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A STUDY OF THE EFFECTS OF ISOACIDS, UREA, AND SULFUR ON THE RATE OF FERMENTATION IN THE RUMEN

By

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ABSTRACT

A STUDY OF THE EFFECTS OF ISOACIDS, UREA AND SULFUR ON THE RATE OF FERMENTATION IN THE RUMEN

By

Maria Esperanza Quispe Salas

Agricultural by-products such as pineapple tops are available as a new alternative ruminant feed. In order to find ways to enhance their fermentation in the rumen, a 2^3 factorial crossover experiment was conducted in Two 4x4 quasi-Latin squares, to study the effects of isoacids (isobutyrate, 2-methyl butyrate, iso-valerate and valerate), urea and sulfur on the rate of fermentation in the rumen. Eight fistulated Tabasco rams divided by body weight into two groups of four. Each ram received four of the diets with different combinations of supplementation with the 3 factors.

The levels of supplementation used were: 0.07 g and 0.14 g of isoacids/Kg body weight; 0 and 0.43 g of urea/Kg body weight, and 0 and 0.086 g of sulfur/Kg body weight. After each of the 8 experimental weekly periods, rumen acetate production was measured using an isotope dilution procedure. Increases in acetate production were found when diets contained higher levels of isoacids (0.14 g/Kg of body weight), in combination with urea and sulfur supplementation (0.43 g and 0.086 g/Kg body weight, respectively).

DEDICATED TO

My parents, whose many sacrifices and encouragement so meaningfully enriched my life.

and

My husband, Paco, whose pacience and understanding made this work posible.

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LIST OF ABREVIATIONS

VFA	Volatile Fatty Acids
MJ	Mega Joules
^{14}C	Carbon-14
N	Nitrogen
PEG	Polyethylene Glycol

INTRODUCTION

Cellulosic by-product materials, such as pineapple tops,are abundant in the Mexican tropics. Since 1905, pineapple by-products have been used to replace traditional forages. These materials can be an important feed for ruminants. For optimum use of pineapple by-products in cattle rations, it is necessary to study factors that limit their fermentation in the rumen.

Many tropical pastures have a high yield of dry matter, but annual animal production is seriously limited by the seasonal nature of this production. The main factor limiting pasture growth is the lack of soil moisture for long periods of the year. During the rainy season there is abundant high quality forage available for grazing. When pasture growth ceases at the end of the rainy season, the pasture consists of a large bulk of mature feed from which some of the leafier parts have already been removed.

Severe weight and production loss of cattle during the dry season is a common phenomenon in the Mexican tropics. Yet, while cattle starve, there are in the same area millions of tons of by-products from the agricultural industry that are wasted. These by-products, as in the case of pineapple residues, could be used as cattle feed.

The feeding practices in the Mexican tropics are based on principles established mainly in the temperate zones using European breeds of

cattle. In Mexico only 20% of the cattle are European breeds. The rest Zebu (22.5%) and other breeds (57.5%). The production potential of the cattle in the tropics cannot be achieved until their nutrient requirements are known. In order to improve cattle production in the Mexican tropics basic research is needed that will lead to methods to increase the digestibility of agricultural by-products.

In Mexico 560,000 metric tons of pineapple fruit were processed in 1978 from six states (25). A great part of the annual production is processed at the food plants. However, these factories can utilize only 15 to 25% of the fruit. The rest is waste which constitutes a pollution problem. If these residues were usable as livestock feed, they would be equivalent to several thousand tons of forage.

Numerous research trials have been conducted in Hawaii, India and Mexico (55, 56, 61, 69, 72) on the use of pineapple plant or pineapple by-products as ruminant feed. Most of the studies dealt with its utilization in combination with the rest of the pineapple residues (stems, leaves and pulp) as silages.

However, pineapple tops have not been fed alone because of the high fiber content. They can be categorized as high-fiber, low nitrogen by-products. The low nitrogen content limits the intake or digestibility Therefore in order to utilize them in a diet a source or non-protein nitrogen should be added to correct the microbial deficit.

All studies that have been reported so far are short-term trials. Long term feeding trials and more research are needed to determine effects on production, reproduction and general health.

This study investigated several factors which might benefit the utilization of cellulosic by-products by ruminants, and which could aid in developing more effective systems for their utilization.

REVIEW OF LITERATURE

Pineapple By-Products Use in Livestock Rations

Pineapple by-products show promise as a source of roughage for cattle. There is an abundant supply throughout the year (48, 50,54) and it is a good source of nutrients (21, 55). The nutritive value of the pineapple residues for bovine feed expressed in dry matter is equivalent to that of cereal grain by-products. These pineapple residues resulted in better milk yields than any other tropical forages due to the high level of total digestible nutrientes (65 - 74%). Most tropical forages are lower than 55% in total digestible nutrients (48).

The two types of residues resulting from pineapple processing are non-pulp (tops, leaves and inner cores), and pulp. The chemical composition of pineapple residues fractions are presented in Table 1. These fractions vary considerably according to the fruit variety, degree of maturity and technology used in the cannery. All contribute to the great variation observed in chemical composition.

Many factors must be taken into account when considering the nutritional value of by products. They often vary in chemical composition, are strictly seasonal, local in production and often contain undesirable contaminants of organic or inorganic origin (61). In the case of pineapple by-products, all of these disadvantages should be considered. However, if a permanent market could be developed, many tropical areas

Table 1. Chemical Composition of Pinneapple Canning By-Products (Dry Matter Basis)

Type of by-product	Proportion of total fruit	Crude protein	Ether extract	Crude fiber	Nitrogen free extract	Ash	Dry matter
	----------	----------	----------	%----------	----------	----------	----------
Hulls	56	6.4	0.92	16.7	71.88	4.1	42
Tops	17	7.2	0.82	25.4	62.88	3.7	38
Leaves	15	7.0	0.84	22.3	65.76	4.1	40
Core	5	7.1	0.96	19.7	69.94	2.3	73
Trimmings	2	6.8	0.91	16.2	73.49	2.6	74
Pulp	5	7.8	1.20	21.9	64.70	4.4	63

Source: Muller (48).

areas would benefit.

In Mexico, only 20% of the cattle are European breeds. The rest are Zebu (22.5%) and other breeds (57.5%). The production potential of the cattle in the tropics cannot be achieved until their nutrient requirements are known. In order to improve cattle production in the Mexican tropics basic research is needed on methods to increase the digestibility of agricultural by-products. In Mexico, 560,000 metric tons of pineapple fruit were processed in 1978 from six states (25). A great part of the annual production is processed at the food plants. However , these factories can utilize only 15 to 25% of the fruit. The remainder is waste, which constitutes a pollution problem. If these residues were usable as livestock feed, they would be equivalent to several thousand tons of forage.

Numerous research trials have been conducted in Hawaii, India and Mexico (55, 56, 61, 69, 72) on the use of the pineapple plant or pineapple by-products as ruminant feeds. Most studies dealt with utilization of pineapple stems, leaves and pulp as silage. However, pineapple tops have not been fed alone because of the high fiber content. They can be categorized as high-fiber, low nitrogen by-products. The low nitrogen content limits the intake or digestability. Therefore, in order to utilize then in a diet a source of non-protein nitrogen should be added to correct any microbial deficit of ammonia.

All the studies that have been reported, thus far, were short-term trials. Long-term feeding trials and more research are needed to determine effects on animal production, reproduction and general health. Two studies have shown that pineapple green chop ensiles without problems, and because of its succulent nature, it is a good localy grown roughage

for both beef and dairy cattle. Yearling dairy heifers weighing approximately 300 Kg will consume 14-16 Kg of pineapple silage daily. These animals produced an average of 24 Kg. (72). Beef cattle weighing an average of 320 Kg consumed 16 to 20 Kg of silage a day plus 2.2 Kg of molasses and 2.2 Kg of protein supplement (81). In both studies (72, 81) silages were low in dry matter and animals would need to consume larger amounts to meet their requirements. Confirming the variability in the product, a range of 0.05 to 0.78 Kg in daily body weight gains was obtained (72).

VFA Production in the Rumen

Discovery of Rumen Volatile Fatty Acids

In 1944, Barcroft, et al (8) demostrated that volatile fatty acids are absorbed from the rumen. Elsden (23) later confirmed that these acids were acetate, propionate and butyrate. Walter (1970) and Hulton (1972), cited by Naga and Harmeyer (51), suggested that microbial yield could be affected by VFA production in the rumen.

The reactions occuring in the rumen and the effects of the end products of these reactions on the metabolism of the ruminant is well established (7). The role of the volatile fatty acids; acetate, propionate and butyrate in the productive processes of domestic animals have been the subject of several studies (10, 27, 82). These acids are the principal source of energy, as well as biosynthetic substrates, for various ruminant tissues. Production rates of these acids are studied to determine the efficiency of utilization of plant materials by ruminants. They also estimate the availability of energy to the animal under certain conditions and diets.

Several studies have examined the relationship in the rumen between production of VFA , synthesis of microbial protein, and carbohydrate fermentation (15, 24, 34). The significance of fermentation of carbohydrates to VFA's was soon recognized (34). It was also shown that microbial protein can replace dietary protein as the main source of amino acids for ruminant tissues (35).

Techiniques of Measuring VFA Production.

Many techniques have been used to measure the production rates of fermentation products. Gray et al (32) described two main approaches for conducting such studies. These are: 1) In vitro techniques and (2) In vivo techniques. Several in vivo studies are summarized in Tables 2, 3 and 4.

As a result of various experimental techniques, there are different ways of expressing production rates of rumen volatile fatty acids. They include (1) methods based on changes in rumen VFA concentrations after feeding, (2) production of VFA in inoculated rumen fluid (in vitro), (3) analysis of the blood draining the rumen (in vivo), (4) isotope dilution techniques applied to either the rumen or the whole animal. The latter probably offers the most accurate means of measuring production of organic acids in the rumen, since the measurements may be made in vivo with minimum disturbance to the animal.

Both Anison (1965) and Wagner (1964), cited by Leng (42), discussed the inherent difficulties of obtaining representative samples of rumen contents and suggest that the rates of production of acids are not uniform throughout the rumen. Total VFA production in the rumen has been measured by an isotope dilution procedure based on the infusion of

Table 2. In Vivo Production of Ruminal VFA by sheep.

Conditions	Daily VFA production (mol)				Total/kg of Feed Intake	Reference
	Acetic	Propionic	Butyric	Total		
900 g alfalfa chaff fed at hourly intervals from 08.00 hrs to 19.00 hrs	3.74	0.91	0.71	5.36	5.96	Leng, Leonard (1965)
800 g alfalfa fed at hourly intervals from 08.00 hrs to 19.00 hrs	5.33	1.41	0.42	7.16	8.95	Leng, Brett (1966)
1 kg of 50% alfalfa and 50% wheaten hay chaff fed at 12 hr intervals	-	-	-	4.51	5.00*	Gray et al. (1966)
1 kg of 40% wheaten hay chaff and 60% alfalfa fed 12 hr intervals	3.87	0.96	0.71	-	5.54*	Weller et al. (1967)
1 kg of 50% alfalfa and 50% wheaten chaff fed at						
1 hr intervals	-	-	-	4.12	4.80*	Gray et al. (1967)
2 hr intervals	-	-	-	4.02	4.45*	
12 hr intervals	-	-	-	4.24	4.90*	
Dried ryegrass						
27% crude protein	-	-	-	5.52	-	Weston, Hogan (1968)
12% crude protein	-	-	-	4.76	-	
6% crude protein	-	-	-	3.39	-	
1.1 kg timothy hay fed at hourly interval for 24 hours continously day and night						
1. "^{14}C labelled VFA mixture" infusion . .	2.75	0.53	0.46	-	-	Present studies
2. "^{14}C labelled VFA mixture" infusion . .	3.00	0.59	0.29	3.87	3.92*	

*On dry feed basis

Source: Kreshna and Ekern (40).

Table 3 . Net Production Rate of VFA (Average of two sheep)

Name of Acid	Production of acid mol/day	Mol/kg dry matter consumed	g/day	Caloric value MJ
Infusion of ^{14}C-labelled individual VFA				
Acetic Acid	2.750	2.790	165.25	2.41
Propionic Acid . . .	0.529	0.535	39.15	0.82
Butyric Acid	0.464	0.470	40.83	1.02
Infusion of ^{14}C-labelled VFA mixture				
Acetic Acid	2.996	3.085	179.76	2.62
Propionic Acid . . .	0.591	0.598	43.73	0.91
Butyric Acid	0.285	0.288	25.08	0.62

Source: Krishna & Ekern (40).

Table 4. Rates of Acetate Production in the Rumen of Sheep.

