differ ($P < .05$).

Table 11. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Cynodom dactylon*.

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	34.28^{a,b}	34.91^{a,b}	28.57^{b}	27.98^{b}	35.68^{a,b}	38.74^{a}	37.54^{a,b}	29.32^{a,b}
LIGD (%)	5.46	9.64	-0.19	2.21	3.92	11.53	6.28	6.11
HEMD (%)	38.83^{a,b}	38.54^{a,b,c}	33.13^{c,d}	31.84^{d}	41.14^{a}	42.15^{a}	41.90^{a}	34.47^{b,c,d}
CELD (%)	39.94^{a,b,c}	39.87^{a,b,c}	34.22^{b,c}	33.03^{c}	41.92^{a,b,c}	44.09^{a}	43.67^{a,b}	33.88^{c}
CWD (%)	36.58^{a,b,c}	36.74^{a,b,c}	30.87^{c}	29.92^{c}	38.44^{a,b}	40.47^{a}	39.74^{a}	31.92^{b,c}
IVTD (%)	54.40^{a,b,c}	54.51^{a,b,c}	50.80^{c}	50.06^{c}	56.04^{a,b}	57.22^{a}	56.68^{a}	51.14^{b,c}
NH_3 (mg/dl)	23.72^{b}	23.76^{b}	23.44^{b}	23.96^{b}	31.34^{a}	31.77^{a}	29.83^{a}	27.71^{a,b}
H_2S (mg/dl)	0.18	0.21	0.23	0.20	0.16	0.16	0.17	0.20
Acetate (mM)	43.57	41.23	47.75	57.71	53.93	48.49	57.41	59.43
Propionate (mM)	12.49	13.18	8.43	8.18	7.91	12.56	9.02	8.54
Isobutyrate (mM)	0.77^{b}	1.03^{a,b}	0.84^{a,b}	1.36^{a}	0.74^{b}	0.84^{a,b}	0.81^{a,b}	1.28^{a,b}
Butyrate (mM)	5.08	5.23	5.02	5.04	4.72	5.50	5.44	5.24
2-methylbutyrate (mM)	0.64	0.86	0.73	1.06	0.62	0.73	0.69	0.96
Isovalerate (mM)	0.82	1.07	0.73	1.20	0.85	0.93	0.92	1.22
Valerate (mM)	1.77	1.98	1.94	2.20	1.73	1.94	1.94	2.24
Total volatile fatty acids (mM)	65.13	64.58	65.42	76.74	70.49	70.94	76.21	78.87
IC4+2MB+IC5+C5 (mM)	3.99	4.94	4.23	5.81	3.93	4.39	4.36	5.68
Acetate/propionate ratio	4.13	3.88	5.72	7.07	6.88	4.49	6.38	6.97

[1] LLL = low NH_3, low S, low (no) isoacid; H is high level of NH_3, S, and isoacid. ^{a,b,c,d} Means in the same row with different superscripts differ ($P < .05$).

Table 12. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Digitaria decumbens*.

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	37.01	36.74	32.71	37.03	45.42	40.78	30.87	39.41
LIGD (%)	12.40	13.46	16.89	4.84	14.07	15.64	10.89	14.24
HEMD (%)	39.37	39.76	35.80	39.81	45.93	37.31	32.53	40.88
CELD (%)	40.63	40.17	35.04	41.78	50.04	44.49	33.81	43.12
CWD (%)	38.00	38.00	33.96	38.16	45.63	39.38	31.54	40.00
IVTD (%)	56.75	57.01	54.19	57.50	62.71	58.04	52.83	58.39
NH_3 (mg/dl)	15.94	15.99	15.31	13.94	16.77	22.42	20.08	18.56
H_2S (mg/dl)	0.16	0.16	0.17	0.13	0.16	0.15	0.16	0.14
Acetate (mM)	25.29	25.95	36.74	25.32	22.32	53.14	50.39	28.19
Propionate (mM)	12.40	14.97	10.16	15.20	13.50	8.42	7.34	15.92
Isobutyrate (mM)	0.69^{b}	$1.06^{a,b}$	$0.74^{a,b}$	1.34^{a}	$0.80^{a,b}$	$0.84^{a,b}$	$0.89^{a,b}$	1.32^{a}
Butyrate (mM)	5.06	5.23	4.72	5.45	3.64	5.14	4.72	4.96
2-methylbutyrate (mM)	$0.43^{b,c}$	$0.69^{a,b,c}$	0.40^{c}	$0.84^{a,b}$	$0.50^{a,b,c}$	$0.63^{a,b,c}$	$0.50^{a,b,c}$	0.88^{a}
Isovalerate (mM)	$0.58^{a,b}$	0.96^{a}	$0.61^{a,b}$	0.99^{a}	$0.61^{a,b}$	$0.85^{a,b}$	0.50^{b}	$0.90^{a,b}$
Valerate (mM)	1.96	2.35	1.29	2.24	1.50	1.84	1.17	2.21
Total volatile fatty acids (mM)	46.39	51.17	54.64	51.35	42.85	70.85	65.50	54.37
IC4+2MB+IC5+C5 (mM)	3.65	5.04	3.03	5.39	3.40	4.15	3.06	5.30
Acetate/propionate ratio	2.09	1.74	5.09	1.67	1.62	6.31	6.87	1.77

[1] LLL = low NH_3, low S, and low isoacid; H is high NH_3, S, or isoacid. ^{a,b,c} Means in the same row with different superscripts differ ($P < .05$).

The effects of the three factors (NH_3, S and Isoacids) on the *in vitro* rumen fermentation of *Cenchrus ciliaris* are summarized in Table 10. IVTD values ranged from 50.53 (LLL) to 56.83% (HLL) which are close to *in vivo* values reported by Minson and Bray (1986) of 58.7% but different from that (66.7%) reported by Yerena et al. (1978). The treatment HLL showed the highest NH_3 concentration (29.10 mg NH_3/dl) which significantly decreased (21.92 mg NH_3/dl) when S was added (treatment HHL). Results for the *in vitro* rumen fermentation of *Cynodom dactylon* are presented in Table 11. Some of the treatments were statistically significant for IVTD. The highest values were found for treatments with high level of NH_3, 57.22, 56.68, and 56.04% for HLH, HHL, and HLL, respectively. Total VFA and isoacid concentration did not differ among treatments.

For *Digitaria decumbens*, IVTD (%) was not different between treatments (Table 12). Once again the highest IVTD value was found for high NH_3 level on treatment HLL (62.71%) and the lowest (52.83%) when high S was added (HHL). Hunter and Siebert (1986) found an apparent organic matter digestibility of 59% with grazing cattle. It was observed that the decreased IVTD due to S supplementation was corrected with the addition of isoacids as shown for treatment HHH (58.39%). Total VFA was not affected by the treatments. The effects of NH_3, S and isoacids on the *in vitro* rumen fermentation of *Gliricidia sepium* are summarized in Table 13. *In vitro* true digestibility did not differ among treatments and ranged from 41.64% (HLH) to 46.09% (HLL). Acetate production was higher ($P < .05$) for treatment HLH (56.36 mM) than from all other treatments which averaged 23.57 mM.

Table 13. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Gliricidia sepium* (stems).

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	25.21	24.14	22.42	23.26	23.82	18.52	23.75	18.83
LIGD (%)	10.44	7.25	8.44	9.60	8.61	11.19	9.36	5.36
HEMD (%)	21.64	16.60	14.05	25.17	26.14	18.51	223.48	28.95
CELD (%)	29.90	29.50	26.86	27.60	28.64	20.85	28.33	23.10
CWD (%)	24.42	22.47	20.58	23.68	24.33	18.52	23.70	21.06
IVTD (%)	45.74	44.31	43.04	45.34	46.09	41.64	45.54	44.50
NH_3 (mg/dl)	18.63	18.06	17.05	13.34	19.24	12.50	20.43	19.16
H_2S (mg/dl)	0.20	0.22	0.22	0.20	0.14	0.17	0.21	0.19
Acetate (mM)	24.66[b]	25.22[b]	21.92[b]	19.47[b]	24.77[b]	56.13[a]	24.32[b]	24.78[b]
Propionate (mM)	8.76	11.93	10.79	8.48	11.25	7.29	12.19	14.95
Isobutyrate (mM)	0.71	1.31	1.70	1.49	1.66	1.14	1.44	2.37
Butyrate (mM)	6.21	5.46	5.46	5.24	3.59	5.04	4.84	4.28
2-methylbutyrate (mM)	0.51	0.79	1.62	0.81	0.83	0.79	0.72	1.31
Isovalerate (mM)	0.76	1.09	1.13	1.11	0.91	0.95	0.95	1.28
Valerate (mM)	2.00	2.45	2.39	2.18	2.15	1.94	2.48	2.69
Total volatile fatty acids (mM)	47.87[a,b]	47.86[a,b]	41.09[b]	39.88[b]	44.02[a,b]	77.17[a]	47.28[a,b]	47.38[a,b]
IC4+2MB+IC5+C5 (mM)	3.98	5.61	6.83	5.58	5.54	4.81	5.59	7.65
Acetate/propionate ratio	1.65	2.32	3.04	1.91	2.92	5.34	2.31	2.83

[1] LLL = low NH_3, low S, and low isoacid; H is high NH_3, S, or isoacid. [a,b] Means in the same row with different superscripts differ ($P < .05$).

