THE EFFECT OF FORMALDEHYDE TREATMENT OF THE FORAGE PORTION OF THE DIET, THE ADDITION OF BRANCHED-CHAIN VOLATILE FATTY ACIDS AND/OR SULPHUR ON THE UTILIZATION OF NITROGEN AND CARBOHYDRATE BY SHEEP

by

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ABSTRACT

Formaldehyde treatment of dietary protein to reduce its degradation in the rumen has been reported to be beneficial in some instances.

Four levels of formaldehyde (0.0%, 0.8%, 1.0% and 1.2% on an air dry basis) were applied to a dehydrated and hammermilled grass-clover forage. In vitro nitrogen digestibility and ammonia-nitrogen production at the microbial stage of incubation were reduced significantly ($p < 0.05$) as the level of formaldehyde was increased. Nitrogen digestibility for the combined microbial and acid-pepsin stages of incubation was significantly ($p < 0.05$) reduced only at the 1.2% level of formaldehyde application compared to the untreated forage.

Ram lambs ranging in body weights of 29kg to 36kg were then used in studies of nitrogen and carbohydrate metabolism. One percent formaldehyde was applied to the grass-clover forage. Each of the five diets (14% C.P. on D.M. basis) contained 50% grass-clover forage, 38% cassava, 11% barley and 1% sheep mineral premix on a dry matter basis. Diet one contained the untreated forage while the others contained the formaldehyde treated forage. Diets three and five were supplemented with isovaleric acid (3.0g/Kg diet) and isobutyric acid (2.3g/Kg diet). Diets four and five were supplemented with sulphur in the form of sodium sulphate.

The apparent digestibility coefficients of acid-detergent fibre and cellulose were increased significantly ($p < 0.05$) by formaldehyde treatment of the forage. The apparent digestibility coefficient of nitrogen was significantly ($p < 0.05$) depressed by formaldehyde treatment of the forage except for the diet supplemented with VFAS (diet three). The apparent digestibility coefficients of dry matter and organic matter were not affected significantly ($p > 0.05$).

Ruminal fluid levels of isovaleric and isobutyric acids were significantly ($p < 0.05$) higher for animals fed diet three than for animals fed diets two and four. Formaldehyde treatment of the forage resulted in significantly ($p < 0.05$) higher levels of valeric acid and lower levels of ammonia in ruminal fluid. Ruminal fluid levels of total volatile fatty acids, acetic, propionic and butyric acids and rumen pH were not affected significantly ($p > 0.05$) by formaldehyde treatment of the forage.

The ratio of microbial protein-nitrogen (estimated from RNA-N) to total abomasal digesta nitrogen was significantly ($p < 0.05$) decreased by formaldehyde treatment of the forage except for the diet supplemented with VFAS (diet three). The concentration of non-protein-nitrogen in abomasal digesta was significantly ($p < 0.05$) reduced by formaldehyde treatment of the forage. Abomasal digesta pH, concentration of total nitrogen, RNA-N, microbial protein-nitrogen, acid-detergent fibre, and cellulose were not affected.

The reduction in the ratio of microbial protein-nitrogen to total abomasal digesta nitrogen and non-protein-nitrogen by formaldehyde treatment of the forage suggests that the treatment reduced microbial degradation of dietary protein except perhaps for the diet supplemented with VFAS (diet three).

Nitrogen balance was significantly ($p < 0.05$) improved by formaldehyde treatment of the forage except for the sulphur supplemented diets.. Sulphur supplementation tended to offset the beneficial effects of formaldehyde protection of the forage protein. Supplementation with VFAS did not further enhance nitrogen utilization.

Formaldehyde treatment of the forage significantly ($p < 0.05$) improved sulphur balance except for the diet supplemented with both sulphur and VFAS (diet five).

Daily feed intake and urine output per unit metabolic body size and growth rate over a seventeen-day period were not significantly ($p > 0.05$) affected by formaldehyde treatment of the forage.

The flow of total digesta, organic matter, dry matter, acid-detergent fibre, cellulose and total nitrogen through the duodenum for a 24-hour period was markedly higher for the diets containing the formaldehyde treated forage. The flow of microbial protein-nitrogen and non-protein-nitrogen however was markedly depressed by formaldehyde treatment of the forage. A sheep fitted with a duodenal re-entrant cannula was used for this study.