Reference	Average Weight (Kg)	Number of animals	Feed	Average Acetate production (Mol/day/sheep)	Method of measurement
Bergman et al (1965)	51	2	Dried grass (continuous feeding)	3.70	Constant infusion
		3	50% Wheat + Hay 50% Lucerne Hay	1.30	Constant infusion
Gray et al. (1967)	-*	4	40% Wheat + Hay 60% Lucerne Hay	1.62	Constant infusion
		2	100% Lucerne Hay	1.46	Constant infusion
Leng & Corbett (1968)	42	5	Pastures (Phalares tuberosa)	2.50	Isotope dilution

* Not mentioned.
Source: Adapted from (10, 30 and 41)

individually ^{14}C-labelled acids (82). When ^{14}C-labelled VFA were introduced into the rumen, the results tended to support the hypothesis of non uniform VFA production. Samples of rumen contents obtained from various sites, after insufficient time for complete mixing, varied up to 20% in specific activity. Such large differences could represent a sampling error.

The mean specific activity (after mixing) is related to the mean production rate of the acid in the rumen. If production rates vary throughtout the rumen, local variations may occur in amount or type of VFA, or in numbers and types of organisms responsible for fermentations. Also, there amy be layering of the food materials. Any attempt to mix rumen fluid uniformly by circulation pumps disturbs the normal milieu within the rumen and affects rates and patterns of fermentation (76).

Factors that Affect VFA Production

It has been established that the VFA, produced in the digestive tract of the ruminant by microbial fermentation processes, represent an important source of energy to the host (74). The amounts and proportions of VFA produced are available depending on the nature of the diet, the time after feeding and the age of the animal (74). Other factors, according to Beeson (1965) cited by J. J. O'Conor, et al (54) affecting molar concentration of VFA's could be:

-Roughage to concentrate ratio

-Physical form of feed

-Buffers

-Salivary output

-Kind and amount of protein

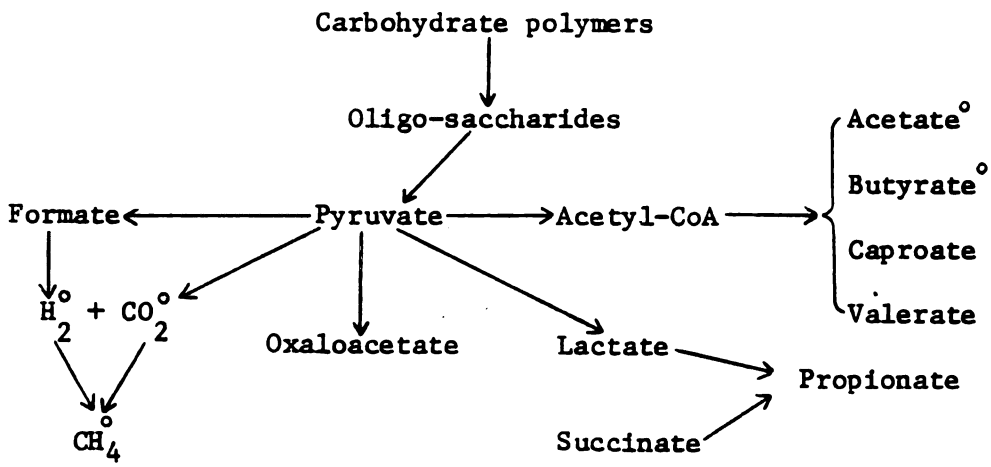
-Frequency of feeding

-Balance of nutrients

Still others might include, level of dry matter intake, and rate of absorption of volatile fatty acids from the rumen (46). A reason for these changes is the intensive microbial degradation of plants, which are primarily composed of carbohydrate polymers, taking place within the rumen.

These polymers initially are hydrolyzed in the rumen to oligosaccharides, which are subsequently fermented to VFA carbon dioxide and methane. (Fig. 1)

GENERALIZED SCHEME FOR RUMINAL DEGRADATION AND FERMENTATION OF CARBOHYDRATES



Source: Russell and Hespell (65).

Quantitative Data on VFA

Numerous studies have quantitated production of VFA in the rumen. In 1958 Stewart (74) by means of an *in vivo-in vitro* technique, measured the VFA production rates, and found concentrations increased from 2 to 6 hours after feeding, while the rates of VFA production were greatest during the first two hours. An average of 2.9 g. were produced per steer/day. Leng and Leonard (43) found that molar proportions of VFA in the rumen remained constant throughout a 24 hour period, that VFA concentrations increased after feeding and reached a plateau around 16 hours post-feeding, but during the subsequent 6 hours a steady decline occurred.

Later studies of simultaneous measurements of the rates of production of VFA in the rumen of sheep suggested that interconversions of the main acids were possible (44). Conversion of acetic acid into butyric accounted for between 40-50% of the butyrate produced, and conversion of butyrate into acetate accounted for 6-13% of the acetic. However, the interconversion between propionate into acetate and butyrate was small.

Weller et al. (83) also measured the rates of production of individual and total fatty acids by infusion of a mixture of ^{14}C-Labelled acids. These studies showed that the proportion of acids produced in the rumen was similar throughout the feeding cycle.

The infusion of certain mixtures of labelled fatty acids showed that the molar composition of the total VFA initially formed in the rumen was acetic 77-83%, propionic 15-18%, and butyric 1-7%. Mean rates of VFA production determined by Weller et al in 1967, (82) in seven sheep during fourteen 3-day periods throughout winter, spring and summer months ranged from 3.4 to 5.3 moles total VFA's per day. Naga and Harmeyer (51),

studied in vitro VFA production at different rates of rumen, microbial protein synthesis, and generally found negative correlations between microbial growth and volatile fatty acid production.

Nutrition of the Rumen Microbiota

Isoacid Requirements

A major problem in ruminant nutrition is to define the nutrients required by rumen microorganisms for maximum fermentation of feedstuffs, particularly for low protein, highly fibrous plant materials. Information is developing on the nutritional requirements of some of the predominant groups of rumen bacteria. Tables 5 and 6 show rumen bacteria grouped on the basis of some nutrient requirements. These organisms are unique in that they synthesize the α-keto acid analogue of several amino acids by direct carboxylation of the corresponding acid (6). For example, isobutyrate is carboxylated to form the α-keto acid analogue of valine. Table 7 shows the VFA required by rumen bacteria, and also 3 other acids that are believed to stimulate the growth of certain rumen bacteria.

When high quality protein is fed to ruminants, the isoacids may be produced in sufficient quantities to satisfy the nutritional requirements of the rumen cullulolytic bacteria. However, when highly fibrous plant materials containing low amounts of poor quality protein are fed, an isoacid (isobutyrate, 2-methyl butyrate, isovalerate and N-valerate) deficiency is undoubtedly a major factor limiting the growth of the rumen bacteria and consequent digestion of cellulose.

Fermentation end products, such as formate, lactate or ethanol may appear in the rumen. Ammonia, carbon dioxide and either short straight,

Table 5 . Some Functions of the Main Nutritional Groups of Rumen Bacteria Based on Energy Sources

ENERGY SOURCES (One or more of the important species in the nutritional group have this function)				
Group 1	Group 2	Group 3	Group 4	Group 5
Cellulose		Cellulose*		
Pentosans	Pentosans	Pentosans		Pentosans
Starch	Starch	Starch	Starch	Starch
		Lactate		
A	A	A	A	A
	B	B		B

*Cellulose digestion by butyrivibrio included in this group
(A) Proteolitic ability
(B) Aminoacid catabolism

Source: Adapted from (13).

Table 6 . Some Functions of the Main Nutritional Groups of Rumen Bacteria based on Nitrogen and Carbon Sources

Essential nitrogen and carbon sources other than energy				
Group 1	Group 2	Group 3	Group 4	Group 5
NH_3	NH_3	NH_3	NH_3	-
VFA*	Peptides	A.a.	-	Peptides & Aa.
	VFA (stimulate)			
19.1%	31.5%	24.7%	5.6%	5.6%

*Require one or more of the acids-Isobutyrate, 2-methyl butyrate or isovalerate; sometimes require N-valerate or longer chain fatty acids.

Source: Adapted from (13).

Table 7 . Volatile fatty acids and other acids required for growth of certain rumen bacteria

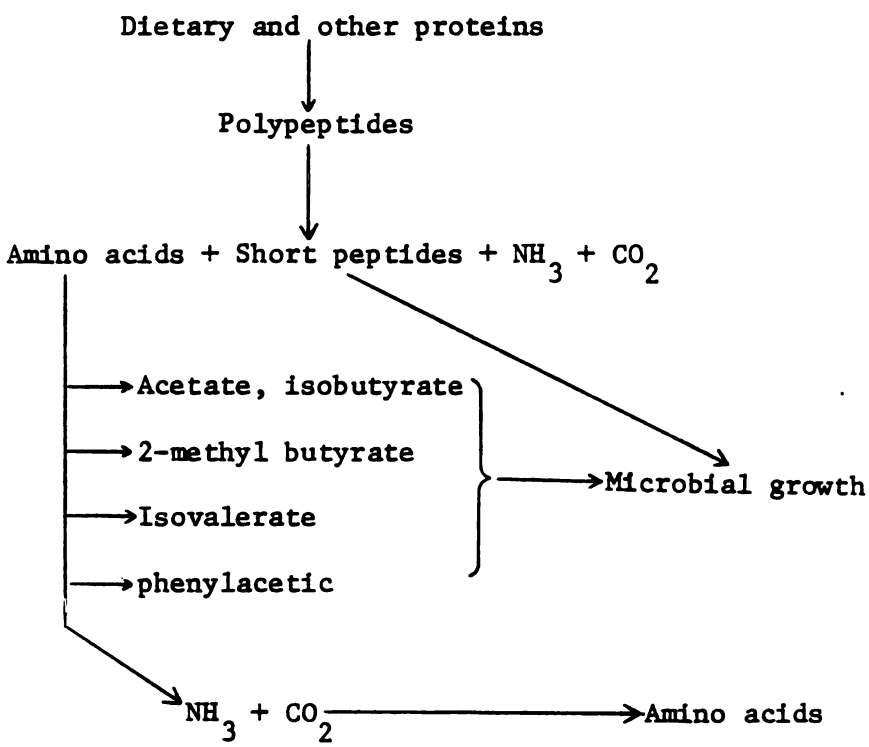
Acid	Source*
N-valeric	CH_2O, proline arginine, lysine
Isovaleric	Leucine
2-methylbutyric	Isoleucine
Isobutyric	Valine
Phenylacetic	Phenylalanine
Indoleacetic	Tryptophan
Imidazoleacetic	Histidine

*These source compounds are catabolized by other bacteria to produce the acids.

Source: Adapted table from (4, 5, 6 and 77).

branched-chain or aromatic fatty acids are formed from protein degradation as shown in Fig. 2 and are used in the production of microbial protein.

Figure 2. FATE OF PROTEINS IN THE RUMEN



Source: Rusell and Hespell (65).

Ammonia requirements

In 1948 McDonald (49) demonstrated that ammonia is produced from the ruminal degradatation of dietary protein. Ammonia can be produced by rumen microbes from both protein and non-protein nitrogeneous substances, and it is probably the most important source of nitrogen for ruminants.

Ammonia (NH_3 and/or NH_4) appears to be incorporated rapidly into rumen bacteria in the form of amide or amino groups and used for amino acid synthesis (62). Ammonia is obligatory for the synthesis of rumen microbial protein as many pure culture studies have shown; the fact that ammonia is the major source of nitrogen for microbial growth also has been confirmed by many *in vivo* studies (16, 52). Generally speaking, bacteria can grow in media with ammonia levels as low as 1 mg/dl. However, according to Satter and Slyter (77), ammonia concentrations of 2 to 5 mg/dl in the rumen are needed for maximum microbial yield. Illinois workers found that it is possible for organisms to grow in a medium of 1.7 mg/dl, however, this level might not give maximum yield of bacterial cells.

When ruminants are fed straw, the rumen ammonia concentration is 1-3 mg NH_3-N/dl (26). Ammonia can be absorbed into the blood from the rumen and converted to urea in the liver. Blood urea can then enter the rumen by diffusion through the rumen wall and by secretion of saliva. This phenomenon is now recognized as the "nitrogen cycle" in ruminants.

Urea can be used to supply ammonia when natural protein is not present in the diet. There is little doubt that entry of plasma urea into the rumen can provide a significant source of nitrogen for microbial growth and enhance survival where dietary nitrogen intake is low. For

sheep and cattle fed low quality hay, endogenous urea may provide 25% of nitrogen available in the rumen.

One way to determine the amount of urea that can be utilized with a particular diet is to monitor rumen ammonia concentration while incresing dietary urea addition. The point at which rumen ammonia accumulates signifies the point of maximum urea utilization (70). This categorization of feed ingredients as to siutability for use with urea still requires more quantitative information.