Table 14. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Leucaena leucocephala* (stems).

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	7.79^{a,b}	10.46^{a}	-1.49^{a,b}	-1.48^{a,b}	2.59^{a,b}	2.14^{a,b}	-0.85^{a,b}	-2.5^{b}
LIGD (%)	7.83	9.27	9.63	9.56	9.77	6.56	7.77	7.05
HEMD (%)	18.70	17.72	11.43	10.51	8.97	13.23	9.09	8.02
CELD (%)	7.77	11.04	-6.87	-6.83	-0.88	0.01	-5.01	-7.12
CWD (%)	10.53^{a,b}	12.29^{a}	1.76^{b}	1.54^{b}	4.19^{a,b}	4.94^{a,b}	1.65^{b}	0.15^{b}
IVTD (%)	33.71^{a,b}	34.82^{a}	27.11^{a,b}	26.88^{a,b}	28.93^{a,b}	29.35^{a,b}	26.87^{a,b}	25.76^{b}
NH_3 (mg/dl)	12.67^{a,d}	17.58^{a}	11.77^{d}	11.75^{d}	14.64^{b,c}	15.94^{a,b}	15.66^{a,b}	15.76^{a,b}
H_2S (mg/dl)	0.19^{a}	0.19^{a,b}	0.19^{a}	0.16^{a,b}	0.13^{a,b}	0.16^{a,b}	0.19^{a}	0.12^{b}
Acetate (mM)	36.32	29.91	21.58	30.69	30.27	19.24	33.74	33.50
Propionate (mM)	5.79	11.09	5.40	8.61	8.74	12.13	5.92	5.59
Isobutyrate (mM)	0.84^{b,c}	0.83^{b,c}	0.84^{b,c}	1.37^{a}	0.75^{c}	1.04^{a,b,c}	0.87^{b,c}	1.12^{a,b}
Butyrate (mM)	4.00^{a,b}	4.26^{a}	3.30^{a,b}	3.21^{a,b}	2.94^{a,b}	2.95^{a,b}	3.09^{a,b}	2.47^{b}
2-methylbutyrate (mM)	0.52^{a,b}	0.60^{a,b}	0.43^{b}	0.84^{a}	0.45^{b}	0.66^{a,b}	0.50^{a,b}	0.67^{a,b}
Isovalerate (mM)	0.69^{b}	0.88^{a,b}	0.67^{b}	1.13^{a}	0.60^{b}	0.87^{a,b}	0.58^{b}	0.79^{a,b}
Valerate (mM)	1.76	1.53	0.94	1.30	1.03	1.36	0.73	0.88
Total volatile fatty acids (mM)	49.85	49.08	33.14	47.12	44.75	38.22	45.40	45.00
IC4+2MB+IC5+C5 (mM)	3.79^{a,b}	3.83^{a,b}	2.87^{b}	4.63^{a}	2.82^{b}	3.92^{a,b}	2.68^{b}	3.46^{a,b}
Acetate/propionate ratio	6.43	3.28	4.43	5.42	4.99	1.77	6.33	8.24

[1] LLL = low NH_3, low S, and low isoacid; H is high NH_3, S, or isoacid. ^{a,b,c,d} Means in the same row with different superscripts differ ($P < .05$).

Table 15. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Panicum maximun*.

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	43.00^{a,b}	41.31^{a,b,c}	36.75^{c,d}	34.09^{d}	39.12^{a,b,c,d}	44.19^{a}	35.29^{d}	38.46^{b,c,d}
LIGD (%)	11.76	13.65	11.38	10.47	14.16	20.25	13.06	16.50
HEMD (%)	46.20^{a,b,c}	45.48^{a,b,c}	39.41^{d}	44.44^{b,c}	49.01^{a}	47.77^{a,b}	45.33^{a,b,c}	43.02^{c,d}
CELD (%)	48.28^{a}	45.99^{a,b}	41.04^{b,c,d}	38.09^{d}	43.34^{a,b,c}	48.24^{a}	39.05^{c,d}	42.18^{b,c,d}
CWD (%)	44.43^{a}	43.17^{a,b}	37.93^{c}	38.70^{c}	43.52^{a,b}	45.78^{a}	39.76^{b,c}	40.49^{b,c}
IVTD (%)	66.54^{a}	65.86^{a,b}	62.61^{c}	62.98^{c}	65.95^{a,b}	67.35^{a}	63.70^{b,c}	64.12^{b,c}
NH_3 (mg/dl)	21.13	21.35	20.38	20.98	21.25	25.12	22.87	25.61
H_2S (mg/dl)	0.18^{a}	0.13^{a,b}	0.12^{b}	0.14^{a,b}	0.13^{a,b}	0.13^{a,b}	0.16^{a,b}	0.16^{a,b}
Acetate (mM)	39.84	44.56	29.81	29.34	25.85	16.13	24.74	30.61
Propionate (mM)	7.61	12.66	15.66	15.66	14.56	10.76	15.14	7.01
Isobutyrate (mM)	0.90^{a,b}	1.38^{a}	0.94^{a,b}	1.35^{a}	0.69^{b}	1.18^{a,b}	0.86^{a,b}	1.33^{a}
Butyrate (mM)	4.89	5.16	4.96	4.77	3.90	5.13	4.40	5.00
2-methylbutyrate (mM)	0.60^{a,b,c}	0.93^{a,b,c}	0.58^{b,c}	0.97^{a,b,c}	0.52^{c}	1.01^{a,b}	0.57^{b,c}	1.06^{a}
Isovalerate (mM)	0.87	1.27	0.93	1.27	0.71	1.25	0.78	1.29
Valerate (mM)	1.39^{a,b}	1.71^{a,b}	1.59^{a,b}	1.88^{a,b}	1.31^{b}	1.89^{a,b}	1.64^{a,b}	2.04^{a}
Total volatile fatty acids (mM)	56.09	67.65	54.44	55.21	47.51	37.34	48.70	48.33
IC4+2MB+IC5+C5 (mM)	3.75^{b,c}	5.28^{a,b}	4.02^{a,b,c}	5.45^{a,b}	3.22^{c}	5.33^{a,b}	3.84^{a,b,c}	5.72^{a}
Acetate/propionate ratio	5.24	4.43	1.91	1.87	1.77	3.40	1.62	3.86

[1] LLL = low NH_3, low S, and low isoacid; H is high NH_3, S, or isoacid. ^{a,b,c,d} Means in the same row with different superscripts differ ($P < .05$).

Table 16. Effects of ammonia, sulfur and isoacids on the *in vitro* rumen fermentation of *Pennisetum purpureum*.

	Treatments[1]							
	LLL	LLH	LHL	LHH	HLL	HLH	HHL	HHH
ADFD (%)	59.38	61.35	60.47	61.15	60.98	60.82	62.68	61.69
LIGD (%)	40.49	30.16	27.71	29.91	34.58	37.38	37.40	34.75
HEMD (%)	61.94	61.30	63.22	63.40	62.94	63.50	65.27	64.77
CELD (%)	61.46	64.78	64.07	64.58	63.88	63.40	65.46	64.66
CWD (%)	60.53	61.33	61.70	62.16	61.85	62.02	63.84	63.07
IVTD (%)	62.90	64.20	64.43	64.79	64.37	64.55	66.24	65.46
NH_3 (mg/dl)	21.10^{a,b}	21.10^{a,b}	18.06^{b}	20.37^{a,b}	23.99^{a,b}	25.87^{a}	20.35^{a,b}	20.98^{a,b}
H_2S (mg/dl)	0.16	0.17	0.17	0.12	0.13	0.12	0.13	0.14
Acetate (mM)	25.65	20.49	22.81	22.76	24.00	38.94	24.26	36.84
Propionate (mM)	13.23	13.01	10.72	14.94	14.34	12.26	12.00	8.46
Isobutyrate (mM)	0.98	0.94	0.85	1.15	0.85	1.29	0.78	1.30
Butyrate (mM)	4.92	3.67	5.06	4.52	4.77	5.11	4.95	5.00
2-methylbutyrate (mM)	1.07	0.90	0.63	0.81	1.18	1.31	0.51	0.87
Isovalerate (mM)	0.93	0.89	0.77	1.09	0.81	1.22	0.61	1.03
Valerate (mM)	1.84	1.51	1.16	1.52	1.78	2.08	0.69	1.26
Total volatile fatty acids (mM)	48.59	41.39	41.97	46.78	47.71	62.18	43.78	54.76
IC4+2MB+IC5+C5 (mM)	4.81	4.24	3.40	4.57	4.61	5.90	2.59	4.46
Acetate/propionate ratio	1.99	1.54	2.16	1.53	1.84	4.08	2.19	4.78

[1] LLL = low NH_3, low S, and low isoacid; H is high level of NH_3, S, or isoacid. [a,b] Means in the same row with different superscripts differ ($P < .05$).