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INTRODUCTION

Studies on the digestion of feedstuffs by some workers have revealed that most of the essential amino acids being made available to the ruminant are from microbial sources (Weller et al., 1958; Bergen et al., 1967; Leibholz, 1972; Phillipson, 1972). The rumen micro-organisms utilize dietary nitrogen for the synthesis of their cellular proteins. They generally prefer de novo synthesis of amino acids from simpler nitrogen sources such as ammonia, and also carbon skeletons, rather than making use of amino acids in the feed (Saeur et al., 1975; Umuna et al., 1975). The micro-organisms hydrolyze the proteins to amino acids with enzymes, de-aminate some portions, and utilize part of the ammonia released to resynthesize amino acids for incorporation into microbial protein (Church, 1975c). A portion of the released ammonia is lost to the animal. The degree of dietary nitrogen loss depends partly on the rate of protein breakdown which in turn depends on protein solubility in the ruminal fluid (Church, 1975c). This also depends on the rate of amino acid synthesis by the rumen micro-organisms. The loss of dietary protein is more than compensated for if the dietary protein is of poor quality, as the microbial protein so formed is of medium quality. The micro-organisms are killed by the abomasal acid. Then the microbial protein is made available to the animal after digestion in the lower parts of the digestive tract.

If, however, the dietary protein is of high quality, there is a loss to the ruminant due to the above processes. Therefore, attempts have been made to by-pass rumen digestion of protein when high quality dietary protein is fed. The by-pass can be in the form of abomasal infusions (Little and Mitchel, 1967; Schelling and Hatfield, 1968) or by reducing the solubility of the protein in the rumen (Ferguson et al., 1967). The common treatments applied to reduce solubility of dietary proteins in the rumen are heat or aldehydes (Phillipson, 1972), although tannic acid and volatile fatty acid treatments have been attempted successfully (Nishimuta et al., 1973; Barker et al., 1973; Candlish et al., 1973; Atwal et al., 1974).

Protection of protein contained in casein from attack by rumen micro-organisms has been reported to consistently give significantly positive responses of nitrogen balance,wool growth and growth rate (Little and Mitchell, 1967; Schelling and Hatfield, 1968; Reis and Tunks, 1969; Phillipson, 1972). This has been attributed to the higher quality of casein protein compared with rumen microbial protein.

Faichney and Davies (1972) treated groundnut (peanut) meal with formaldehyde and obtained a slight but non-significant response in nitrogen balance compared to untreated groundnut meal. Treatment of soybeam meal has been reported to give variable responses. Some workers (Schmidt et al., 1971; Schmidt et al., 1974) obtained negative responses in nitrogen balance while Peter et al. (1970), Peter et al. (1971), Nimrick et al. (1972) and Amos et al. (1974) obtained positive responses in nitrogen

balance. Little and Mitchell (1967) obtained increased nitrogen retention when soybean was infused abomasally.

There have been limited reports on formaldehyde treatment of dehydrated forages (Hemsley et al., 1970; Dinius et al., 1975; Beever et al., 1976).

Experiments, reported later, were conducted to study the effect of formaldehyde treatment of the grass-legume forage portion of the diet on nitrogen and carbohydrate utilization by sheep.

Isovaleric and isobutyric acids were added to some of the diets containing the formaldehyde treated forage. Protection of the dietary protein may result in deficiency of these acids in the ruminal fluid. el-Shazly (1952a and 1952b) reported that these volatile fatty acids result from the deamination of valine and leucine. Allison et al. (1962) reported that Ruminococcus flavefaciens strain C94, a rumen cellulolytic micro-organism requires isobutyric and isovaleric acids for growth.

Sulphur was also supplemented to some of the diets. Kennedy et al. (1975) reported that the extent of incorporation of recycled urea-nitrogen into microbial protein may be limited by the quantity of recycled sulphur. Recycling of sulphur into the rumen was reported by these workers to be less than recycling of nitrogen into the rumen. Sulphur deficiency may become more acute with the protection of dietary protein. About ninety percent (90%) of the sulphur in most plants was reported to be present in the sulphur-containing amino acids (Beaton et al., 1968). Nitrogen, on the other hand, may not be limiting in the rumen with the protection of dietary protein. Langlands (1973b) and Faichney (1974) reported adequate amounts of recycled urea-nitrogen into the rumen with formaldehyde treatment of diets for sheep.