Reported values for rumen ammonia levels that give maximum microbial growth have ranged from 1 to 25 mg/dl. Satter and Roffler (70) reported that an ammonia level of 5 mg NH_3-N/dl is the upper limit for ammonia utilization by the rumen microbiota. They proposed that needs above this value must come from supplementary feed protein that bypasses rumen fermentation. However, Miller (47) and Orskov et al (58) reported that 23 mg NH_3-N/dl of rumen fluid is the upper limit for ammonia utilization by the rumen microbiota.

These studies have not considered the need for other intermediates for amino acid biosynthesis by rumen microbiota, such as isoacids and sulfur, eventhough feeding trials have shown an increase in nitrogen retention when isoacids were added to diets (17, 26, 57). The benefits of using both urea and natural protein to supplement corn silage for balancing rations for high producing cows have been studied extensively (Huber and Thomas, 1971; Conrad and Mugerwa, 1970; cited by Felix, 1976). However, the optimum level of urea which can be successfully fed, especially if the ration is comprised of highly fibrous roughages, is still controversial and requires more investigation.

Sulfur requirements

In addition to ammonia and isoacids, sulfur is an essential nutrient for the synthesis of rumen microbial protein, and thus for optimum fermentation of substrates. Practically all of the sulfur present in protein is in S-containing amino acids cystine, methionine and cystathionine, or in tissues as metabolic derivatives which accounts for about 1% of the total S.

In the rumen, dietary sulfur is converted to hydrogen sulfide. Sulfide is the key intermediate between breakdown of ingested or recycled sulfur and subsequent utilization of sulfur by rumen microbiota. Based on findings of many workers (9, 11, 29), it is obvious that ruminant animals require sulfur for systemic metabolism. However, if normal rumen function is to take place, rumen microorganisms must also be supplied with adequate sulfur.

Without adequate sulfur, rumen microbes have a reduced ability to function normally, thus digestibilities and nitrogen retention are decreased (76). In sheep, dry matter digestibility increased as sulfur in rumen fluid increased from 0.07 to 4.2 ug/dl. The precise level at which rumen sulfide concentrations limit rumen fermentation has not been determined, particularly if urea is a major source of supplemental nitrogen.

Dietary Nitrogen to Sulfur Ratios

Bray et al, (12) found that sulphate supplementation of a sheep increased crude fiber digestility and nitrogen and sulfur retention. Also, Bray (12) showed that inorganic ^{35}S (sulphate) was transferred to

the rumen by passage of sulphate was apparently increased by influx of water into the rumen. Only 0.3 to 1.4 % of the injected sulphate used this route over a 4 h. period. Therefore, it was suggested that the utilization of recycled urea nitrogen may be severely limited in sheep on low sulfur and low nitrogen intakes, unless sulfur is recycled to the rumen by routes other than across the rumen wall or in other forms.

In most temperate zone forages, the sulfur is in the protein component which has an average N:S ratio of about 15:1. However, the total N:S ratio of the fodder can vary from 4:1 to 50:1. The desirable N:S is reported to be 10-13.5:1 for sheep and 13.5-15:1 for cattle in the temperate zones (45). A number of researchers (9, 16, 20, 29, 45, 84) have questioned the meaning of dietary nitrogen to sulfur ratios. Potentially, there are a multitude of correct dietary nitrogen to sulfur ratios depending on the availabilities of dietary nitrogen and sulfur (45).

When the diet consists of low protein, fibrous plant materials, supplementation with urea requires simultaneous supplementation with sulfur. Sulfur supplements used are elemental sulfur, various sulphate salts, and, in some cases, s-amino acids and methionine hydroxy analogue. A dietary sulfur deficiency restricts dry matter digestibility. The effects of sulfur on the fermentation of carbohydrates has been reviewed (84). Jones and Haag (37) observed a growth response in dairy heifers fed a basal ration of low sulfur hay plus grain when 3% urea and 1% sodium sulphate were added. Lassiter, et al (41) and Brown et al (14) also observed a growth response to a sulfur supplementation of rations for dairy heifers. Other studies with sulfur supplementation have given inconsistent results. This probably indicates that levels of sulfur in the basal diets were sufficient for the production levels achieved.

Based upon published values (53) for nitrogen and sulfur content of feed, it can be shown that the use of 0.5% urea-nitrogen and 0.5% sulfur in simple concentrate mixes of practical diets for dairy cattle can result in N:S of 18-20:1 . The National Research Council (53) reports that the sulfur requirement of lactating cows is 0.2% of the total diet, which implies a N:S ratio of 12:1 for medium producing cows (15% protein in the total diet dry matter). Moreover, Rending and Weir (64) studied effects of S fertilization of a S-deficient soil on the nutritional quality of forage produced for lambs and showed consistent, though not always significant, trend towards higher gains in lambs when S fertilization was practiced.

In S-fertilization experiments in New Zealand, McNaught and Chrisstoffels (50) reported N:S ratios of 17 to 18.5 for white clover and 11 to 12 in grasses gave maximum yields. Pumphrey and Moore (63) found a N:S ratio of 11 or less indicated an adequate S supply for digestibility and growth of alfalfa. Thus the N:S ratios found desirable for optimum growth of plants are slightly higher than the N:S ratio of 10:1 to 15:1 suggested as optimum for ruminants (20). Moreover, plants growing at an optimum rate may not always be of ideal nutritional quality for ruminants (1).

Practical Feeding Trials Using Iso-acids

Metabolic studies have been conducted to determine the effect that short-chain VFA have on the utilization of various dietary components. A conventional balance trial was conducted with 8 lambs consuming a purified diet (39% cellulose and urea as the sole N source). The addition of a short chain VFA mixture significantly increased the apparent N

digestibility (17). Umunna et al (80) showed an increase in nitrogen retention and decrease in urinary nitrogen loss when animals on urea and high roughage rations were ruminally infused with isobutyric and/or iso-valeric acids. Infusion of these acids did not affect dry matter or protein digestibility. Oltjen et al (55) studied the influence of branched-chain VFA on the rumen microbial population and fermentation patterns as well as nitrogen utilization by steers fed urea or isolated protein supplemented-diets, they found no difference in rumen protozoa numbers, but nitrogen retention was greater with isoacid supplementation, but most of this change was observed with isolated soy protein, suggesting the importance of dietary amino acid balance.

An in-vivo experiment with dairy cows and heifers showed a positive effect on milk production, body weight, feed intake and nitrogen balance, when isoacids were added to a urea-based diet (27, 28).

In the present study , an in-vivo rumen fermentation trial with Tabasco rams was carried out in order to evaluate fibrous materials as a potential feed source for ruminants. The effects of urea, sulfur and isoacid supplementation on the rate of rumen fermentation of chopped dried pineapple tops was studied. Our specific objective was to determine the levels of rumen ammonia, rumen sulfur and rumen isoacids (iso-butyrate, 2-methyl butyrate, iso-valerate and valerate) that yields maximum fermentation of pineapple tops.

MATERIALS AND METHODS

The experiment was conducted at Centro Experimental Pecuario "La Posta", Paso del Toro, Veracruz, Mexico during the months of January, February and March of 1980.

The state of Veracruz is located on the east coast of Mexico, between 17 08' and 22 28' north latitude, and stretches along the coastline of the Gulf of Mexico.

The experimental procedure was carried out in Mexico at the station and the chemical analysis and supportive work at Michigan State University.

Animals and Management

Eight Tabasco sheep were sorted by weight into 2 groups. Four lighter sheep, each weighing approximately 25 Kg, were separated and identified (S_2, S_3, S_4, S_5). A second group of four heavier sheep weighing 35 Kg were also identified (S_6, S_7, S_8, S_{10}). All sheep were fitted with rumen cannulae and housed in individual metabolic cages.

Sheep were fed basal high fiber diet consisting of pineapple tops plus normal minerals. A daily dry-matter intake of approximately 1 Kg was maintained during the experiment. The animals were gradually adapted to urea. Water was provided ad libitum.

Ration Formulations

Pineapple tops were obtained from a regional canning plant located at the small village of "Los Robles" near the experimental station. The pineapple tops were processed through a silage chopper (1-1½ in. long) at the station and then spread on the ground to dry to 20% moisture.

Part of this material was further dried and finely ground for use in a premix to prepare the chemical supplements. Eight different rations were prepared to provide combinations of isoacids, crude protein- and sulfur, each at 2 levels. On the basis of prior cattle experimentation, isoacids were administered at 0.14 g/kg body weight. Thus, at the high level 3.5 g and 4.9 g of isoacids were fed to the lightest and heaviest groups of sheep, respectively. One half of this amount was fed at the low level.

In order to achieve two levels of ammonia in the rumen (about 5 and 15 mg/ 100 ml), pineapple tops were fed alone or supplemented with urea. For both groups, urea was offered at 0.43 g/Kg body weight (11 g for the light sheep and 15 g for the heavy sheep).

Sulfur addition to the diets was determined on the basis of N:S ratios. Four different nitrogen/sulfur treatment combinations were used: low nitrogen/high sulfur, high nitrogen/ high sulfur, low nitrogen/low sulfur, and high nitrogen/ low sulfur, yielding N:S ratios of 3:1, 5:1, 8:1 and 12:1.

For the low sulfur rations, no additional sulfur was provided, but for the high sulfur level 0.086 g /Kg body weight of added sulfur resulted in intakes of 2.17 g for the lightest group and 3.01 g for the heaviest group. Mineral supplementation was estimated at 0.8 g/Kg body weight, therefore 20 g and 27 g were supplied to the respective groups.

Rations and proximate analysis of pineapple tops are described in Tables 8 and 9.

Experimental Design

A three-factor 2^3 crossover experiment was performed in 2-4 x 4 quasi-Latin squares (ABC interaction confounded with squares) . Each experimental diet was fed for a one week period. After this period the last day was devoted to experimental and sampling procedures. The 4 animals in each square were given a different diet for each of 4 weeks as shown in Table 10. Treatment effects were estimated by measuring the rate of acetate production in the rumen on the last day of each experimental period.

Chemical Analysis

On the sampling day, all 4 animals were fed rations ad libitum, plus water free choice. Thirty minutes after feeding, rumen fluid samples were taken every 20 minutes and blood samples at 1, 2 and 3 hours.

Rumen fluid dilution rate was determined by measuring the rate of disappearance from the rumen of Polyethylene glycol, a water soluble marker (36). This method consisted of adding 10 g of PEG (M. W. 4000, Sigma Chemical Company) dissolved in 150 ml of water to the rumen through a perforated plastic tube (15 mm x 20 cm long), which achieved a better distribution of the solution throughout the rumen. This plastic tube was fitted to one end of a dosing syringe which allowed infusion of liquid solutions into the rumen as well as collection of the samples.

In vivo acetate production rates were determined by the single injection radioisotope technique. Each animal received approximately 100 uCi of Na (1-^{14}C) acetate (New England Nuclear Corp. Boston, MA)

Table 8. Chemical Composition of Treatment Rations Fed to Sheep.

Ingredients	Treatments							
	Lighest Animals				Heaviest Animals			
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Pineapple tops (g)	1215	1215	1215	1215	1700	1700	1700	1700
Isoacids (ml)	2.8	5.6	5.6	2.8	3.9	3.9	7.8	7.8
Urea (g)	-*	11.0	-	11.0	-	15.0	-	15.0
Sulfur (g)	-	-	2.2	2.2	3.0	-	-	3.0
Minerals (g)	20.0	20.0	20.0	20.0	27.0	27.0	27.0	27.0

* No urea or sulfur added to the ration.

Table 9. Proximate Analysis of Pineapple Tops Used in Feeding Trials

	Wet basis (%)	Dry basis (%)
Moisture[1]	83.6	0
Crude Protein	1.0	6.6
Sulfur	0.021	0.133

[1]Determined by forced aire oven drying at 100-110 C for 24 hours.

Table 10. Experimental Design for Rations

Square (4 lightest animals)					Square 2 (4 heaviest animals)				
	Periods					Periods			
Animal	P_1	P_2	P_3	P_4	Animal	P_5	P_6	P_7	P_8
S_5	T_1	T_2	T_3	T_4	S_6	T_5	T_6	T_7	T_8
S_2	T_2	T_3	T_4	T_1	S_7	T_6	T_7	T_8	T_5
S_3	T_3	T_4	T_1	T_2	S_8	T_7	T_8	T_5	T_6
S_4	T_4	T_1	T_2	T_3	S_{10}	T_8	T_5	T_6	T_7

*Treatment definition

T_1: $A_L B_L C_L$ T_5: $A_L B_L C_H$

T_2: $A_H B_H C_L$ T_6: $A_L B_H C_L$

T_3: $A_H B_L C_H$ T_7: $A_H B_L C_L$

T_4: $A_L B_H C_H$ T_8: $A_H B_H C_H$

A: Level of isoacids.
B: Level of urea
C: Level of sulfur

L: Low level
H: High level.

intraruminally, which was infused with 10 g of PEG. The radio-labeled acetate was dissolved in a 0.001 M solution of sodium acetate and infused into the rumen of each animal after the morning feeding.