For *Leucaena leucocephala*, IVTD values were lower than all other species and differed slightly between treatments. Values obtained ranged from 25.76% (HHH) to 34.82% (LLH). Vadiveloo (1989) and Yerena et al. (1978) found dry matter digestibility values of 58.5 and 59.7% when Leucaena leaves were fed to goats and lambs, respectively. Treatment with addition of high S and isoacids (LHH) showed the highest isoacid concentration which was 4.63 mM (Table 14). IVTD values of *Panicum maximum* were found to differ ($P < .05$) with treatments HLH and LHL showing the highest (67.35%) and lowest (62.61%) values, respectively. However, IVTD (%) value obtained for this species was the highest (64.89%) of all species studied. Treatment with high NH_3, S and isoacids (HHH) gave the highest value for total isoacid concentration (5.72 mM). However, when S and isoacids were low (HLL), it decreased to 3.22 mM (Table 15). Results for *Pennisetum purpureum* showed no significant differences among treatments for any of the parameters studied (Table 16). A value of 64.61% was found for IVTD.

Apparently, S exerted a toxic effect on the digestibility of *Panicum maximum* (Table 15). This differs from previous studies with herbage diets where increased digestion of organic matter or dry matter and ADF (*in vivo*) were found as a result of the addition of S to low S herbage (Weston et al., 1988; Rees et al., 1974; Guardiola et al., 1983; Bray and Hemsley, 1969; Kennedy and Siebert, 1972). On the other hand, Kennedy (1974) reported that digestion of tropical spear grass was not limited by S intake although VFA concentration was increased by sulphate supplementation accompanied by a small decrease in propionic and isovaleric acids.

DISCUSSION

There are several interesting results from this study. First, IVTD was highly variable among species. By increasing NH_3, IVTD was increased for all grasses. This suggests that NH_3 may be limiting in the early stages of fermentation. At the end of the 48 h fermentation NH_3 levels were about the same for all treatments within a grass species. This may be due to the fermentation of trypticase which would result in the levels of ammonia observed. Isoacids are produced from trypticase. However, when the experimental treatments included high levels of isoacids, the levels of total and individual isoacids were about doubled compared to low isoacids treatments at 48 h for most of the forage species. This shows that an increase in isoacid levels was accomplished by adding isoacids to the fermentation media.

The high NH_3 level accompanied by high isoacids decreased IVTD. However, when the high level of isoacid was added to treatments with low levels of NH_3, IVTD was increased. It may be that the first limiting factor in the fermentation was NH_3 with isoacids a second limiting factor because adding isoacids to low NH_3 treatments increased digestibility. But adding isoacids when requirements for NH_3 were met decreased IVTD. It is recognized that the explanation of treatment effects on IVTD is confounded by the addition of trypticase to the media. Since high isoacids along with low levels of NH_3

increased IVTD, it may be that these effects occurred early in the incubation before trypticase was extensively fermented. Cline et al. (1966) found that cellulose and dry matter digestibility was improved by the addition of isoacids in lambs fed a purified diet containing 39% cellulose and urea. Ruminal NH_3 levels were lower than in the present study. Similar results were obtained *in vitro* by Cummins and Papas (1985). However, Hefner et al. (1985) observed that when urea and isoacids were both supplemented the tendency was to decrease the extent of digestion concluding that isoacid deficiency is not the first limiting factor affecting digestion in ruminants.

Variation in IVTD among all forages was basically due to differences in the fiber fractions. Grasses averaged 41.12% NDF, 23.04% ADF and 4.28% lignin while values for legumes were 63.87%, 49.47% and 14.62% for NDF, ADF and lignin, respectively. This explains the large differences in digestibility between legumes and grasses. Within grasses *Panicum* (35.5% NDF, 20.62% ADF) showed the highest digestibility value (64.89%) and *Cenchrus* (45.37% NDF, 25.87% ADF) had the lowest digestibility (53.40%).

Concerning the digestibility of the different fiber fractions, it was observed that *Pennisetum* (Table 16) had the highest values for ADFD, CWD, HEMD and CELD. Digestibility of those fractions were similar within all other grasses (Table 9, 11, 12, 15). However, ADFD, CWD, HEMD and CELD values for *Cenchrus* were slightly lower (Table 10). It was clear that digestibility of hemicellulose was closely related to that of cellulose for all forages and negatively correlated with lignin. The legumes, *Gliricidia* and *Leucaena* were very high in lignin and, therefore, digestibility of cellulose

and hemicellulose was less than the grasses. There was no significant difference between treatments in the digestibility of the fiber fractions of *Brachiaria, Cenchrus, Digitaria, Gliricidia* and *Pennisetum*. However, there were slight differences between treatments on fiber fractions for *Cynodon, Leucaena* and *Panicum*.

Volatile fatty acid accumulation varied among species perhaps due to differences in fiber content. When acetate concentration was lower, propionate was higher as expected. *In vitro* true digestibility of forages was more positively correlated with propionate than with acetate for *Digitaria, Gliricidia, Leucaena, Panicum* and *Pennisetum*. However, the opposite trend was observed for *Brachiaria, Cenchrus* and *Cynodon*. For *Brachiaria, Cenchrus* and *Panicum* high levels of NH_3 decreased acetate concentration but increased IVTD. However, for *Cynodon, Digitaria, Gliricidia* and *Pennisetum* the increase in NH_3 accounted for an increase in propionate concentration which increased IVTD. The levels of VFA in the 10 ml of rumen fluid used to inoculate the media were low (e.g. 40 mM acetate) and were not subtracted from the 48 h VFA values because they would not affect treatment differences.

Isoacid addition increased total VFA accumulation and for some species increased acetate concentration (*Brachiaria, Digitaria* and *Gliricidia*). Treatments with high levels of NH_3 and isoacids decreased propionate concentration. This decrease in propionate could be a possible explanation for the decrease in IVTD discussed above. NH_3 concentration differed between species probably due to differences in available protein.

Of major interest was the S inhibition of IVTD. This observation was unexpected. High levels of S were found to decrease IVTD. When high S and isoacids

were combined, IVTD further decreased. However, adding high NH_3 to a system high in S and isoacids partially prevented the inhibitory effect of S and isoacids. Bird (1972b) reported that sulfate supplementation without urea decreased N balance and depressed digestion of feed in ruminants. As discussed above, adding NH_3 to the system increased digestibility. This may explain why NH_3 tends to alleviate the inhibitory effect of S and isoacids. However, this does not explain why there is a negative synergistic effect between S and isoacids on IVTD. Since the trend was for S to increase total isoacid levels at 48 h, it may be that S stimulated trypticase fermentation at the expense of fiber digestion.

Kennedy (1974) found that S supplementation inhibited propionate fermentation in his experiment which could explain a decrease in digestibility and Morrison et al. (1990) observed that S increased acetate but lowered propionate. However, in our study there is no evidence that S affected propionate concentration.

For *Cenchrus*, treatments with low S increased NH_3 concentration but treatments with high S decreased digestibility and affected NH_3 levels. The highest digestibility for *Digitaria* was found when the S level was low. The same pattern was found for most species with the exception of *Pennisetum*. This is evidence that S inhibited the fermentation. Another possible explanation is that S level and reducing potential may have been confounded. Sulfide acts as a reducing agent. Treatments with high levels of S had three times more sulfide added than did low S treatments. There may have been significant differences in reducing potential early in the incubation period that affected both trypticase fermentation and IVTD.

SUMMARY

- There were three two-way interactions, species x NH_3, species x S and isoacids x NH_3 which affected IVTD. High NH_3 increased IVTD for all forages except the legumes.

- High levels of S tended to decrease IVTD for all forages, except *Pennisetum*.

- The addition of isoacids (15 mg/dl) to low NH_3 increased digestibility, but decreased IVTD when NH_3 was high. Also, addition of isoacids increased total VFA accumulation.

CONCLUSION

In the 48 h *in vitro* ruminal fermentation end point assay using the standard trypticase addition to the media, it is concluded that to prevent inhibition of IVTD, S levels must be less than 6 mg/dl. Also, added NH_3 levels of 10 mg/dl improved digestibility. IVTD can also be increased by adding isoacids at 15 mg/dl along with NH_3 at 5 mg/dl.

APPENDIX

Table 17. Analysis of variance of the effects of ammonia, sulfur and isoacids on isoacid concentration after a 48 h *in vitro* rumen fermentation of eight tropical forages.