LITERATURE REVIEW

Rumen microbiology

The ruminant is able to subsist on roughages because of the micro-organisms present in the reticulorumen that can ferment feeds. There are many types of micro-organisms in the rumen, the major ones being bacteria and ciliated protozoa. Other types observed at times in the rumen are yeast-like organisms, phages and flagellated protozoa (Church, 1975b).

Rumen bacteria

There are a number of ways rumen bacteria are classified. Methods of classification include morphology, gram-stain reaction and various products of metabolism. The use of any one of these methods alone has limitations. Rumen bacteria are too similar to identify solely on the basis of morphology. Some strains within the same species give both positive and negative reactions to the gram stain. There is usually a great deal of overlapping with different species of bacteria with regard to source of energy, substrates attacked and fermentation end-products and by-products (Church, 1975b).

In view of the problems listed above concerning identification of rumen bacteria, classification has been difficult. The classification used by Hungate (1966), based partially on substrates and partially on the end-products in *in vitro* culture systems, has been used in this literature review.

There are about ten (10) classification groups known using this system.

(a) Cellulolytic bacteria: Have the enzyme cellulase and may also be able to degrade cellobiose. These types are in greatest concentration when animals are fed fibrous rations. The most important of these cellulolytic species include Bacteroides succinogenes, Ruminococcus flavefaciens, Ruminococcus albus, Clostridium loch headii, and Cillobacterium cellulosolvens.

(b) Hemicellulose digesting bacteria: Most organisms which can hydrolyze cellulose are also able to utilize hemicellulose. A number of organisms which can utilize hemicellulose, however, cannot utilize cellulose. Some of the species which utilize hemicellulose are Butyrivibrio fibrisolvens, Lachnospira multiparus, and Bacteroides ruminicola.

(c) Amylolytic bacteria: Some cellulolytic organisms can digest starch; however, there are some organisms which are purely amylolytic and cannot digest cellulose. Amylolytic bacteria are in the greatest concentrations when rations containing large amounts of starch are fed. Examples of amylolytic organisms are: Bacteroides amylophilus, Succinimonas amylolytica, Butyrivibrio fibrisolvens, B. alactacidigens, Bacteroides ruminicola, Selenomonas ruminantium, Selenomonas lactilytica and Streptococcus bovis.

(d) Bacteria utilizing sugars: Most of the bacteria utilizing polysaccharides are also able to utilize sugars. The sugars may originate from plants, dead and lysing bacteria cells, or from capsular material

(mostly carbohydrates of bacterial cell walls). High concentrations of micro-organisms dependent on lactose for energy are present in the rumens of young animals. An example of bacteria utilizing sugars is Eubacterium ruminantium.

(e) Bacteria utilizing organic acids: The organic acids which are probably utilized to the greatest extent are lactic, succinic, malic and fumaric. Others including formic, acetic and oxalic are also utilized by some organisms. The latter may not be the only source of energy to the micro-organisms utilizing them. Some lactic acid utilizing organisms are: Veillonella gazogenes, V. alacalescens, Peptostreptoccus elsdenii, Propionibacterium sp., Desulphovibrio sp., and Selenomonas lactilytica.

(f) Proteolytic bacteria: Bacteria that utilize amino acids as the primary energy sources. Bacteroides amylophilus, Clostridium sporogenes and Bacillus licheniformis are the best known to have proteolytic capability.

(g) Ammonia producing bacteria: These produce ammonia from various sources. Examples of these are Selenomonas ruminantium, Peptostreptococcus elsdenii, Bacteroides ruminicola, and certain strains of Butyrivibrio sp.

(h) Methanogenic bacteria: These produce methane gas. They are obligate anaerobes and are therefore difficult to culture. Since normally about 25% of the gas in the rumen is methane (Church, 1975b), a large number of them are indicated. Important examples of these are Methanobacterium ruminantium, and M. formicium. Other species of less importance are Methanobacterium sohngenii, M. suboxydans and Methanosarcina sp.

(i) Lipolytic bacteria: These utilize glycerol and hydrolyze glycerol from lipids. Some lypolytic micro-organisms hydrogenate unsaturated fatty acids and may produce positional isomerization in fatty acids. Certain lipolytic micro-organisms may metabolize long chain fatty acids into ketones. Viviani (1970) reported of one lypolytic bacterium, Anaerovibrio lypolytica. Hobson and Mann (1961) reported Selenomonas ruminantium strain lactilyticans as a glycerol fermenter.