Rumen fluid samples were collected every 20 minutes throughout a 3 hour period to study changes in dilution rates, volatile fatty acid concentrations, specific radioactivity of acetate, ammonia concentrations and hydrogen sulfide concentrations. At each time a 30 ml sample was obtained from the rumen, strained through 3 layers of cheesecloth, inmediately acidified with 50% sulfuric acid (v/v), frozen at -16 °C and stored for subsequent analysis.

Determination of Rumen Volume

A 4 ml aloquot of strained rumen fluid was assayed for PEG, using a modified version of the method of Smith (68), which was as follows:

1. Replicate 2 ml samples of strained rumen fluid were diluted with 1.5 ml of distilled water in 12-15 ml centrifuge tubes.
2. Two ml of 0.3 N $Ba(OH)_2$ was added to the centrifuge tubes followed by 2 ml of 5% $FeSO_4 \cdot 7 H_2O$ (w/v) and mixing through.
3. Subsequently, 0.5 ml of 10 % $BaCl_2 \cdot 2H_2O$ (w/v) solution was added to the centrifuge tubes and mixed.
4. After 5 min., the mixture was centrifuged at 3,600 x G for 5 minutes.
5. 0.25 ml of the resulting supernatant was combined with 2.25 ml of distilled water in a spectrophotometer cuvette to a total volume of 2.5 ml. (This final dilution was obtained from preliminary tests in order to adjust absorbance at a measurable range).
6. 2.5 ml solution containing 30% TCA-5.9% $BaCl_2 \cdot 2H_2O$ was rapidly added to the sample using a finn pipette (H. Thomas Co, No 7734-W05). The

sample was inmediately mixed using a vortex mixer.

7. Finally, the reaction was allowed to proceed for 3 minutes, after which absorbance was measured at 500 nm using a Beckman Model 6/20 spectrophotometer.
8. A standard curve for polyethylene glycol was prepared using a range of 0.10 to 0.50 absorbance.

Analysis for Volatile Fatty Acid Concentrations

1. Six ml of strained rumen fluid were centrifuged at 3,600 x G for 10 min. and a 1 ml aliquot of supernatant was used for analysis.
2. The aliquot was acidified with 200 µl of redistilled 88% Formic Acid and 0.1 molar solution of phosphoric acid.
3. The VFA analysis was performed on a Hewlett-Packard 5730 A gas chromatograph equipped with a flame ionization detector, a 7671 A autosampler and a 3380 A integrator. Temperature of 125 C was set for the column and flow rate at 40 ml/min.
4. A silanized glass column (approximately 6 foot x 2 mm ID) was packed with Carbopack c/0. 3%·CW 20W/0.1 % H_3PO_4 (Supelco 1-1825). Phosphoric acid-treated glass wool was used in the column ends.
5. Data output from this equipment was given in mmoles/100 ml of rumen fluid.

Determination of Specific Activity of Acetate

1. Three ml of strained rumen fluid was used for determining the specific activity of acetic acid. This amount of sample was centrifuged at 39,400 x G for 20 minutes in a Sorvall, Model RC 2-B, in order to remove microbial protein and feed particles.
2. A 2 ml aliquot was deprotenized by the addition of 2 ml of 0.3 N

Barium Hydroxide (Sigma # 14-3)* and 2 ml of 0.3 N Zinc Sulfate (Sigma # 14-4)*, and centrifuging for 20 minutes at 39,400 x G.

3. This second supernatant was filtered through a filter paper (Munktells' # 52-80150).
4. The clear filtrate from 3 was mixed with 50 ul of 10 M NaOH (final pH of filtrate was approximately 10) in order to prevent volatilization of the volatile fatty acids during the freeze-drying process. Acetate recoveries from rumen fluid were of approximately 90-95%.
5. Dehydration of the sample was performed in a Lyophilizer (Vertis, Model 25 SRC) until sample was completely dry.
6. Each sample was reconstituted with 1.9 ml of a 1% solution of H_3PO_4 in deionized water and the pH was adjusted to 2.0 using 100 ul of 18 M H_2SO_4.
7. A 500 ul. aliquot was taken for analysis using a high pressure liquid chromatograph apparatus which consisted of a mini pump (Laboratory Data Control, Model N51-33R), a Ryodine sample injector (Ryodine, Model 7120), a pressure dispenser (Laboratory Data Control, Model 110), a UV detector (Laboratory Data Control, Model 1203) with a 214 filter, and a chart recorder (Perkin-Elmer, Model 0-23) providing 0.018 absorbance units full scale.
8. Individual volatile fatty acids were separated using a C_8 4.6 mm x 25 cm LiChromasorb, 10 um Hibar II analytical column (E.M. Reagents # A00/2/04) and a 4.6 nm x 70 mm Perisorb RP-8 guard column (E.M. Reagents # 910436-94) fitted in front of the analytical column.
9. The mobile phase used was 1% H_3PO_4 in double distilled deionized water which was degassed under vacuum just before use.
10. The flow rate was set at 2.0 ml/min, requiring a pressure of 1500 to

2000 psi.

11. After each injection, the injector was placed in the load position so that the guard column could be washed with the following sequence of solvents: 10 ml of 65% aqueous acetonitrile, 10 ml of deionized water and 10 ml of 1% (v/v) aqueous phosphoric acid in order to prevent VFA carryover to the analytical column.
12. 500 µl aliquots from standards containing similar amounts and proportions of acetic acid as found in the samples to be essayed were injected onto the column to prepare a standard curve. See Figs. 3 and 4.
13. The 0.8 ml fractions from the column were collected in 7 ml scintillation vials for determination of specific radioactivity using a fraction collector (Isco, Model 328).
14. Five ml of Aqueous Scintillation counting cocktail, (ACS Amershan Corporation)* were added to each vial and then assayed for radioactivity using a Liquid Scintillation Spectrophotometer (Searle Analytic Inc, ISOCAP/ 300 Model 68708) with data output to a Model 8491 teletype.
15. Samples were counted for 10 minutes using the channel ratio method, (Program 2, ^{14}CSCR), to determine efficiency. Recovery of radioactivity from the column was essentially 100% for radioactive acetic acid . No appreciable radioactivity was detected after running non radioactive acetic through the column immediately following a radioactive sample. This shows that there was not a carryover between samples.

Determination of Rumen Hydrogen Sulfide Concentration

Ruminal hydrogen sulfide concentration was determined with a sulfide

*Amershan Corporation, 2636 S. Clearbrook Dr., Arlington Heights, 1160005.

Figure 3. Separation of VFA Standards by High Pressure Liquid Chromatography

Column: 4.6 mm x 25 cm Li-Chromasorb C_8 , eluent 1% phosphoric acid in double distilled deionized water, flow rate 2 ml/min, injection of a mixture of VFA standards (formic: 0.7 mM, acetic: 7 mM, and propionic: 1mM)in 250 µl, sample pH 2.
VFA concentrations were determined by absorbance at 210 nm.

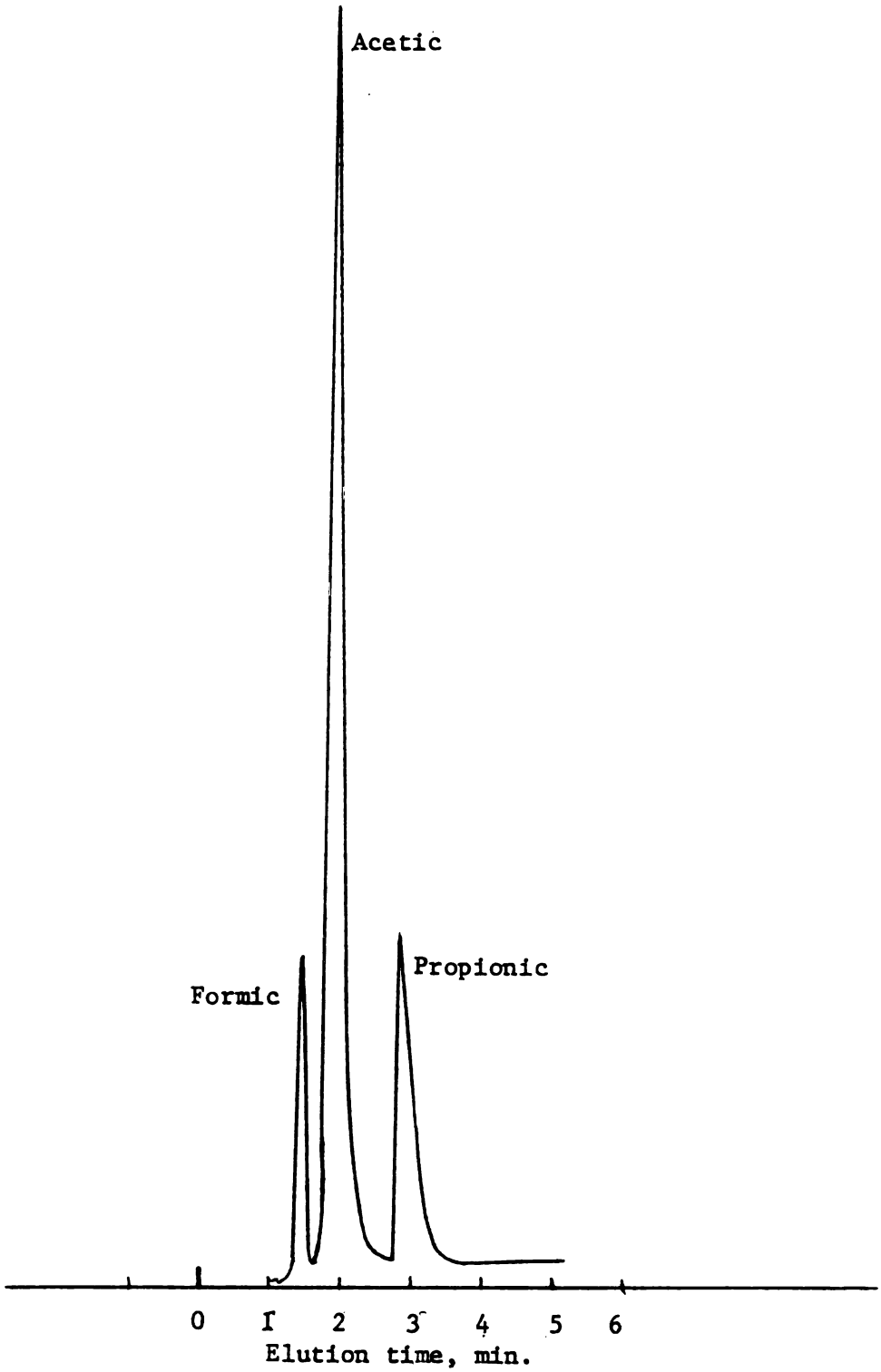
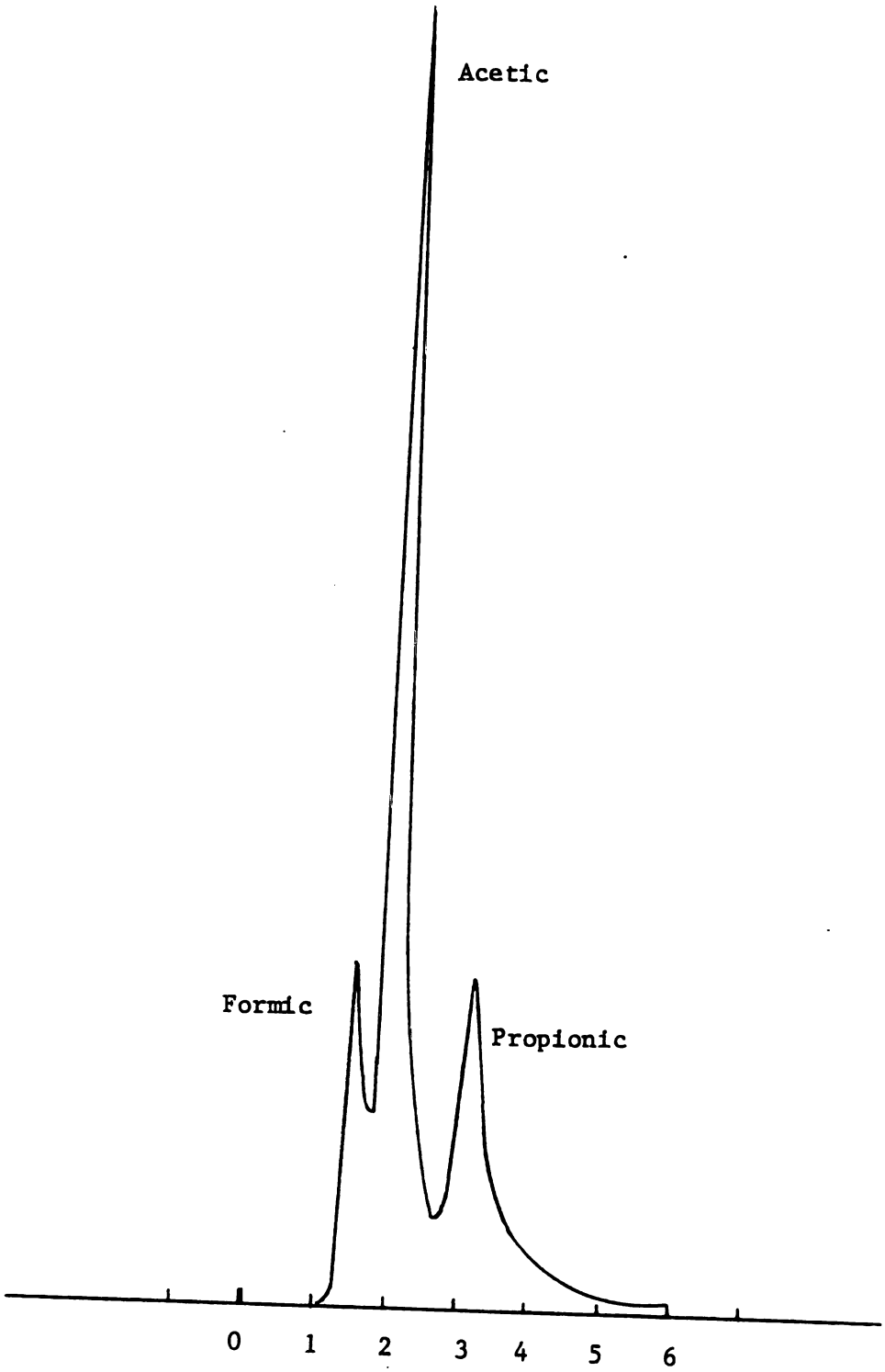