	Total isoacids			Isobutyrate			Isovalerate		
Source	df	MS	P	df	MS	P	df	MS	P
Species (SP)	7	6.24	.001	7	0.49	.001	7	0.19	.001
Isoacids (I)	1	41.55	.001	1	3.36	.001	1	2.66	.001
Nitrogen (N)	1	0.14	.627	1	0.02	.512	1	0.04	.253
Sulfur (S)	1	2.67	.039	1	1.41	.001	1	0.07	.109
SP x I	7	0.78	.260	7	0.03	.729	7	0.04	.187
SP x N	7	0.43	.654	7	0.10	.034	7	0.03	.378
SP x S	7	3.23	.001	7	0.12	.014	7	0.07	.021
I x N	1	0.04	.799	1	0.04	.760	1	0.02	.367
I x S	1	2.47	.046	1	0.26	.019	1	0.13	.034
N x S	1	0.09	.693	1	0.02	.851	1	0.04	.228
I x N x S	1	0.59	.325	1	0.11	.128	1	0.02	.464
SP x I x N	7	0.51	.550	7	0.05	.393	7	0.04	.196
SP x I x S	7	0.29	.844	7	0.02	.836	7	0.02	.726
SP x N x S	7	0.53	.524	7	0.03	.771	7	0.02	.523
SP x I x N x S	7	1.38	.036	7	0.20	.004	7	0.03	.447
Error	64	0.60		64	0.04		64	0.03	
Grasses vs legumes	1	1.39	.132	1	1.12	.001	1	0.03	.293

"Table 17 (cont'd)."

	2-M-Butyrate			Valerate		
Source	df	MS	P	df	MS	P
Species (SP)	7	0.26	.001	7	1.74	.001
Isoacids (I)	1	1.65	.001	1	2.86	.001
Nitrogen (N)	1	0.03	.001	1	0.15	.221
Sulfur (S)	1	0.13	.751	1	0.04	.544
SP x I	7	0.06	.026	7	0.14	.207
SP x N	7	0.02	.021	7	0.11	.329
SP x S	7	0.21	.750	7	0.78	.001
I x N	1	0.04	.001	1	0.05	.486
I x S	1	0.07	.221	1	0.20	.152
N x S	1	0.06	.106	1	0.01	.793
I x N x S	1	0.06	.143	1	0.01	.806
SP x I x N	7	0.06	.119	7	0.04	.889
SP x I x S	7	0.02	.039	7	0.09	.515
SP x N x S	7	0.03	.475	7	0.16	.135
SP x I x N x S	7	0.11	.304	7	0.08	.571
Error	64		.006	64	0.10	
Grasses vs legumes	1	0.03	.736	1	0.06	.427

Table 18. Comparison of the effects of low levels (LLL) of ammonia (5 mg NH_3/dl), sulfur (2 mg H_2S/dl) and isoacids (0 mg/dl) on *in vitro* rumen fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	37.05	28.52	34.28	37.01	25.21	7.79	43.00	59.38
LIGD (%)	-8.91	9.35	5.46	12.40	10.44	7.83	11.76	40.49
HEMD (%)	44.53	24.90	38.83	39.37	21.64	18.70	46.20	61.94
CELD (%)	43.36	30.98	39.94	40.63	29.90	7.77	48.28	61.46
CWD (%)	40.62	26.97	36.58	38.00	24.42	10.53	44.43	60.53
IVTD (%)	61.39	50.53	54.40	56.75	45.74	33.71	66.54	62.90
NH_3 (mg/dl)	14.86	23.20	23.72	15.94	18.63	12.67	21.13	21.10
H_2S^* (mg/dl)	0.25	0.19	0.18	0.16	0.20	0.19	0.18	0.16
C2 (mM)	27.20	48.86	43.57	25.29	24.66	36.32	39.84	25.65
C3 (mM)	6.16	7.75	12.49	12.40	8.76	5.79	7.61	13.23
IC4 (mM)	0.62	0.83	0.77	0.69	0.71	0.84	0.90	0.98
C4 (mM)	3.24	4.51	5.08	5.06	6.21	4.00	4.89	4.92
2MB (mM)	0.39	0.58	0.64	0.43	0.51	0.52	0.60	1.07
IC5 (mM)	0.64	0.83	0.82	0.58	0.76	0.80	0.87	0.93
C5 (mM)	1.00	1.57	1.77	1.96	2.00	1.76	1.39	1.84

* H_2S values were below limits of detection.

Table 19. Comparison of the effects of different levels (LLH) of ammonia (5 mg NH_3/dl), sulfur (2 mg H_2S/dl) and isoacids (15 mg/dl) on *in vitro rumen* fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	38.93	31.86	34.91	36.74	24.14	10.46	41.31	61.35
LIGD (%)	-7.05	1.27	9.64	13.46	7.25	9.27	13.65	30.16
HEMD (%)	44.93	30.98	38.54	39.76	16.60	17.72	45.48	61.30
CELD (%)	45.23	35.78	39.87	40.17	29.50	11.04	45.99	64.78
CWD (%)	41.79	31.48	36.74	38.00	22.47	12.29	43.17	61.33
IVTD (%)	62.10	53.83	54.51	57.01	44.31	34.82	65.86	64.20
NH_3 (mg/dl)	22.02	24.49	23.76	15.99	18.06	17.58	21.35	21.10
H_2S^* (mg/dl)	0.23	0.20	0.22	0.16	0.21	0.19	0.13	0.17
C2 (mM)	39.50	47.72	41.23	25.95	25.22	29.91	44.56	20.49
C3 (mM)	8.90	7.73	13.18	14.97	11.93	11.09	12.66	13.01
IC4 (mM)	1.31	0.90	1.03	1.06	1.31	0.83	1.38	0.94
C4 (mM)	4.75	4.66	5.23	5.23	5.46	4.26	5.16	3.67
2MB (mM)	0.82	0.68	0.86	0.69	0.79	0.60	0.93	0.90
IC5 (mM)	1.16	0.99	1.07	0.96	1.09	0.58	1.27	0.89
C5 (mM)	1.67	1.65	1.98	2.35	2.45	1.53	1.71	1.51

* H_2S values were below limits of detection.

Table 20. Comparison of the effects of different levels (LHL) of ammonia (5 mg NH_3/dl), sulfur (6 mg H_2S/dl) and isoacids (0 mg/dl) on *in vitro* rumen fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	30.49	28.18	28.57	32.71	22.42	-1.49	36.75	60.47
LIGD (%)	-10.08	5.84	-0.19	16.89	8.44	9.63	11.38	27.71
HEMD (%)	35.79	26.29	33.13	35.80	14.05	11.43	39.41	63.22
CELD (%)	36.04	31.04	34.22	35.04	26.86	-6.87	41.04	64.07
CWD (%)	33.02	27.37	30.87	33.96	20.58	1.76	37.93	61.70
IVTD (%)	56.19	51.15	50.80	54.19	43.04	27.11	62.61	64.43
NH_3 (mg/dl)	26.99	22.23	23.44	15.31	17.05	11.77	20.38	18.06
H_2S^* (mg/dl)	0.22	0.23	0.24	0.17	0.22	0.19	0.12	0.17
C2 (mM)	46.43	48.44	47.75	36.74	21.92	21.58	29.81	22.81
C3 (mM)	8.48	7.47	8.43	10.16	10.79	5.40	15.66	10.72
IC4 (mM)	0.94	0.84	0.84	0.74	1.70	0.84	0.94	0.85
C4 (mM)	5.25	4.47	5.02	4.72	5.46	3.30	4.96	5.06
2MB (mM)	0.58	0.59	0.73	0.40	1.62	0.43	0.58	0.63
IC5 (mM)	0.89	0.73	0.73	0.61	1.13	0.87	0.93	0.77
C5 (myMol/l)	1.73	1.33	1.94	1.29	2.39	0.94	1.59	1.16

* H_2S values were below limits of detection.

Table 21. Comparison of the effects of different levels (LHH) of ammonia (5 mg NH_3/dl), sulfur (6 mg H_2S/dl) and isoacids (15 mg/dl) on *in vitro rumen* fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	26.99	31.58	27.98	37.03	23.26	-1.48	34.09	61.15
LIGD (%)	-19.10	10.08	2.21	4.84	9.60	9.56	10.47	29.91
HEMD (%)	31.37	28.02	31.84	39.81	25.17	10.51	44.44	63.40
CELD (%)	33.31	34.34	33.03	41.78	27.60	-6.83	38.09	64.58
CWD (%)	29.08	30.06	29.92	38.16	23.68	1.54	38.70	62.16
IVTD (%)	53.50	52.78	50.06	57.50	45.34	26.88	62.98	64.79
NH_3 (mg/dl)	29.85	22.17	23.96	13.94	13.34	11.75	20.98	20.37
H_2S^* (mg/dl)	0.22	0.29	0.20	0.13	0.20	0.16	0.14	0.12
C2 (mM)	49.59	47.85	57.71	25.32	19.47	30.69	29.34	22.76
C3 (mM)	8.01	7.62	8.18	15.20	8.48	8.61	15.66	14.94
IC4 (mM)	1.39	1.18	1.36	1.34	1.49	1.37	1.35	1.15
C4 (mM)	5.16	4.67	5.04	5.45	5.24	3.21	4.77	4.52
2MB (mM)	0.95	0.84	1.06	0.84	0.81	0.84	0.97	0.81
IC5 (mM)	1.40	1.12	1.20	0.99	1.11	0.60	1.27	1.09
C5 (mM)	1.98	1.70	2.20	2.24	2.18	1.30	1.88	1.52

* H_2S values were below limits of detection.