(j) Vitamin synthesizers: Some rumen bacteria are able to synthesize B- complex vitamins including cobalamin (vit. B12) provided they are supplied with adequate cobalt (Phillipson, 1975). The vitamin synthesizers have not been studied extensively.

Rumen Protozoa

Rumen protozoa are classified according to morphology. They are easier to classify by that method compared to bacteria because of their larger size. Counts of protozoa in the rumen vary from none to as high as five (5) million per ml of rumen fluid. The size of rumen protozoa varies from thirty eight microns in length and fifteen microns in width for Charon equi to one hundred and ninety five microns in length and one hundred and nine microns in width for Metadinium medium. Rumen protozoa are mainly ciliates although at times such flagellates as Monocercomonas ruminantium, Callimastix frontalis, Tetratichomonas sp., Pentatrichomonas hominis, Monocercomonas bovis and Chilomastix sp. are observed.

The most important rumen ciliates belong to subclasses holotrichia and spirotrichia. Examples of the holotrichia are Dasytricha ruminantium, Isotricha intestinalis, Isotricha prostoma and also Charon equi, which is rare. Examples of the spirotrichia subclass belonging to the order entodinimorpha (oligotrichs) are Diplodinium spp., Eudiplodinium spp., Polyplastron spp., Elystroplastron spp., Ostracodinicum spp., and Enoploplastron spp. Rumen protozoa are obligate anaerobes.

Other rumen micro-organisms

Other rumen micro-organisms include bacteriophages (bacterial viruses) yeast-like organisms, and facultatively anaerobic fungi. There are about one hundred and twenty five (125) morphologically distinct types of bacteriophages observed in the rumen. Some have been observed inside rumen bacteria. The bacteriophages may exceed rumen bacteria in numbers by two to ten times. Pun and Satter (1975) reported the existence of nitrogen fixing bacteria in the rumen but believed these bacteria were not of any significant value.

Factors affecting populations of rumen micro-organisms

Many kinds of micro-organisms are present in the rumen. There are many ways by which micro-organisms may arrive in the rumen. Methods of entering the rumen include being carried by food, water, licking and drenching with drugs (Church, 1975b). Micro-organisms that are normally

not part of the rumen microbial population may be present at times. Gall and Hubtanen (1950) listed the following as criteria to be satisfied if micro-organisms are to be considered typical of the rumen environment:

(a) the organism must be able to live anaerobically;

(b) it should be able to produce the types of end-products characteristic of the rumen, and

(c) the rumen should contain not less than one million per gram of the organisms.

This last criterion applies to bacteria but not protozoa.

It should also be noted that animals on the same or similar diets might have different microbial populations.

One of the factors which can affect type and number of micro-organisms in the rumen is cyclical variations. Inhibition by volatile fatty acids on the growth of some others such as Escherichia coli has been reported. Restriction in growth of one type of microbe by toxins or antibiotics produced by another type has been postulated. Those micro-organisms producing the toxins and antibiotics are not affected (Church, 1975b). Hungate (1970) reported that mycoplasma which can kill bacteria and protozoa have been observed free in the rumen. The mycoplasma secretes an enzyme or enzymes capable of digesting Butyrivibrio sp., Ruminococcus sp., and Escherichia coli but not the gram-positive Streptococcus bovis.

Diet can affect microbial populations in the rumen. The diet may also be influenced by geographic location or season. Some types of micro-organisms may be present in some ruminant species but not others. For instance a large oval form of bacteria (Quinn's oval) is common in sheep but not cattle. Perhaps the most important influencing factor is the diet. Church (1975b) indicated that the optimum pH for growth of rumen micro-organisms is between 5.5 and 7 while the optimum temperature range is 39-41^{o}C.

Eadie and Mann (1970) reported that high soluble carbohydrate levels in the diet rapidly results in low pH from the production of excess acid. This in turn changes the micro-flora and fauna. Under such conditions, the only ciliates found are Entodinia sp. (Eadie and Mann, 1970). Hobson (1971) however was of the opinion that protozoa are absent below pH 6. Church (1975b) also reported that diets high in soluble carbohydrates generally result in a depression in the numbers of cellulolytic organisms. Eadie and Mann (1970) however reported that with diets high in soluble carbohydrates viable bacteria counts may be higher than with roughage diets. These workers also reported flagellate protozoa and large bacteria such as Selenomonas sp. to be present in high concentration in animals fed high carbohydrate diets. Slyter et al. (1970) also reported that the level of feeding of high carbohydrate diets affected the types of rumen microbes. Full feeding of a high carbohydrate diet reduced the number of protozoa while restricted feeding did not. These workers also reported that feeding of high carbohydrate diets caused more unstable rumen conditions than feeding

roughage diets. These unstable conditions resulted in greater animal variations even with identical twins with respect to stability of pH, and volatile fatty acid production. They also indicated that animals with least ability to stabilize rumen conditions were the first to lose the ciliate protozoa.