Acetic
Formic
Propionic
0
1
2
3
4
5
6
Elution time, min.

Figure 4. Quantitation of Acetate in Rumen Fluid by High Pressure Chromatography

Column: 4.6 mm x 25 cm Li-Chromasorb C_8, eluent 1% phosphoric acid in double distilled deionized water, flow rate 2 ml/min, inection of 250 ul of sample (7 mM acetate), sample pH 2.
Acetic acid concentration was determined by absorbance at 210 nm.


Acetic
Formic
Propionic
0
1
2
3
4
5
6
Elution time, min.

hydrogen sulfide sensing electrode, Lazar Model GS-136 connected to a Lazar Model digital potentiometer.

1. An aliquot of 5 ml of strained rumen fluid was combined with 2.5 ml of an antioxidant buffer* and 2.5 ml of deionized water.
2. The standarization procedure was carried out using a series of dilutions in a range of 0.5 to 10 ppm obtained from primary solution**. The diluent was a mixture of 25% (v/v) antioxidant buffer and 75% (v/v) distilled water.
3. The electrode was equilibrated in a 10 ppm sulfide solution ($NaSO_4$) for 30 minutes before being used.
4. Standards were read by immersing the electrode to a depth of 2 cm and stirring at a constant rate until a constant reading was achieved.
5. The electrode was allowed to stabilize for a few minutes before millivolt readings were recorded. Rinsing of the electrode with distilled water between samples was found to be critical.
6. Potential readings (millivolts) were plotted vs. sulfide concentration on semilog paper, (concentration was plotted on logarithmic axis) in order to obtain a linear standard curve.
7. Sample readings proceeded in a similar manner and values were converted to mg of sulfur/100 ml of rumen fluid.

Determination of Ruminal Ammonia Concentration

Determination of ruminal ammonia concentration was performed with an ammonia sensing electrode (Orion, Model 95-10) attached to an Orion Model potentiometer

*Buffer: 250 g of sodium salicilate, 65 g of ascorbic acid, 85 g of NaOH, and distilled water up to 600 ml.

1. Two ml of previously strained rumen fluid were centrifuged at 27,000 x G for 5 minutes.
2. One ml of the resulting supernatant was mixed with 9 ml of deionized water and 50 ul of 40% NaOH and read.
3. Standards were prepared in a range between 2-20 mg of ammonia/100 ml of solution. To obtain best results, samples and standard were analyzed at the same temperature (22°C).
4. The electrode was allowed to stabilize for 3 minutes between samples. The potential values (mv) were compared against the ones of the standard curve in order to determine the ammonia concentration (mg ammonia/ 100 ml rumen fluid).

Analysis of Urea and Glucose

Blood samples were taken from the jugular vein at 1, 2 and 3 hours after the beginning of the sampling period. Samples were collected in heparinized blood collection tubes for urea and glucose analysis.

Determination of Blood Glucose

Based on an enzymatic colorimetric procedure (Sigma Technical Bulletin No 510).

1. Samples were first deprotenized by mixing 0.5 ml of blood with 5.5 ml of of water, 2 ml of Zinc Sulfate (Sigma No. 14-3) and 2 ml of Barium Hydroxide (Sigma No. 14-3).
2. The mixture was then centrifuged at 39,400 x G for 15 minutes.
3. Duplicate 0.5 ml subsamples of the clear filtrate were analyzed for

**Primary sulfide standard solution: 7.5 g of sodium sulfide crystal to 250 ml of buffer and enough distilled water to make 1 liter. This makes 1000 ppm standard.

glucose content, along with a reagent blank (6 ml of distilled water) and 0.5 ml of a glucose standard (Sigma, stock solution No 635-100), 5 ml of Combined solution Reagent "A" were added to each tube. Then all tubes were incubated at 37 °C in a water bath for 30 ± 5 minutes or at room temperature for 45 minutes.

4. At the end of the incubation period, readings of absorbance at 450 nm of the standard and sample were determined using a spectrophotometer (Coleman Junior I, Model 6/20).

5. Test values were calculated using the following formula:

$$\text{Serum Glucose(mg/100 ml)} = \frac{\text{A test}}{\text{Astandard}} \times 100$$

Determination of Urea Nitrogen

Quantitation of urea nitrogen in blood was carried out using a highly sensitive, colorimetric procedure.

1. Whole blood samples were deprotenized by mixing 1.8 ml of cold 3 % (w/v) Trichloroacetic acid with 0.2 ml of whole blood followed by vortex mixing.
2. Samples were then centrifuged at 39,400 x G for 15 minutes.
3. A reagent blank (0.2 ml of 3% (w/v) TCA), a urea nitrogen standard (0.2 ml of 1:10 dilution of urea standard stock solution # 535-30 in 3% TCA) and duplicate 0.2 ml subsamples of the clear supernatant were subjected to the analytical procedure.
4. A premix consisting of 7 parts of BUN Acid Reagent (Sigma, Stock # 535-3)[a] in 5 parts of BUN Color Reagent (Sigma, Stock # 535-5)[b] was prepared and 4.8 ml were added to each tube simultaneously.

*SIGMA Chem. C. 3500 De Dekab St., St. Louis, Missouri 63118

5. All tubes were placed in a boiling water bath for exactly 10 minutes-
6. Then, they were removed and placed in a container of cold water for 3-5 minutes.
7. The contents were transferred to cuvettes and absorbance was measured at 525 nm using a Coleman Junior, Model 6/20 Spectophotometer.
8. Urea concentrations were determined directly from a standard curve prepared following the described procedure using different dilutions (1:1, 1:2, 1:4, 1:6, 1:8, 1:10) of urea standard in water. Values were expressed in mg urea-nitrogen/100 ml of solution.

(a) Contains Ferric chloride, phosphoric and sulfuric acids.

(b) Contains 0.18% (w/v) Diacetyl monoxide and thiosemicarbazide.

Statistical Analysis

Analysis of variance and single degree of freedom comparisons (28) were used to test for differences between treatment means of rumen and blood parameters. Comparisons of the ruminal response were made over the effects of isoacids (Factor A), nitrogen (Factor B), and sulfur (Factor C), or within various combinations, depending on which interactions were significant. For each variable measured, Bonferroni-t tests were used to compare the differences between treatments.

RESULTS AND DISCUSSION

In this investigation, the production rate of acetate is defined as the rate at which acetate enters the pool in the rumen as determined by an isotope, dilution technique. To calculate the total amount of acetatate, the rumen fluid volume of each animal was determined using the PEG method.

Differences in rumen fluid volume were found between individual sheep on the same ration and between sheep on different rations. The range of values was from 4.2 to 8.5 liters. Also, heavier animals showed a slight increase in the rumen fluid volume compared to lighter animals See Table 11.

Variations in rumen fluid volume of the same animals under different treatments might be due to variations in the eating or drinking patterns in response to that treatment or to the level of each treatment factor (isoacids, sulfur or urea) in the diet. It was observed that during the experiment animals drank rather large amounts of water. This could have definetely contributed to changes in the dilution rate of the marker.

Measurements on sheep of a Swedish native breed, weighing approximately 50 Kg and fitted with permanent rumen fistulae, showed a mean rumen fluid volume of 4.5 L (36). Additional studies have shown rumen volume values in a range of 4.7 to 6.0 L. in cross-bred wethers (10).

Table 11. Rumen Fluid Volume of Experimental Sheep

Sheep No.	Sheep Wt. (kg)	Treatments*	Rumen Fluid Volume (liter)
S_5	27.6	T_1	5.410
		T_2	5.989
		T_3	7.574
		T_4	4.544
		Average:	5.879
S_2	25.3	T_1	5.834
		T_2	6.644
		T_3	5.617
		T_4	4.935
		Average:	5.757
S_3	25.5	T_1	6.456
		T_2	4.700
		T_3	7.636
		T_4	6.890
		Average:	6.420
S_4	27.5	T_1	5.688
		T_2	4.509
		T_3	8.174
		T_4	6.872
		Average:	6.310
S_6	34.5	T_5	5.652
		T_6	5.324
		T_7	4.700
		T_8	8.628
		Average:	6.070
S_7	34.5	T_5	5.150
		T_6	4.935
		T_7	7.052
		T_8	7.764
		Average:	6.225
S_8	35.0	T_5	4.351
		T_6	7.896
		T_7	5.105
		T_8	6.946
		Average:	6.074
S_{10}	36.0	T_5	5.806
		T_6	4.140
		T_7	8.471
		T_8	5.989
		Average:	6.100

*Details of treatments are given on page 31. Sheep were fed ad libitum during the sampling period.

Similar results were obtained in a study using Merino ewes and wethers; values in the study of Leng, ranged from 4 to 5.4 L (43).

Another study on the absorption of volatile fatty acids from the reticulo-rumen showed a higher rumen volume ranging from 5.9 to 7.2 L for mature Merino sheep (44), and ours from 4.2 to 8.5 L. Therefore, the rumen volume results obtained were similar to those, but slightly higher than others (10, 36, 43).

The effects of isoacids, urea nitrogen and sulfur on the concentrations of volatile fatty acids are summarized in Table 13. There were four observations per treatment(see Table 27). The statistical analysis of variance for VFA production is presented in Table 12. There were no significant differences among treatments in the ruminal acetate, propionate and iso-butyrate concentrations ($P < 0.05$).

Mean ruminal butyrate concentrations are shown in Table 13. Isoacids and urea interacted as shown in Table 12. Ruminal butyrate concentration was slightly increased with joint supplementation of high levels of isoacids and urea. However, this effect was not significant ($P > 0.10$, Table 14).