Table 22. Comparison of the effects of different levels (HLL) of ammonia (10 mg NH_3/dl), sulfur (2 mg H_2S/dl) and isoacids (0 mg/dl) on *in vitro* rumen fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	37.80	37.76	35.68	45.42	23.82	2.59	39.12	60.98
LIGD (%)	-7.60	20.28	3.92	14.07	8.61	9.77	14.16	34.58
HEMD (%)	41.78	35.07	41.14	45.93	26.14	8.97	49.01	62.94
CELD (%)	44.03	40.01	41.92	50.04	28.64	-0.88	43.34	63.88
CWD (%)	39.70	36.61	38.44	45.63	24.33	4.19	43.52	61.85
IVTD (%)	60.15	56.83	56.04	62.71	46.09	28.93	65.95	64.37
NH_3 (mg/dl)	24.44	29.10	31.34	16.77	19.26	14.64	21.25	23.99
H_2S^* (mg/dl)	0.21	0.21	0.16	0.16	0.14	0.13	0.13	0.13
C2 (mM)	32.75	49.23	53.93	22.32	24.77	30.27	25.85	24.00
C3 (mM)	7.67	8.08	7.91	13.50	11.25	8.74	14.56	14.34
IC4 (mM)	0.67	0.86	0.74	0.80	1.66	0.75	0.69	0.85
C4 (mM)	3.63	4.73	4.72	3.64	3.59	2.94	3.90	4.77
2MB (mM)	0.47	0.63	0.62	0.50	0.83	0.45	0.52	1.18
IC5 (mM)	0.72	1.31	0.85	0.61	0.91	1.13	0.71	0.81
C5 (mM)	1.15	1.77	1.73	1.50	2.15	1.03	1.31	1.78

* H_2S values were below limits of detection.

Table 23. Comparison of the effects of different levels (HLH) of ammonia (10 mg NH_3/dl), sulfur (2 mg H_2S/dl) and isoacids (15 mg/dl) on *in vitro rumen* fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	37.64	35.29	38.74	40.78	18.52	2.14	44.19	60.82
LIGD (%)	-4.28	10.46	11.53	15.64	11.19	6.56	20.25	37.38
HEMD (%)	46.05	34.32	42.15	37.31	18.51	13.23	47.77	63.50
CELD (%)	43.39	38.47	44.09	44.49	20.85	0.01	48.24	63.40
CWD (%)	41.66	34.87	40.47	39.38	18.52	4.94	45.78	62.02
IVTD (%)	61.72	55.84	57.22	58.04	41.64	29.35	67.35	64.55
NH_3 (mg/dl)	25.14	27.08	31.77	22.42	12.50	15.94	25.12	25.87
H_2S^* (mg/dl)	0.19	0.17	0.16	0.15	0.17	0.16	0.13	0.12
C2 (mM)	42.90	49.85	48.49	53.14	56.13	19.24	16.13	38.94
C3 (mM)	7.37	8.77	12.56	8.42	7.29	12.13	10.76	12.26
IC4 (mM)	0.72	1.30	0.84	0.84	1.14	1.04	1.18	1.29
C4 (mM)	4.22	4.73	5.50	5.14	5.04	2.95	5.13	5.11
2MB (mM)	0.55	0.92	0.73	0.63	0.79	0.66	1.01	1.31
IC5 (mM)	0.79	1.07	0.93	0.85	0.95	0.67	1.25	1.22
C5 (mM)	1.34	2.00	1.94	1.84	1.94	1.36	1.89	2.08

* H_2S values were below limits of detection.

Table 24. Comparison of the effects of different levels (HHL) of ammonia (10 mg NH_3/dl), sulfur (6 mg H_2S/dl) and isoacids (0 mg/dl) on *in vitro rumen* fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	36.46	32.10	37.54	30.87	23.75	-0.85	35.29	62.68
LIGD (%)	-4.03	14.25	6.28	10.89	9.36	7.77	13.06	37.40
HEMD (%)	41.47	31.87	41.90	32.53	23.48	9.09	45.33	65.27
CELD (%)	42.01	34.39	43.67	33.81	28.33	-5.01	39.05	65.46
CWD (%)	38.85	32.01	39.74	31.54	23.70	1.65	39.76	63.84
IVTD (%)	59.94	53.76	56.68	52.83	45.54	26.87	63.70	66.24
NH_3 (mg/dl)	27.98	21.92	29.83	20.08	20.43	15.66	22.87	20.35
H_2S^* (mg/dl)	0.19	0.18	0.17	0.16	0.21	0.19	0.16	0.13
C2 (mM)	42.82	37.62	57.41	50.39	24.32	33.74	24.74	24.26
C3 (mM)	10.31	11.02	9.02	7.34	12.19	5.92	15.14	12.00
IC4 (mM)	0.98	0.95	0.81	0.89	1.44	0.87	0.86	0.78
C4 (mM)	5.45	5.72	5.44	4.72	4.84	3.09	4.40	4.95
2MB (mM)	0.62	0.57	0.69	0.50	0.72	0.50	0.57	0.51
IC5 (mM)	0.83	0.75	0.92	0.50	0.95	0.88	0.78	0.61
C5 (mM)	1.88	1.00	1.94	1.17	2.48	0.73	1.64	0.69

* H_2S values were below limits of detection.

Table 25. Comparison of the effects of different levels (HHH) of ammonia (10 mg NH_3/dl), sulfur (6 mg H_2S/dl) and isoacids (15 mg/dl) on *in vitro rumen* fermentation of tropical forages at 48 h.

	Species							
	Brachiaria decumbens	*Cenchrus ciliaris*	*Cynodon dactylon*	*Digitaria decumbens*	*Gliricidia sepium*	*Leucaena leucocephala*	*Panicum maximum*	*Pennisetum purpureum*
ADFD (%)	31.69	27.85	29.32	39.41	18.83	-2.5	38.46	61.69
LIGD (%)	-10.67	11.36	6.11	14.24	5.36	7.05	16.50	34.75
HEMD (%)	36.53	32.35	34.47	40.88	28.95	8.02	43.02	64.77
CELD (%)	37.50	29.97	33.88	43.12	23.10	-7.12	42.18	64.66
CWD (%)	34.00	29.77	31.92	40.00	21.06	0.15	40.49	63.07
IVTD (%)	56.94	52.50	51.14	58.39	44.50	25.76	64.12	65.46
NH_3 (mg/dl)	27.21	23.94	27.71	18.56	19.16	15.76	25.61	20.98
H_2S^* (mg/dl)	0.23	0.16	0.20	0.14	0.19	0.12	0.16	0.14
C2 (mM)	36.92	41.00	59.43	28.19	24.78	33.50	30.61	36.86
C3 (mM)	9.57	10.62	8.54	15.92	14.95	5.59	7.01	8.46
IC4 (mM)	1.36	1.17	1.28	1.32	2.37	1.12	1.33	1.30
C4 (mM)	5.43	4.74	5.24	4.96	4.28	2.47	5.00	5.00
2MB (mM)	0.95	0.80	0.96	0.88	1.31	0.67	1.06	0.87
IC5 (mM)	1.19	1.00	1.22	0.90	1.28	0.69	1.29	1.03
C5 (mM)	2.14	1.53	2.24	2.21	2.69	0.88	2.04	1.26

* H_2S values were below limits of detection.

Table 26. Fiber fraction (%) of tropical forages after a 48 h *in vitro* rumen fermentation.

	NDF (%)	ADF (%)	LIG (%)
Brachiaria decumbens	41.25	22.38	4.25
Cenchrus ciliaris	45.37	25.87	4.00
Cynodon dactylon	45.75	23.50	5.34
Digitaria decumbens	42.87	25.50	4.75
Gliricidia sepium	56.00	43.75	12.25
Leucaena leucocephala	71.75	55.25	17.00
Panicum maximum	35.50	20.62	4.00
Pennisetum purpureum	36.00	20.37	3.37

Table 27. Dry matter and crude protein of tropical forages.

	Dry matter (%)	Crude Protein (%)
Brachiaria decumbens	95.61	6.80
Cenchrus ciliaris	96.62	5.06
Cynodon dactylon	96.32	6.20
Digitaria decumbens	95.64	7.80
Gliricidia sepium	94.28	
Leucaena leucocephala	94.62	6.00
Panicum maximum	96.38	5.28
Pennisetum purpureum	92.73	7.70

Table 28. Hemicellulose and cellulose fractions (%) of tropical forages after a 48 h *in vitro* rumen fermentation.

	Hemicellulose (%)	Cellulose (%)
Brachiaria decumbens	18.28	17.46
Cenchrus ciliaris	19.62	21.99
Cynodon dactylon	22.38	18.01
Digitaria decumbens	17.06	21.14
Gliricidia sepium	12.42	27.34
Leucaena leucocephala	16.63	38.39
Panicum maximum	14.90	16.41
Pennisetum purpureum	15.63	16.98

LIST OF REFERENCES

Allen, M.S., and D.R. Mertens. 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. 118:261.