Certain rumen micro-organisms also require some factors which are produced by others. Factors which inhibit the growth of those producing these factors inhibit the population of those requiring these factors (Church, 1975b). Bacteroides succinogenes, R. albus, and Butyrivibrio fibrisolvens require B vitamins produced by other microbes (Church, 1975b). Some ammonia utilizing bacteria, especially Bacteroides succinogenes and also R. flavefaciens require isovaleric, N-valeric iso-butyric and 2-methyl butyric acids, for their growth (Allison et al., 1962; Allison and Bryant, 1963; Hemsley and Moir, 1963; Allison et al., 1966). Allison and Bryant (1963) thought that the mechanism for the synthesis of the isopropyl moiety in the branched-chain fatty acids was inadequate in these micro-organisms. el-Shazly (1952a and 1952b) reported that the branched-chain fatty acids resulted from the de-amination of proteins. Sulphur is also required by some micro-organisms (Kennedy et al., 1975). Hobson (1971) claimed that facultative anaerobes such as Streptococcus bovis and Veillonella gazogenes take up the small amount of oxygen existing in the rumen, this enables the methanogenic bacteria and protozoa which are completely anaerobic to survive. This also helps Bacteroides amylophilus and Selenomonas ruminantium which cannot tolerate oxidation-reduction potential of even - 45 mv. Methane inhibitors such as bromochloromethane and unsaturated fatty acids have been used to

suppress methane production (Johnson et al., 1972; Sawyer et al., 1974). These may also adversely affect the populations of methanogenic bacteria.

Cyclical variations in protozoal numbers are more pronounced than bacterial numbers. Highest protozoal concentrations have been recorded two hours after feeding (Clarke, 1965) and bacteria numbers are highest about 4-8 hours after feeding. (Moir and Sommers, 1956; Bryant and Robinson, 1968). Seasonal variations are due mainly to changes in feed. Nitrogen deficiency or mineral deficiency due to seasonal effects on diet, can limit growth (Schwartz and Gilchrist, 1975; Church, 1975b; Amos et al., 1976a).

Microbial protein-nitrogen composition and microbial protein synthesis

The nitrogen content of rumen bacteria is about 10.5% of the cell dry matter (i.e. about 65% CP). Amino nitrogen is about 75% of the total nitrogen in mixed organisms from the rumen (bacteria, protozoa and others). Rumen bacteria contain about 86% of their total nitrogen as amino nitrogen (Allison, 1970). The non-amino-nitrogen content of rumen bacteria is mainly nucleic acids, DNA and RNA. Nucleic acid may account for 14-19% of the total nitrogen of rumen micro-organisms. Most of this is RNA since DNA accounts for 2.2 - 4.1% of the total nitrogen (Allison, 1970). Little has been done to characterize the walls of rumen bacteria. The cell-wall of several non-rumen bacteria accounts for 10-20% of the mass ofthe cell and the cell-wall material in these organisms is 5-10% N (Allison, 1970). If rumen bacteria have similar proportions of walls

and wall nitrogen and if the total cells have a nitrogen content of 10.5% then cell-wall nitrogen would be 5-19% of the total cell nitrogen. (Allison, 1970). The bacterial cell walls may have peptidoglycan or mucoprotein as major components. These are resistant to trypsin and pepsin attack and may not be useful to the host. Non-amino-nitrogen in the cell wall is mainly in the form of neucleic acids. Muramic acid and α, ϵ-diaminopimelic acids are unique to bacterial cell walls while 2-aminoethylphosphonic acid is also unique to protozoal cell walls (Work, 1951; Work and Dewey, 1958; Allison, 1970). Bergen et al. (1967) reported that the limiting amino acid in pooled cellulolytic strains was methionine and for non-cellulolytic strains was leucine. Protozoal proteins are more digestible than bacterial proteins and protozoa have a higher lysine content than bacteria (Church, 1975b).