Increase in butyrate concentration with increasing dietary urea concentration could be caused by a stimulus of the rumen cellulolitic bacterial population in response to increasing ammonia levels

Treatment effects on ruminal 2-methyl butyrate concentrations are presented in Table 13. The concentration of this branched acid was increased when high isoacid was given instead of low isoacid in combination with urea and no sulfur ($P < 0.01$), as shown on paired treatment comparisons in Table 15. This might be due to degradation of this acid

Table 12. Analysis of Variance for VX Concentration in Rumen Fluid

Table 12. Analysis of Variance for VFA Concentration in Rumen Fluid

	FACTORS			Interactions				Periods	Sheep	
	A=Isoac.	B=Urea	C=Sulfur	AB	BC	AC	ABC	Square	Square	Error
Acetate										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.924	0.017	0.164	4.562	0.652	2.236	6.772	1.500	0.479	1.295
Significance							*			
Proplonate										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.002	0.000	0.007	0.408	0.011	0.086	0.978	0.118	0.217	0.154
Significance							*			
Iso-Butyrate										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.00024	0.00056	0.00047	0.00402	0.00119	0.00302	0.00237	0.00146	0.00165	0.00213
Significance										
Butyrate										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.00352	0.01306	0.00403	0.22726	0.01301	0.05710	0.03444	0.00750	0.05175	0.03444
Significance				*						
2 Methyl-Butyrate										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.00345	0.00051	0.00112	0.00267	0.00056	0.00154	0.00415	0.00071	0.00055	0.00025
Significance	*			**		*	**			
Iso-Valerate										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.00070	0.00000	0.00003	0.00140	0.00001	0.00004	0.00202	0.00013	0.00009	0.00015
Significance				**			**			
Valerate										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.00245	0.00036	0.00021	0.00020	0.00318	0.00096	0.00344	0.00037	0.00120	0.00050
Significance						*	*			

* (P < 0.05)

** (P < 0.01)

Table 13. Effects of Treatment Combinations on VFA Concentrations in the Rumen

Treatment Combination	MEANS* (mg/dl of Rumen Fluid)						
	Acetate	Propionate	Butyrate	Iso-Butyrate	2-methyl Butyrate	Iso-Valerate	Valerate
T_1: $A_LB_LC_L$	6.576	1.324	0.630	0.077	0.301	0.030	0.054
T_2: $A_HB_HC_L$	7.204	1.483	0.735	0.106	0.081	0.043	0.056
T_3: $A_HB_LC_H$	6.546	1.187	0.544	0.087	0.034	0.027	0.082
T_4: $A_LB_HC_H$	5.437	1.108	0.439	0.066	0.016	0.013	0.027
T_5: $A_LB_LC_H$	7.593	1.786	0.583	0.099	0.017	0.013	0.029
T_6: $A_LB_HC_L$	7.315	1.553	0.525	0.048	0.010	0.003	0.032
T_7: $A_HB_LC_L$	6.795	1.502	0.373	0.055	0.018	0.009	0.026
T_8: $A_HB_HC_H$	7.738	1.661	0.600	0.064	0.240	0.022	0.049
** SEM	±0.568	±0.196	±0.092	±0.023	±0.007	±0.006	±0.011

Table 14. Pooled Treatment Comparisons of The Effects of Isoacids (A) and Urea (B) on Butyrate Concentration in The Rumen.

Comparison	Treatment Contrasts	Mean Difference* (mg/ dl of Rumen Fluid)	Significance
A/B_L	$A_HB_LC_H + A_HB_LC_L$ vs $A_LB_LC_H + A_LB_LC_L$	0.148	$P > 0.10$
A/B_H	$A_HB_HC_L + A_HB_HC_H$ vs $A_LB_HC_H + A_LB_HC_L$	0.186	$P > 0.10$
B/A_L	$A_LB_HC_H + A_LB_HC_L$ vs $A_LB_LC_L + A_LB_LC_H$	0.124	$P > 0.10$
B/A_H	$A_HB_HC_L + A_HB_HC_H$ vs $A_HB_LC_H + A_HB_LC_L$	0.209	$P > 0.10$

*SED = $\pm$ 0.093

when there is low ammonia in the rumen. Sulfur showed a negative effect on the concentration of this acid ($P < 0.01$), as indicated in the last comparison in Table 15.

Iso-valerate concentrations for various treatments are shown on Table 13. For this acid 4 contrasts were made from the interaction AB (isoacids-nitrogen) which was found to be significant ($P < 0.01$), Table 12. When isoacids were increased in combination with urea, the concentration of iso-valerate increased ($P < 0.001$). In the absence of urea the effect of isoacids was minimal (Table 16).

This result was supported by the work of other investigators which reported pronounced depressions in iso-valeric acid concentration when ruminants were fed diets essentially free of protein. Moreover these comparisons agree with the results of Cline et al (17), in the sense that the decrease in rumen iso-valerate indicates that when supplementary urea was added to the diet more of the available ammonia was being converted into microbial protein resulting in an increase of this acid.

Mean differences of ruminal valerate concentrations are presented in Table 17. When rations were supplemented with sulfur, there was a positive response to increased isoacids ($P < 0.05$).

The effects of the dietary parameters on liquid turn over time were estimated by the regression analysis of 1/turn over rate. Average turn over times for each treatment are shown in Table 18, and the analysis of variance for this variable is presented in Table 19. There were not significant interactions between treatment factors. Some decrease in turn over time was noticed with increasing levels of isoacids and with sulfur in the ration, but the differences were not significant ($P > 0.10$), because of massive error variance.

Table 15. Paired treatment Comparisons of 2-Methyl Butyrate Concentration in the Rumen

Treatment Contrasts	Mean Difference* (mg/dl of Rumen Fluid)	Significance
A/B_LC_L: $A_LB_LC_L$ vs $A_HB_LC_L$	0.012	**
A/B_LC_H: $A_HB_LC_H$ vs $A_LB_LC_H$	0.017	**
A/B_HC_L: $A_HB_HC_L$ vs $A_LB_HC_L$	0.071	(positive effect of isoacids, urea present, sulfur absent, P< 0.01)
A/B_HC_H: $A_LB_HC_H$ vs $A_HB_HC_H$	0.008	**
B/A_LC_L: $A_LB_LC_L$ vs $A_LB_HC_L$	0.020	**
B/A_LC_H: $A_LB_HC_H$ vs $A_LB_LC_H$	0.001	**
B/A_HC_L: $A_HB_HC_L$ vs $A_HB_LC_L$	0.063	(positive effect of urea, high level of isoacids, no sulfur, P< 0.01)
B/A_HC_H: $A_HB_LC_H$ vs $A_HB_HC_H$	0.010	**
C/A_LB_L: $A_LB_LC_L$ vs $A_LB_LC_H$	0.013	**
C/A_LB_H: $A_LB_HC_H$ vs $A_LB_HC_L$	0.006	**
C/A_HB_L: $A_HB_LC_H$ vs $A_HB_LC_L$	0.016	**
C/A_HB_H: $A_HB_HC_L$ vs $A_HB_HC_H$	0.057	(negative effect of sulfur, high level of isoacids, urea present, P< 0.10)

* SED = 0.011
**(P > 0.10)

Table 16. Pooled Treatment Comparisons of the Effects of Isoacids (A) and Urea (B) on Isovalerate Concentration in the Rumen

Comparison	Treatment Contrasts	Mean Difference* (mg/dl of Rumen Fluid)	Significance
A/B_L	$A_HB_LC_H + A_HB_LC_L$ vs $A_LB_LC_H + A_LB_LC_L$	0.004	$P > 0.10$
A/B_H	$A_HB_HC_L + A_HB_HC_H$ vs $A_LB_HC_H + A_LB_HC_L$	0.025	(positive effect of iso acid, when urea high, $P < 0.001$)
B/A_L	$A_LB_HC_H + A_LB_HC_L$ vs $A_LB_LC_L + A_LB_LC_H$	0.014	$P > 0.10$
B/A_H	$A_HB_HC_L + A_HB_HC_H$ vs $A_HB_LC_H + A_HB_LC_L$	0.015	$P > 0.10$

*SED = $\pm$ 0.006

Table 17. Pooled Treatment Comparisons of the Effects of Isoacids (A) and Sulfur (C) on Valerate Concentration in the Rumen.

Comparison	Treatment Combination	Mean Difference* (mg/dl of Rumen Fluid)	Significance
A/C_L	$A_HB_HC_L + A_HB_LC_L$ vs $A_LB_LC_L + A_LB_HC_L$	0.0180	$P > 0.10$
A/C_H	$A_HB_LC_H + A_HB_HC_H$ vs $A_LB_HC_H + A_LB_LC_H$	0.0375	(positive effect of isoacids, with sulfur, $P < 0.05$)
C/A_L	$A_LB_HC_H + A_LB_LC_L$ vs $A_LB_LC_H + A_LB_HC_H$	0.0125	$P > 0.10$
C/A_H	$A_HB_LC_H + A_HB_HC_L$ vs $A_HB_HC_H + A_HB_LC_L$	0.0245	$P > 0.10$

*SED = $\pm$ 0.011

Table 18. Effects of Treatment Combinations on Acetate Turnover Time in the Rumen.

Treatment Combination	Means* (minutes)
T_1: $A_LB_LC_L$	125.480
T_2: $A_HB_HC_L$	114.898
T_3: $A_HB_LC_H$	86.805
T_4: $A_LB_HC_H$	75.961
T_5: $A_LB_LC_H$	83.134
T_6: $A_LB_HC_L$	148.609
T_7: $A_HB_LC_L$	94.040
T_8: $A_HB_HC_H$	78.017

* SEM = $\pm$ 29.90

Table 19. Analysis of Variance of Acetate Production, Turnover Time, Hydrogen Sulfide Concentration, and Ammonia in the Rumen

	FACTORS			Interactions				Periods	Sheep	
	A=Isoac.	B=Urea	C=Sulfur	AB	BC	AC	ABC		Square	Error
							Squares			
Acetate Production										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.06498	0.01674	0.02376	0.00536	0.01882	0.05330	0.01178	0.00662	0.01324	0.01003
Significance	*					*				
Turnover										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	7240	883	3967	2188	7303	0	1895	3877	1441	3585
Significance										
Hidrogen Sulfide										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	20.566	9.660	207.731	0.266	0.629	1.007	3.387	8.116	7.196	1.749
Significance	**	*	**					*	*	
Ammonia- Nitrogen										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	1.057	499.318	0.582	1.103	23.158	10.165	0.004	16.087	12.183	6.747
Significance		**								

* (P < 0.05)

**(P< 0.01)

Means of acetate production in the rumen are shown in Tables 20 and 21. All mean acetate production values appear normal and found to be consistant except Treatment 5 ($A_LB_LC_H$) which was 0.172 moles/hr x sheep and less than Treatment 1 ($A_LB_LC_L$) value which was 0.20 moles/hr x sheep. This resulting low mean could have been caused by the lower dry matter intake shown by 2 of the sheep at 2 of the sampling times. This resulted in a decrease of VFA production.

Because of significant interaction AC ($P< 0.01$), Bonferroni-t tests were made on low isoacids/sulfur supplemented treatments compared with treatments with the same level of sulfur but higher isoacid levels. Results from these analyses indicated that acetate production was increased when isoacids were increased with sulfur present ($P< 0.05$). The same was found when sulfur was added in presence of high isoacids ($P< 0.10$) See Table 22.

When treatments with different N:S ratios were compared, a tendency of increasing acetate production in the rumen was found with 5:1 ratios, compared to ratios of 3:1, 8:1, or 12:1. This effect is clearly seen in Figure 3. Ruminal ammonia concentration and nitrogen to sulfur ratios relationships with acetate production were compared. It was found that ruminal ammonia concentration had a very low correlation with respect to acetate production (0.18) as presented in Figure 4. Moreover there is evidence that N:S ratios are more important in predicting the production of acetate ($R^2= 14.6\%$), as compared to absolute ammonia concentrations in the rumen ($r^2= 3.2\%$) See Figures 3 and 4. The curve predicting acetate production from N:S ratio indicates an optimum ratio of 5:1. The decrease in acetate production shown at 10:1 was not represented by data and resulted from the curve being forced low by required