Allison, M.J. 1969. Biosynthesis of amino acids by ruminal microorganisms. J. Anim. Sci. 29:797.

Allison, M.J., and M.P. Bryant. 1958. Volatile fatty acid growth factors for cellulolytic cocci of bovine rumen. Science 28:474.

Allison, M.J., M.P. Bryant, and R.N. Doetsch. 1962. Studies on the metabolic function of branched-chain volatile fatty acids, growth factors for ruminococci. I. Incorporation of isovalerate into leucine. J. Bacteriol. 83:523.

Allison, M.J., and J.L. Peel. 1971. The biosynthesis of valine from isobutyrate by *Peptostreptococcus elsdenii* and *Bacteroides ruminicola*. Biochem. J. 121:431.

Allison, M.J., I.M. Robinson, and A.L. Baetz. 1974. Tryptophan biosynthesis from Indole-3-acetic acid by anaerobic bacteria from the rumen. J. Bacteriol. 117(1):175.

Armstrong, D.G., K.L. Blaxter, N. McGraham, and F.W. Wainman. 1958. The utilization of the energy of two mixtures of steam-volatile fatty acids by fattening sheep. Br. J. Nutr. 12:177.

Barcroft, J., R.A. McAnally and T. Phillipson. 1944. Absorption of volatile acids from the alimentary tract of the sheep and other animals. J. Exp. Biol. 20:120.

Bartley, E.E., A.D. Davidovich, G.W. Barr, G.W. Griffel, A.D. Dayton, C.W. Deyoe and R.M. Bechtle. 1976. Ammonia toxicity in cattle.I. Rumen and blood changes associated with toxicity and treatment methods. J. Anim. Sci. 43(4):835.

Bergen, W.C., and M.T. Yokoyama. 1977. Productive limits to rumen fermentation. J. Anim. Sci. 46:573.

Bergen, W.G. 1979. Factors affecting growth yields of microorganisms in the rumen. Trop. Anim. Prod. 4:13.

Bergman, E.N., R.S. Reid, M.G. Murray, J.M. Brockway and F.G. Whitelaw. 1965. Interconversions and production of volatile fatty acids in the sheep rumen. Biochem. J. 97:53.

Bird, P.R. 1972a. Sulphur metabolism and excretion studies in ruminants. IX. Sulphur, nitrogen, and energy utilization by sheep fed a sulphur-deficient and a sulphate-supplemented, roughages-based diet. Aust. J. Biol. Sci. 25:1073.

Bird, P.R. 1972. Sulphur metabolism and excretion studies in ruminants. XII. Intake and utilization of wheat straw by sheep and cattle. Aust. J. Agric. Res. 25:631.

Bouchard, R. and H.R. Conrad. 1973a. Sulfur requirement of lactating dairy cows. I. Sulfur balance and dietary supplementation. J. Dairy Sci. 56(10);1276.

Bouchard, R. and H.R. Conrad. 1973b. Sulfur requirement of lactating dairy cows. II. Utilization of sulfates, molasses, and lignin sulfonate. J. Dairy Sci. 56(11):1429.

Bouchard, R. and H.R. Conrad. 1973c. Sulfur requirement of lactating dairy cows. III. Fate of sulfur-35 from sodium and calcium sulfate. J. Dairy Sci. 56(11):1435.

Bray, A.C. and J.A. Hemsley. 1969. Sulphur metabolism of sheep. IV. The effect of a varied dietary sulphur content on some body fluid sulphate levels and on the utilization of urea supplemented roughages by sheep. Aust. J. agric. Res. 20:759.

Bray, A. C. and A. R. Till. 1975. Metabolism of sulfur in the gastro intestinal tract. *In* Digestion and metabolism in the ruminant. ED. I.W. McDonald, Univ. of New England Publishing, Armindale, Australia. pp. 244-250.

Brondani, V.A., R. Towns, K. Chou, R.M. Cook and H. Barradas. 1991. Effects of isoacids, urea, and sulfur on ruminal fermentation in sheep fed high fiber diets. J. Dairy Sci. 74: 2724.

Bryant, M.P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Federation Proc. 32:1809.

Bryant, M.P., and R.N. Doetsch. 1955. Factors necessary for the growth of *Bacteroides succinogenes* in the volatile acid fraction of rumen fluid. J. Dairy Sci. 38:340.

Bryant, M.P., and I.M. Robinson. 1961. Studies on nitrogen requirements of some ruminal cellulolytic bacteria. Appl. Microbiol. 9(2):96.

Bush, K.J., R.W. Russel and J.W. Young. 1979. Quantitative separation of volatile fatty acids by high pressure liquid chromatography. J. Liquid Chrom. 2(9):1367.

Butterworth, M.H. 1964. The digestible energy content of some tropical forages. J. Agric. Sci. 63:319.

Chalupa, W. 1973. Utilization of non-protein nitrogen in the production of animal protein. Proc. Nutr. Soc. 32:99.

Chaney, A.L., and E.P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8(2):130.

Child, R.D., J.N. Njuki, R.M. Hansen and D.L. Whittington. 1982. Digestibility of protein content of Leucaena leucocephala. E. Afr. agric. For. J. 48(2):32.

Cline, T.R., T.V. Hershberger, and O.G. Bentley. 1958. Utilization and/or synthesis of valeric acid during the digestion of glucose, starch and cellulose by rumen microorganisms in vitro. J. Anim. Sci. 17:284.

Cline, T.R., U.S. Garrigus and E.E. Hatfield. 1966. Addition of branched and straight-chain volatile fatty acids to purified lamb diets and effects on utilization of certain dietary components. J. Anim. Sci. 25:734.

Cook, R.M., and R. Towns. 1987. Isoacids for dairy and beef cattle. Proc. Southeast Nutr. Conference manage., Tempe, AZ.

Cotta, M.A., and J.B. Russell. 1982. Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture. J. Dairy Sci. 65:226.

Cummins, K.A., and A.H. Papas. 1985. Effect of isocarbon-4 and isocarbon-5 volatile fatty acids on microbial protein synthesis and dry matter digestibility in vitro. J. Dairy Sci. 68:2588.

Dehority, B. A., Scott, H. W. and Kowalut, P. 1967. Volatile fatty acid requirements of cellulolytic rumen bacteria. J. of Bacteriology, 94(3):537-543.

Delgado, I. and P. F. Randel. 1989. Supplementation of cows grazing tropical grass swards with concentrates varying in protein level degradability. J. Dairy Sci. 72: 995-1001.

Elliot, R., A.J. Ash, F. Calderon-Cortes, and B.W. Norton. 1987. The influence of anaerobic fungi on rumen volatile fatty acid concentrations in vivo. J. Agric. Sci. (Camb) 109:13.

Emery, R.S., C.K. Smith, and C.F. Huffman. 1957a. Utilization of inorganic sulfate by rumen microorganisms. I. Incorporation of inorganic sulfate into amino acids. Appl. Microbiol. 5:360.

Emery, R.S., C.K. Smith, and L. Faito. 1957b. Utilization of inorganic sulfate. Appl. Microbiol. 5:363.

Falen, L.R., J.P. Baker, R.M. Cook, D.O. Everson, and R.C. Bull. 1968. Some factors affecting intake and digestion of low-quality forages by ruminants. Proc. Western Amer. Soc. Anim. Sci. 19:193.

Felix, A., R.M Cook, and J.T. Huber. 1980. Isoacid and urea as a protein supplement for lactating cows fed corn silage. J. Dairy Sci. 63:1098.

Felix, A. 1976. Effect of supplementing corn silage with isoacids and urea on performance of high producing cows. Ph. D. thesis. Michigan State University.

Garrigus, U.S. 1970. The need for sulfur in the diet of ruminants. *In* Symposium: Sulfur in nutrition. Ed. Muth and Oldfield. Westport, CT. pp. 126-152.

Gill, J. L. 1978. Design and analysis of experiments, Vol. 1, 2,and 3. Iowa St. Univ. Press, Ames, IA. pp. 203-205.

Goodrich, R.D. and Garret, J.E. 1986. Sulfur in livestock nutrition. *In* Sulfur in agriculture. Ed. M.A. Tabataibai, Wisconsin. pp. 617-629.

Goering, H. K. and P. J. Van Soest. 1970. Forage and fiber analysis. Agricultural Handbook No. 379. U.S.D.A., Washington D. C.

Gorosito, A.R., J.B. Russel, and P.J. Van Soest. 1985. Effect of carbon-4 and carbon-5 volatile fatty acids on digestion of plant cell wall. J. Dairy Sci. 68:840.

Gray, F.V., Weller, R.A., A.F. Pilgrim, and G.B. Jones. 1966. The rates of production of volatile fatty acids in the rumen. III. Measurement of production *in vivo* by two isotope dilution procedures. Aust. J. Agric. Res. 17:69.