Figure 3. EFFECTS OF N:S RATIOS ON MEAN ACETATE PRODUCTION

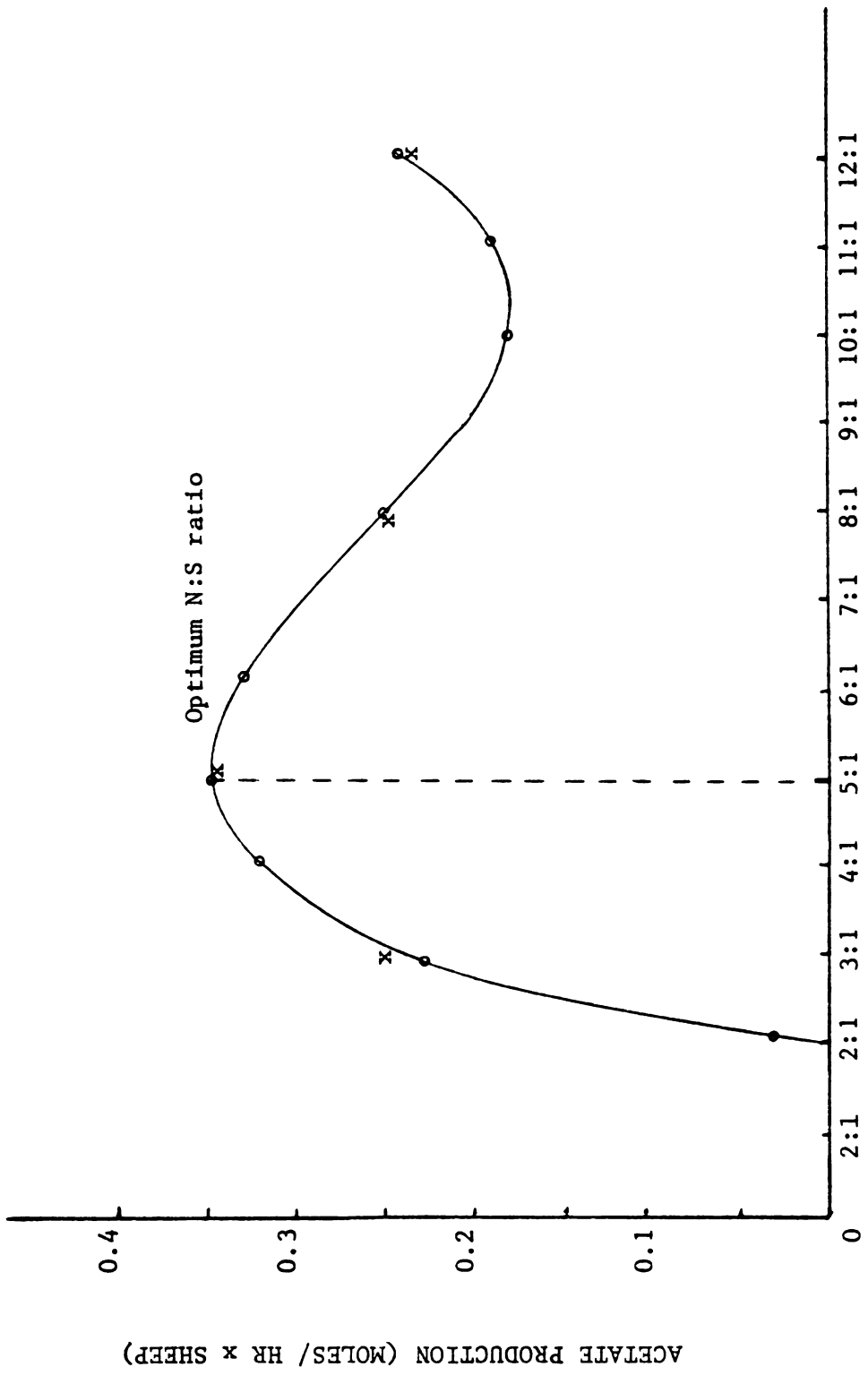
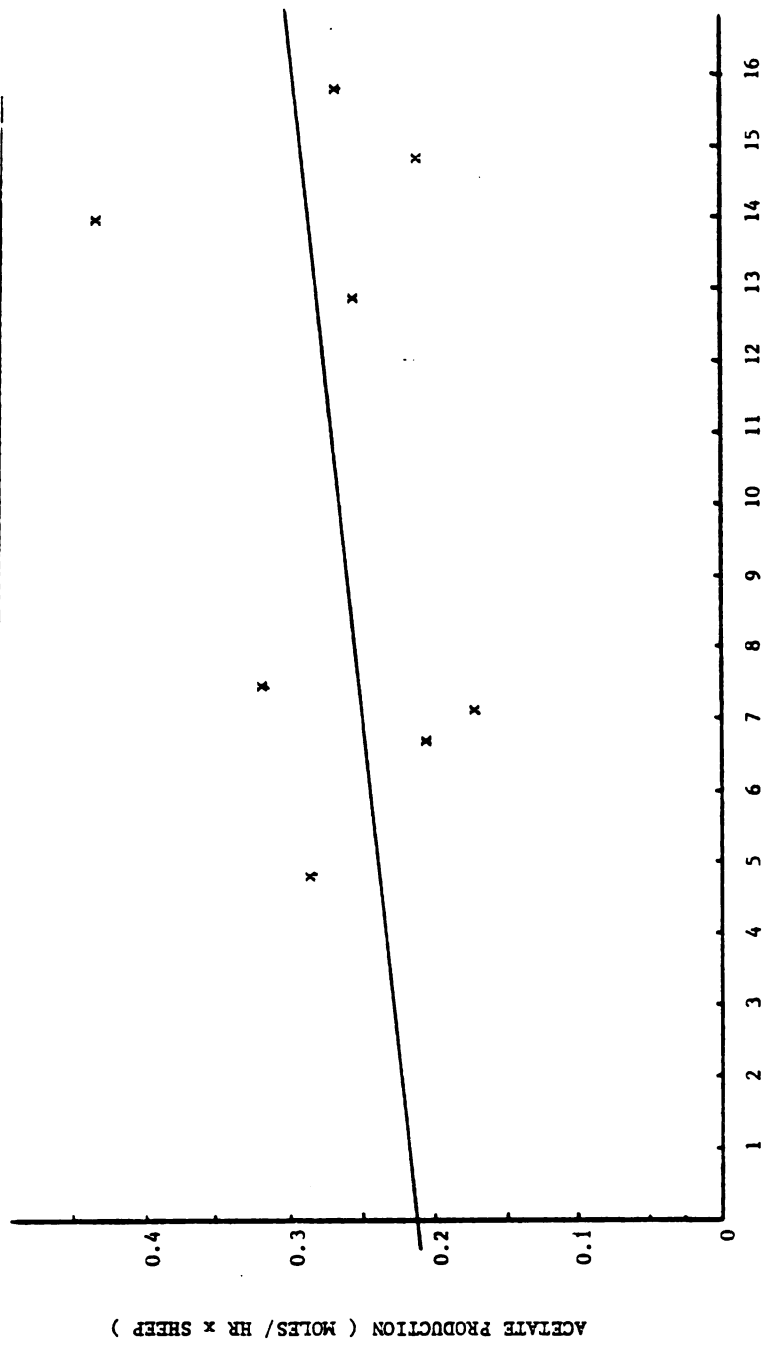


Figure 4. EFFECT OF AMMONIA CONCENTRATION IN THE RUMEN ON ACETATE PRODUCTION



regularity of a low-degree polynomial function.

Acetate production in sheep was estimated by Leng and Leonard (42), to be 0.231 moles/hr x sheep. When lucerne chaff was fed values of 3.7 to 4.2 moles/12 hours were found by Bergman et al (10) in sheep. Weller et al (82), fed sheep on roughage diets (lucerne and wheaten hay) for 24 hours, obtained an acetate production rate of 3.7 moles/ 24 hr. The literature values given in Tables 2 and 4 agree with the values found in the present study.

Table 20. Effects of Isoacids (A), Urea (B) and Sulfur (C) on Acetate Production in the Rumen.
(4 lightest animals)

Treatment Combination	Animal No.	Acetate Production (moles/hr/sheep)
Low isoacids	S_5	0.268
Low urea	S_2	0.172
Low sulfur	S_3	0.107
(T_1: $A_LB_LC_L$)	S_4	0.290
		Mean:0.209*
High isoacids	S_5	0.162
High urea	S_2	0.282
Low sulfur	S_3	0.256
(T_2: $A_HB_HC_L$)	S_4	0.160
		Mean:0.215*
High isoacids	S_5	0.278
Low urea	S_2	0.204
High sulfur	S_3	0.449
(T_3: $A_HB_LC_H$)	S_4	0.394
		Mean:0.331*
Low isoacids	S_5	0.193
High urea	S_2	0.300
High sulfur	S_3	0.260
(T_4: $A_LB_HC_H$)	S_4	0.262
		Mean:0.253*

*SEM = $\pm$ 0.050

Table 21. Effects of Isoacids (A), Urea (B) and Sulfur (C) on Acetate Production in the Rumen
(4 Heaviest animals)

Treatment Combination	Animal No.	Acetate Production (moles/hr/sheep)
Low isoacids	S_6	0.261
Low urea	S_7	0.067
High sulfur	S_8	0.248
(T_5: $A_LB_LC_H$)	S_{10}	0.112
		Mean: 0.172*
Low isoacids	S_6	0.141
High urea	S_7	0.135
Low sulfur	S_8	0.450
(T_6: $A_LB_HC_L$)	S_{10}	0.357
		Mean: 0.270*
High isoacids	S_6	0.277
Low urea	S_7	0.251
High sulfur	S_8	0.147
(T_7: $A_HB_LC_L$)	S_{10}	0.453
		Mean: 0.282*
High isoacids	S_6	0.472
High urea	S_7	0.445
High sulfur	S_8	0.347
(T_8: $A_HB_HC_H$)	S_{10}	0.486
		Mean: 0.438*

*SEM = $\pm$ 0.050

Mean ruminal ammonia levels are presented in Table 23. The concentrations of ammonia in the rumen contents ranged from 13.9 to 15.9 mg of ammonia-N/dl of rumen fluid when diets were supplemented with urea. They ranged from 4.8 to 7.4 mg/dl of rumen fluid when diets were not supplemented with urea. This main effect of urea was significant ($P < 0.01$). Both, rumen ammonia and hydrogen sulfide concentration primary data are presented in Tables 28 and 29.

Table 22. Pooled Treatment Comparisons of The Effects of Isoacids (A) and Sulfur (B), and N:S Ratios on Acetate Production in the Rumen.

Comparison	Treatment Contrast	Mean Difference* (moles/hr x sheep)	Significance
A/C_L	$A_HB_HC_L + A_HB_LC_L$ vs $A_LB_LC_L + A_LB_HC_L$	0.009	P > 0.10
A/C_H	$A_HB_LC_H + A_HB_HC_H$ vs $A_LB_HC_H + A_LB_LC_H$	0.172	(positive effect of isoacids, when sulfur present, P< 0.05)
C/A_L	$A_LB_HC_H + A_LB_LC_H$ vs $A_LB_LC_L + A_LB_HC_L$	0.027	P > 0.10
C/A_H	$A_HB_LC_H + A_HB_HC_H$ vs $A_HB_HC_L + A_HB_LC_L$	0.136	(positive effect of sulfur, when isoacids high, P< 0.10)
3:1 vs 5:1**	$A_HB_LC_H + A_LB_LC_H$ vs $A_LB_HC_H + A_HB_HC_H$	0.094	P > 0.10
8:1 vs 5:1	$A_LB_LC_L + A_HB_LC_L$ vs $A_LB_HC_H + A_HB_HC_H$	0.100	P > 0.10
12:1 vs 5:1	$A_HB_HC_L + A_LB_HC_L$ vs $A_LB_HC_H + A_HB_HC_H$	0.103	P > 0.10

* SED = $\pm$ 0.011
** N:S Ratios

Means of hydrogen sulfide concentration are presented in Table 23. When sheep were fed sulfur supplemented rations, the concentrations of hydrogen sulfide in the rumen were approximately 7 to 9 ug/ml of rumen fluid; compared to 0.7 to 4 ug/ml of rumen fluid when no sulfur was a added.

When mean N:S ratios of rations were compared with N:S ratios of rumen contents, the same general pattern was found (Table 24). Ratios of low N:S ratios, such as 3:1 and 5:1 resulted in nitrogen to hydrogen sulfide ration in the rumen of 9 to 10:1 and 16 to 18:1 respectively. Ratios of high N:S ratios, such as 8:1 and 12:1 gave ratios of 14:1 and 36 to 54:1 respectively. A ratio of 90:1 on treatment 1, found to be rather wide and resulted from a very low hydrogen sulfide value found in the rumen. For the rest of the treatments, this difference in N:S ratios between rations and rumen could be due to a decrease in ruminal H_2S production during the experiment.

In general, it was found that addition of sulfur to the diet resulted in higher rumen hydrogen sulfide concentrations ($P < 0.01$), Table 19.

Mean blood urea and glucose concentration in blood are represented in Table 25. Analysis of these two variables is shown in Table 26. From these analysis, significant effects of Urea (B), ($P < 0.01$) and sulfur (C), ($P < 0.05$) were found in blood urea. Blood urea increased greatly ($P < 0.01$) when diet was supplemented with urea, even when ratios were low in isoacids and without addition of sulfur.

Blood glucose levels are shown in Table 25. There was no effect of treatments on the concentration of glucose in blood.

Table 23. Effects of Treatment Combinations on Hydrogen Sulfide and Ammonia Concentration in the Rumen.

Treatment Combination	Means*	
	Sulfur ug/ml	Ammonia mg/dl
T_1: $A_LB_LC_L$	0.738	6.709
T_2: $A_HB_HC_L$	4.075	14.820
T_3: $A_HB_LC_H$	7.813	7.406
T_4: $A_LB_HC_H$	7.384	12.842
T_5: $A_LB_LC_H$	7.043	7.035
T_6: $A_LB_HC_L$	2.950	15.961
T_7: $A_HB_LC_L$	3.520	4.819
T_8: $A_HB_HC_H$	9.110	13.998
*SEM	$\pm$0.661	$\pm$1.298

Table 24. N:S Ratios in The Rumen and Rations

	Treatments							
	T_1 ($A_LB_LC_L$)	T_2 ($A_HB_HC_L$)	T_3 ($A_HB_LC_H$)	T_4 ($A_LB_HC_H$)	T_5 ($A_LB_LC_H$)	T_6 ($A_LB_HC_L$)	T_7 ($A_HB_LC_L$)	T_8 ($A_HB_HC_H$)
Rumen	90:1	36:1	9:1	18:1	10:1	54:1	14:1	16:1
Ration	8:1	12:1	3:1	5:1	3:1	12:1	8:1	5:1

Table 25. Effects of Treatment Comabinations on Urea and Glucose Con-Centration in Blood.

Treatment Combination	Means ± SEM*	
	Urea	Glucose
	(mg/dl of Whole Blood)	
T_1: $A_L B_L C_L$	4.710	37.965
T_2: $A_H B_H C_L$	7.458	43.429
T_3: $A_H B_L C_H$	7.166	43.130
T_4: $A_L B_H C_H$	8.498	40.330
T_5: $A_L B_L C_H$	7.457	33.276
T_6: $A_L B_H C_L$	11.207	31.767
T_7: $A_H B_L C_L$	5.623	38.461
T_8: $A_H B_H C_H$	9.998	40.330
*SEM	±0.929	±5.852

Table 26. Analysis of Variance of Urea and Glucose Concentrations in Blood

	FACTORS			Interactions				Periods Square	Sheep Square	Error
	A=Isoac.	B=Urea	C=Sulfur	AB	BC	AC	ABC Squares			
Glucose										
Degrees of Freedom	1	1	1	1	1	1	1	6	6	12
Mean square	211.3	1.4	8.0	0.3	5.8	10	250.3	145.4	93.2	137
Significance										
Urea										
Degrees of freedom	1	1	1	1	1	1	1	6	6	12
Mean square	0.314	46.287	21.405	0.043	1.762	1.323	7.502	1.371	5.140	3.455
Significance		**	*							

* (P< 0.05)

* *(P< 0.01)

Values found were in the range of 37 to 44 mg/dl of whole blood. This compares with reported ranges in sheep of 30 to 50 mg/dl (78). These values were only determined to find out the levels of glucose in animals in the tropics (See Table 30).

CONCLUSIONS

Ruminal concentrations of acetate, propionate and iso-butyrate were not affected by treatments. Butyrate, 2-methyl butyrate, iso-valerate and valerate levels in the rumen fluid were affected by level of iso-acids fed, or interactions of isoacids with urea and sulfur. Isovalerate and 2-methyl butyrate increased with high level of isoacids in the presence of urea, and valerate was increased by isoacids in the presence of sulfur.

Turn over time of acetate was not affected by treatments, but acetate production rates were increased when the high level of isoacids was fed (0.14 g/kg of body weight), in combination with sulfur supplementation.

Rumen ammonia and blood urea were greater when animals were fed urea. Ruminal hydrogen sulfide concentrations were increased when sulfur was added. Blood glucose levels were not affected by any treatment.

The results of this study suggest that N:S ratios are more important than absolute concentrations of ammonia in the rumen. The N:S for an optimum fermentation as measured by acetate production was 5:1. Amount of 0.43 g of urea/Kg body weight and 0.086 g of sulfur/Kg body weight gave this ratio.

Therefore, it is concluded from this study that for optimum rumen fermentation of pineapple tops by sheep the ration should be supplemented with 0.14 g of isoacids, 0.43 g of urea and 0.086 g of sulfur per Kg of body weight.

APPENDIX

Table 27. Effects of Isoacids, Urea and Sulfur on Volatile Fatty Acid Concentration in the Rumen*

Variables (acids)	Animal	Treatments							
		$A_LB_LC_L$	$A_HB_HC_L$	$A_HB_LC_H$	$A_LB_HC_H$	$A_LB_LC_H$	$A_LB_HC_L$	$A_HB_LC_L$	$A_HB_HC_H$
Acetic Acid	S_5	8.2640	7.5790	7.5750	5.5940				
	S_2	5.9306	6.4280	6.0390	5.0348				
	S_3	5.3667	8.2660	6.6250	6.2830				
	S_4	6.7429	6.6054	5.9452	4.8386				
	S_6					8.5642	6.3040	7.5620	8.1770
	S_7					6.9405	7.5757	7.4873	7.2849
	S_8					8.0690	6.4648	4.0197	7.6275
	S_{10}					6.8000	8.9160	8.1128	7.5634
	Average	6.5760	7.2196	6.5460	5.4374	7.5936	7.3151	6.7954	7.6630
Propionic	S_5	1.8848	1.1738	1.4149	1.0565				
	S_2	1.0800	1.3900	1.3310	1.4231				
	S_3	1.1412	1.4459	1.0647	1.0159				
	S_4	1.1914	1.9253	0.9400	0.9377				
	S_6					2.1908	1.2876	1.7399	1.3946
	S_7					1.2516	1.1618	1.8120	1.9502
	S_8					2.1652	1.3839	0.7220	1.2653
	S_{10}					1.5367	2.3804	1.7348	2.0342
	Average	1.3243	1.4837	1.1876	1.1083	1.7860	1.5534	1.5021	1.6610
I-Butyric	S_5	0.0733	0.1417	0.1412	0.0596				
	S_2	0.0697	0.1079	0.1030	0.1191				
	S_3	0.0551	0.0935	0.0610	0.0602				
	S_4	0.1106	0.0810	0.0447	0.0262				
	S_6					0.2397	0.0437	0.0546	0.0593
	S_7					0.0335	0.0592	0.0501	0.0516
	S_8					0.0413	0.0984	0.0251	0.0564
	S_{10}					0.0553	0.0601	0.0873	0.0567
	Average	0.0771	0.1060	0.0874	0.0663	0.0924	0.0653	0.05428	0.0560

Table 27 (cont'd.).

Variables (Acids)	Animal	Treatments							
		$A_LB_LC_L$	$A_HB_HC_L$	$A_HB_LC_H$	$A_LB_HC_H$	$A_LB_LC_H$	$A_LB_HC_L$	$A_HB_LC_L$	$A_HB_HC_H$
Butyric	S_5	0.8772	0.5248	0.5320	0.5082				
	S_2	0.5329	0.8323	0.5829	0.5901				
	S_3	0.5881	0.7770	0.6341	0.4018				
	S_4	0.5222	0.8084	0.4293	0.2567				
	S_6					0.7018	0.4049	0.4029	0.4054
	S_7					0.4267	0.4029	0.7017	0.6387
	S_8					0.7391	0.4502	0.2643	0.5773
	S_{10}					0.4660	0.8447	0.0368	0.7817
	Average	0.6301	0.7358	0.5446	0.4392	0.5836	0.5256	0.3739	0.6008
2-Methyl Butyrate	S_5	0.0418	0.1355	0.0322	0.0183				
	S_2	0.0261	0.1201	0.0552	0.0238				
	S_3	0.0289	0.0340	0.0259	0.0236				
	S_4	0.0238	0.0355	0.0253	-				
	S_6					0.0259	0.0121	0.0139	0.0195
	S_7					0.0105	0.0183	0.0309	0.0188
	S_8					0.0136	-	0.0140	0.0299
	S_{10}					0.0210	0.0135	0.0141	0.0233
	Average	0.0301	0.0814	0.0346	0.0164	0.0177	0.0109	0.0182	0.0243

Table 27 (cont'd.).

Variables (Acids)	Animal	Treatments							
		$A_LB_LC_L$	$A_HB_HC_L$	$A_HB_LC_H$	$A_LB_HC_H$	$A_LB_LC_H$	$A_LB_HC_L$	$A_HB_LC_L$	$A_HB_HC_H$
Iso-Valerate	S_5	0.0343	0.0603	0.0268	0.0120				
	S_2	0.0241	0.0503	0.0431	0.0188				
	S_3	0.0461	0.0261	0.0186	0.0239				
	S_4	0.0192	0.0266	0.0210	-				
	S_6					0.0196	-	-	0.0034
	S_7					-	0.0146	0.0237	0.0155
	S_8					0.0313	-	-	0.0261
	S_{10}					0.0048	0.004	0.0141	0.0233
	Average	0.0309	0.0408	0.0274	0.0137	0.0139	0.0038	0.0095	0.0221
Valerate	S_5	0.0597	0.0530	0.1539	0.0120				
	S_2	0.0469	0.0559	0.0446	0.0598				
	S_3	0.0717	0.0495	0.0412	0.0385				
	S_4	0.0383	0.0657	0.0915	-				
	S_6					0.0433	0.0473	0.0293	0.0547
	S_7					-	-	0.0488	0.0463
	S_8					0.0412	0.0278	-	0.0456
	S_{10}					0.0342	0.0554	0.0257	0.0509
	Average	0.0541	0.0560	0.0828	0.0270	0.0296	0.0326	0.0261	0.0494

* mg/dl of Rumen Fluid.

Table 28. . Rumen Ammonia Concentrations in Sheep Fed Diets with and without Urea Supplementation

Sheep No.	Treatments							
	Non Supplemented				Supplemented			
	$A_LB_LC_L$	$A_HB_LC_H$	$A_LB_LC_H$	$A_HB_LC_L$	$A_HB_HC_L$	$A_LB_HC_H$	$A_LB_HC_L$	$A_HB_HC_H$
	mg/dl							
S_5	8.944	5.968			20.921	13.580		
S_2	6.780	6.560			15.030	9.480		
S_3	4.737	9.769			13.500	12.826		
S_4	6.378	7.329			9.832	15.482		
S_6			8.753	4.430			14.710	12.836
S_7			5.352	4.937			13.695	14.498
S_8			8.635	6.262			19.069	22.010
S_{10}			5.403	3.848			16.373	6.650
Average	6.709	7.406	7.035	4.819	14.820	12.842	15.961	13.998

Table 29. Rumen Hydrogen Sulfide Concentration in Sheep Fed Diets with and without Sulfur Supplementation.

	Treatments							
	Non Supplemented				Supplemented			
Sheep No.	$A_LB_LC_L$	$A_HB_HC_L$	$A_LB_HC_L$	$A_HB_LC_L$	$A_HB_LC_H$	$A_LB_HC_H$	$A_LB_LC_H$	$A_HB_HC_H$
	ppm							
S_5	1.401	5.145			12.770	6.180		
S_2	0.150	3.417			7.737	10.185		
S_3	0.615	2.161			4.450	7.161		
S_4	0.787	5.580			6.297	6.012		
S_6			2.840	1.803			4.421	8.163
S_7			4.140	6.297			8.122	9.350
S_8			1.833	4.428			10.141	12.345
S_{10}			2.985	1.589			5.490	6.588
Average	0.738	4.075	2.950	3.529	7.813	7.384	7.043	9.111

Table 30. Effects of Treatment Combinations on Urea and Glucose Concentrations in Blood.

Treatment	Animal	Blood Urea (mg/dl)	Blood Glucose (mg/dl)
T_1	S_5	6.83	33.30
	S_2	4.33	56.02
	S_3	3.00	31.35
	S_4	5.00	31.19
Means		4.79	37.96
T_2	S_5	6.16	56.02
	S_2	9.66	38.88
	S_3	9.00	43.90
	S_4	5.00	34.91
Means		7.45	43.42
T_3	S_5	5.00	31.35
	S_2	10.00	38.53
	S_3	7.00	56.73
	S_4	6.66	45.90
Means		7.16	43.12
T_4	S_5	10.33	31.19
	S_2	6.33	57.71
	S_3	9.00	44.10
	S_4	8.33	28.32
Means		8.49	40.33
T_5	S_6	7.55	28.66
	S_7	8.33	50.33
	S_8	6.00	27.11
	S_{10}	8.00	27.00
Means		7.47	33.27
T_6	S_6	8.33	31.89
	S_7	8.66	34.07
	S_8	16.33	36.11
	S_{10}	11.50	25.00
Means		11.20	31.76
T_7	S_6	4.16	35.33
	S_7	6.33	35.17
	S_8	4.66	30.44
	S_{10}	7.33	52.90
Means		5.62	38.46
T_8	S_6	12.00	31.11
	S_7	9.33	31.69
	S_8	11.33	33.88
	S_{10}	7.33	57.57
Means		9.99	38.56

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