Gray, F.V., Weller, R.A., A.F. Pilgrim, and G.B. Jones. 1967. The rates of production of volatile fatty acids in the rumen. V. Evaluation of fodder in terms of VFA produced in the rumen of the sheep. Aust. J. Agric. Res. 18:625.

Guardiola, C.M., G.C. Fahey, J.W. Spears, and U.S. Garrigus. 1983. The effects of sulphur supplementation on cellulose digestion in vitro and on nutrient digestion, nitrogen metabolism and rumen characteristics of lambs fed on good quality fescue and tropical star grass hays. Anim. Feed Sci. Technol. 8:189.

Harrison, D.G., and A.B. McAllan. 1980. Factors affecting microbial growth yields in the reticulo-rumen. *In* Digestive physiology and metabolism in ruminants. Ruckebush and Thivens Eds., MTP Press Ltd., Lancaster, England. pp. 205-226.

Hefner, D.L., L.L. Berger, and G.C. Fahey. 1985. Branched-chain fatty acid supplementation of corn crop residue diets. J. Anim. Sci. 61(5):1264.

Hemsley, J.A., and R.J. Moir. 1963. The influence of higher volatile fatty acids on the intake of urea supplemented low quality cereal hay by sheep. Aust. J. Agric. Res. 14:509.

Hill, K., J.R. Wilson, and H.M. Shelton. 1989. Yield, persistence and dry matter digestibility of some C_3, C_4 and C_3/C_4 Panicum species. Tropical Grassland 23(4): 240-249.

Horino, M., L.J. Machlin, F. Hertelendy, and D.M. Kipnis. 1968. Effect of short chain fatty acids on plasma insulin in ruminant and nonruminant species. Endocrinology 83:118.

Houpt, T.R. and K.A. Houpt. 1968. Transfer of urea nitrogen across the rumen wall. Amer. J. Phys. 214(6): 1296-1303.

Huber, J.T., and L. Kung Jr. 1981. Protein and nonprotein nitrogen utilization in dairy cattle. J. Dairy Sci. 64:1170.

Hume, I.D., R.J. Moir, and M. Somers. 1970. Synthesis of microbial protein in the rumen. I. Influence of the level of NH_3 intake. Aust. J. Agric. Res. 21:283.

Hume, I.D. 1970a. Synthesis of microbial protein in the rumen. II. A response to higher volatile fatty acids. Aust. J. Agric. Res. 21:297.

Hume, I.D. 1970b. Synthesis of microbial protein in the rumen. III. The effect of dietary protein. Aust. J. Agric. Res. 21:305.

Hume, I.D., and P.R. Bird. 1970. Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulphur. Aust. J. Agric. Res. 21:315.

Hungate, R.E. 1966. The rumen and its microbes. Academic Press. New York.

Hunter, R.A., and B.D. Siebert. 1986. Digestion of mature pangola grass (Digitaria decumbens) by Bos taurus and Bos indicus cattle. Aust. J. Agric. Res. 37:665.

Huntington, G.B. 1986. Uptake and transport of nonprotein nitrogen by the ruminant gut. Federation Proc. 45:2272.

Johnson, W.H., Goodrich, R.D. and J.C. Meiske. 1970. The role of sulfur in ruminant nutrition. IMC, Tech. release. pp. 1-16.

Kandylis, K. 1983. Transfer of plasma sulfate from blood to rumen. J. Dairy Sci. 66:2263.

Kandylis, K. 1984. The role of sulfur in ruminant nutrition. Livestock Prod. Sci. 11:611.

Kandylis, K. 1984. Toxicology of sulfur in ruminants. J. Dairy Sci. 67:2179.

Kayongo-Male, H., J.W. Thomas, D.E. Ullrey, R.J. Deans, and J.A. Arroyo-Aguilu. 1976. Chemical composition and digestibility of tropical grasses. J. Agr. Univ. P.R. 60(2):186.

Kennedy, P.M., and B.D. Siebert. 1972. The utilization of spear grass (Heteropogon contortus). III. The influence of the level of dietary sulphur on the utilization of spear grass by sheep. Aust. J. Agric. Res. 24:143.

Kennedy, P.M. 1974. The utilization and excretion of sulphur in cattle fed on tropical roughages. Aust. J. Agric. Res. 25:1015.

Kennedy, P.M., and L.P. Milligan. 1978. Quantitative aspects of the transformations of sulfur in sheep. Br. J. Nutr. 39:65.

Kiddle, P., R.A. Marshall and A.T. Phillipson. 1951. A comparison of the mixtures of acetic, propionic and butyric acids in the rumen and in the blood leaving the rumen. J. Physiol. 113:207.

Knox, K.L., A.L. Black, and M. Kleiber. 1967. Some kinetics characteristics of rumen short-chain fatty acids as measured by the isotope dilution method. J. Dairy Sci. 50(10):1716.

Kone, P. 1987. A study on the effect of the combination of monensin and isoacids on rumen fermentation *in vitro*. M.S. thesis. Michigan State University.

Kone, P., P.F. Machado, and R.M. Cook. 1989. Effect of the combination of monensin and isoacids on rumen fermentation *in vitro*. J. Dairy Sci. 72;2767.

Krishna, G., and A. Ekern. 1974a. Volatile fatty acid metabolism in sheep. 3. Effect of intraruminal infusion of VFA on the utilization of nutrients and some biochemical constituents, rectal temperature and pulse rate. Z. Tierphysiol. Tierernahrg. u. Futtermittelkde. 33:323.

Krishna, G., and A. Ekern. 1974b. Volatile fatty acid metabolism in sheep. 1. Studies on the VFA production and availability of energy by using "Isotope dilution technique". Z. Tierphysiol. Tierernahrg. u. Futtermittelkde. 33:275.

Lassiter, C.A., R.S. Emery, and C.W. Duncan. 1958. Effect of alfalfa ash and valeric acid on growth of dairy heifers. J. Dairy Sci. 41(1):552.

Leng, R.A., J.L. Corbett and D.J. Brett. 1968. Rates of production of volatile fatty acids in the rumen of grazing sheep and their relation to ruminal concentrations. Br. J. Nutr. 22:57.

Leng, R.A. and D.J. Brett. 1966. Simultaneous measurements of the rate of acetic, propionic and butyric acids in the rumen of sheep on different diets and the correlation between production rates and concentrations of these acids in the rumen. Br. J. Nutr. 20:541.

Masson, M.J., and A.T. Phillipson. 1951. The absorption of acetate, propionate and butyrate from the rumen of sheep. J. Physiol. 113:189.

McDonald, I.W. 1948. The absorption of ammonia from the rumen of the sheep. J. Sci. 42:584.

McLeod, M.N., and D.J. Minson. 1988. Breakdown of large particles in forage by simulated digestion and detrition. J. Anim. Sci. 66(4):1000.

McLeod, M.N., and B.R. Smith. 1989. Eating and ruminating behavior in cattle given forages differing in fibre content. Anim. Prod. 48:503.

Mehrez, A.Z., E.R. Orskov, and I. McDonald. 1977. Rates of rumen fermentation in relation to ammonia concentration. Br. J. Nutr. 38:447.

Miller, E.L. 1973. Symposium on nitrogen utilization by the ruminant: Evaluation of foods as sources of nitrogen and amino acids. Proc. Nutr. Soc. 32:79.

Minson, D.J., and R.A. Bray. 1986. Voluntary intake and *in vivo* digestibility by sheep of five lines of *Cenchrus ciliaris* selected on the basis of preference rating. Grass and Forage Sc. 41:47.

Moir, R.J. 1970. Implications of the N:S ratio and differential recycling. *In* Symposium: Sulfur in nutrition. Ed. Muth and Oldfield. Westport, CT. pp. 165-181.

Moir, K.W. 1974. The estimation of the metabolizable energy of forage from its cell-wall content and digestible cell wall. J. Agric. Sci. (Camb) 82:423.

Morrison, M., R.M. Murray, and A.N. Boniface. 1990. Nutrient metabolism and rumen microorganisms in sheep fed a poor quality tropical grass hay supplemented with sulphate. J. Agri. Sci. (Camb.) 115:269.

Naga, M.A., and J.H. Harmeyer. 1975. Gas and volatile fatty acid production at different rates of rumen microbial protein synthesis in vitro. J. Anim. Sci. 40(2):374.

National Academic of Sciences. 1976. Urea and other Non Protein Nitrogen compounds in animal nutrition. Washington, D.C. pp. 17-37, 73-74.

National Research Council. 1989. Nutrient requirements of dairy cattle. 6th Ed., Washington, D.C. pp. 90-110, 147.

Nolan, J.V., B.W. Norton, and R.A. Leng. 1973. Nitrogen cycling in sheep. Proc. Nutr. Soc. 32:93.

Oltjen, R.R., L.L. Slyter, E.E. Williams, JR. and D.L. Kern. 1971. Influence of the branched-chain volatile fatty acids and phenylacetate on ruminal microorganisms and nitrogen utilization by steers fed urea or isolated soy protein. J. Nutr. 101:101.

Onwuca, C.F and A.O. Akinsoyinu. 1989. Effect of elemental sulphur levels on urea-nitrogen utilization by goats and sheep. Tropical Agric. (Trinidad), 66:158.

Orskov, O.R. 1982. Protein nutrition in ruminants. Academic Press. New York.

Orskov, E.R., and D.M. Allen. 1966. Utilization of salts of volatile fatty acids by growing sheep. 4. Effects of type of fermentation on the basal diet on the utilization of salts of volatile fatty acids for nitrogen retention and body gains. Br. J. Nutr. 20:519.

Otterby, D.E., D.G. Johnson, R. Towns, R.M. Cook, R.A. Erdman, H.H Van Horn, J.A. Rogers, and W.A. Clark. 1990. Dose response of dairy cows to ammonium salts of volatile fatty acids. J. Dairy Sci. 73:2168.

Panditharatne, S., V.G. Allen, J.P. Fontenot, and M.C.N. Jayasuriya. 1988. Effect of stage of growth and chopping length on digestibility and palatability of guinea 'A' grass silage. J. of Anim. Sci. 66:1005.

Papas, A.M., S.R. Ames, R.M. Cook, C.J. Sniffen, C.E. Polan, and L. Chase. 1984. Production responses of dairy cows fed diets supplemented with ammonium salts of Iso C-4 and C-5 acids. J. Dairy Sci. 67:276.

Peck, H.D. 1970. Sulfur requirements and metabolism of microorganisms. *In* Symposium: Sulfur in nutrition. Ed. Muth and Oldfield. Westport, CT. pp. 61-79.

Peters, J.P., J.A.Z. Leedle, and J.B. Paulissen. 1989. Factors affecting the in vitro production of volatile fatty acids by mixed bacterial populations from the bovine rumen. J. Anim. Sci. 67:1593.

Pittman, K.A. and M.P. Bryant. 1964. Peptides and other nitrogen sources for growth of *Bacteroides Ruminicola*. J. Bacteriol. 88(2):401.

Poole, D.A., and D.M. Allen. 1979. Utilization of salts of volatile fatty acids by growing sheep. 5. Effects of type of fermentation of the basal diet on the utilization of salts of acetic acid for body gains. Br. J. Nutr. 24:695.

Preston, T.R. and R.A. Leng. 1975. Utilization of tropical feeds by ruminants. *In* Digestive physiology and metabolism in ruminants. p.p. 621-640.

Quispe, M. E. 1982. A study of the effects of isoacids, urea and sulfur on the rate of fermentation in the rumen. M.Sc. Thesis, Michigan State University.

Quispe, M.E., H. Barradas, and R.M. Cook. 1991. Effects of isoacids, urea and sulfur on ruminal fermentation in sheep fed pineapple tops. Small Rum. Res. 6:49.

Rees, M.C., D.J. Minson and F.W. Smith. 1974. The effect of supplementary and fertilizer sulphur on voluntary intake, digestibility, retention time in the rumen, and site of digestion of pangola grass in sheep. J. Agric. Sci. (Camb) 82:419.

Reid, R.L., G.A. Jung, and W.V. Thayne. 1988. Relationship between nutritive quality and fiber components of cool season and warm season forages: a retrospective study. J. Anim. Sci. 66:1275.

Robertson, J. B. and P. J. Van Soest. 1977. Dietary fiber estimation in concentrate feedstuffs. J. Anim. Sci. (Abstract).

Robinson, I.M., and M.J. Allison. 1969. Isoleucine biosynthesis from 2-methylbutyric acid by anaerobic bacteria from the rumen. J. Bacteriol. 97(3):1220.

Roffler, R.E. and L.D. Satter. 1975a. Relationship between ruminal ammonia and NPN utilization by ruminants. I. Development of a model for predicting NPN utilization. J. Dairy Sci. 58(12):1880.

Roffler, R.E. and L.D. Satter. 1975b. Relationship between ruminal ammonia and NPN utilization by ruminants. II. Application of published evidence to the development of a theoretical model for predicting NPN utilization. J. Dairy Sci. 58(12):1889.

Rogers, J.A., W.A. Clark, D.N. Ferraro, A.C. Spearow, V.E. Blumer, C.H. Dougherty Jr., R.M. Cook, A.A. Jimenez, D.D. Dildey, and L.E. Armentano. 1989. Milk production of dairy cows fed ammonium and calcium salts of volatile fatty acids on 43 commercial dairy farms. J. Dairy Sci. 72:270.

Rogers, J.A., and C.L. Davis. 1982. Effects of intraruminal infusions of mineral salts on volatile fatty acid production in steers fed high-grain and high-roughage diets. J. Dairy Sci. 65:953.

Russell, J.B., and R.B. Hespell. 1981. Microbial rumen fermentation. J. Dairy Sci. 64:1153.

Russell, J. B. and C.J. Sniffen. 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro. J. Dairy Sci. 67:987.

Satter, L.D. and L.L Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199.

Slyter, I. L. and J. M., Weaver. 1971. Growth factor requirements of ruminal cellulolytic bacteria isolated from microbial populations supplied diets with or without rapidly fermentable carbohydrate. Appl. Microbiol. 22:5, pp. 930-932.

Soofi, R., G.C. Fahey Jr., L.L. Berger, and F.C. Hinds. 1982. Effect of branched chain volatile fatty acids, trypticase, urea, and starch on in vitro dry matter disappearance of soybean stover. J. Dairy Sci. 65;1748.

Spedding, C.R.W. 1971. The individual plant. *In* Grassland ecology. Oxford Univ. Press, London. pp. 8-14.

Tamminga, S. 1986. Utilization of naturally occurring NPN-compounds by ruminants. Arch. Anim. Nutr. 36:169.

Thomas, W.E., Loosli, J.K., Williams, H.H., and L.A. Maynard. 1951. The utilization of inorganic sulfate and urea nitrogen by lambs. J. Nutr. 43:15.

Tinnimit, P. 1974. Forage evaluation using various laboratory techniques. Ph D. Thesis, Michigan State University.

Tsai, Y.C., L.S. Castillo, W.A. Hardison, and W.J.A. Payne. 1967. Effect of dietary fiber level on lactating dairy cows in the humid tropics. J. Dairy Sci. 50(7):1126.

Umunna, N.N., T. Klopfenstein, and W. Woods. 1975. Influence of branched-chain volatile fatty acids on nitrogen utilization by lambs fed urea containing high roughage rations. J. Anim. Sci. 40(3):523.

Vadiveloo, J. 1989. The intake and digestibility in goats of *Leucaena leucocephala* supplemented with dehydrated palm oil mill effluent. Anim. Feed Sc. Techn. 24:45.

Van Soest, P.J., and W.C. Marcus. 1964. Method for the determination of cell-wall constituents in forages using detergent and the relationship between this fraction and voluntary intake and digestibility. J. Anim. Sci. 47(1):704 (Abstract).

Van Soest, P.J. 1973. The uniformity and nutritive availability of cellulose. Fed. Proc. 32(7):1804.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. Cornell Univ. Press., Ithaca, N.Y. p.p 95,276.

Varga, G.A., W.H. Hoover, L.L. Junkins, and B.J. Shriver. 1988. Effects of urea and isoacids on *in vitro* fermentation of diets containing formaldehyde-treated or untreated soybean meal. J. Dairy Sci. 71:737.

Waldo, D.R. 1968. Symposium: Nitrogen utilization by the ruminant. Nitrogen metabolism in the ruminant. J. Dairy Sci. 51(2):265.

Weller, R.A., F.V. Gray, A.F. Pilgrim and G.B. Jones. 1967. The rates of production of volatile fatty acids in the rumen. IV. Individual and total volatile fatty acids. Aust. J. Agric. Res. 18:107.

Weller, R.A., A.F. Pilgrim and F.V. Gray. 1969. Volatile fatty acid production in the rumen of the grazing sheep: its use as an indicator of pasture value. Br. J. Nutr. 21:97.

Whanger, P.D., and G. Matrone. 1970. Effects of sulfur deficiency on metabolism in sheep. *In* Symposium: Sulfur in nutrition. Ed. Muth and Oldfield. Westport, CT. pp. 153-164.

Wilson, J.R., and P.W. Hattersley. 1989. Anatomical characteristics and digestibility of leaves of Panicum and other grass genera with C_3 and different types of C_4 photosynthetic pathway. Aust. J. Agric. Res. 40:125.

Weston, R.H., J.R. Lindsay, D.B. Purser, G.L.R. Gordon, and P. Davis. 1988. Feed intake and digestion responses in sheep to the addition of inorganic sulfur to a herbage diet of low sulfur content. Aust. J. Agric. Res. 39:1107.

Yerena, F., H.M. Ferreiro, R. Elliot, R. Godoy, and T.R. Preston. 1978. Digestibility of Ramon (*Brosimun alicastrum*), *Leucaena leucocephala*, buffel grass (*Cenchrus ciliaris*), sisal pulp and sisal bagasse (*Agave fourcroydes*). Trop. Anim. Prod. 3:27.