Hogan and Weston (1970) indicated that about 10-12g dry weight of microbes are synthesized per mole of ATP but DM microbial weight synthesis could increase to about 20g per mole ATP, with an increase in dilution rate from 0.1 hr^{-1} to 0.3 to 0.5 hr^{-1}. They indicated that 2.37g of bacteria nitrogen are produced per mole of volatile fatty acid produced. Walker and Nader (1968), reported 13-14g dry weight of microbial cells synthesized per mole of ATP. Hume (1970) reported an increase in microbial protein synthesis with the addition of 2-methylbutyric, isovaleric and n-valeric acids to diets containing non-protein-nitrogen. Other workers (Hemsley and Moir, 1963; Umuna et al., 1975) have also reported increases in microbial protein synthesis when some branched-chain fatty acids and valeric acid were added to diets containing urea.

Beever et al. (1977) reported microbial protein synthesis per 100g O.M. digested in the rumen of 16.7g for a grass silage diet and 6.6g for a formaldehyde treated grass silage diet. Hume (1970) reported a negative correlation between acetic acid proportions in rumen volatile fatty acids and protein synthesis ($r = -0.62$, $p < 0.025$). He concluded that the efficiency with which energy was used for microbial growth was diminished as acetic acid proportions in rumen fluid increased. Schwartz and Gilchrist (1975) and Ishaque et al. (1971) also reported that propionic acid fermentation was more efficient for microbial protein synthesis than acetic or butyric acid fermentation. Harrison et al. (1976) however reported acetic acid fermentation to be more efficient for microbial protein synthesis than propionic acid fermentation. These workers infused artificial saliva at the rate of four litres per day to alter propionic acid fermentation to acetic acid fermentation. The effect of the increased flow rate was not delineated.

A number of methods have been used to assess microbial protein synthesis. These include measuring diaminopimelic acid (DAP) and aminoethylphosphonic acid (AEP) in the sample (Hogan and Weston, 1970; Amos, et al., 1976a); precipitation of microbial protein with trichloroacetic, perchloric, picric and tungistic acids and measuring the amount of the precipitated protein (Hemsley and Moir, 1963; Hume, 1970; Barr et al., 1975); labelling the rumen pool with radioactive sulphur, ^{35}S, and measuring radioactivity level of sulphur in sulphur-containing amino acids (Walker and Nader, 1975; Hume, 1974); and the measurement of ribonucleic acid-nitrogen (Smith, 1975).

All the methods other than the RNA-N, labelling with ^{35}S and the combination of DAP and AEP measure mainly rumen bacterial protein synthesis. The DAP measures bacterial protein synthesis and AEP measures protozoal protein synthesis (Work, 1951; Work and Dewey, 1958; Church, 1975b). The RNA-N method measures both bacterial and protozoa protein synthesis without apportioning fractions to each type of organism (Smith, 1975). With the ^{35}S method, problems of infusion to maintain steady state conditions exist. Smith (1975) believes that the DAP method has disadvantages. One of these is that DAP released into the rumen from cell lysis due to recycling of bacteria is resistant to degradation and thus increases measured protein synthesis. The AEP method according to Smith (1975) is unreliable and unsatisfactory. The technical bulletin of the International Atomic Energy Agency (IAEA) (1970) indicates that DAP is restricted to gram-negative bacteria and bacteria contents of DAP vary greatly. Hogan and Weston (1970) estimated that bacterial contents of DAP vary from 35-46 mg/g DM bacterial nitrogen with an average of 41 mg/g.

Smith (1975) believes that the RNA-N method is more reliable since the ratio of RNA-N to total nitrogen is about 0.075 ± 0.010 and for DAP-N to total nitrogen is 0.5 - 1.1 for different bacterial species. RNA is associated with ribosomes and the quantity of ribosomes in bacterial cells is positively correlated with the rate of protein synthesis (Church, 1975b). Although there is about 30% recycling of RNA in the rumen, RNA is broken down quickly and may not affect measured protein synthesis (McAllan and Smith, 1973; Smith, 1975; Smith and Smith, 1977). Dietary RNA and other sources of RNA introduced into the rumen are also quickly broken down (Smith, 1975; Smith and Smith, 1977).

Metabolism of nitrogen

There is quite a variation in the nitrogenous compounds presented to the rumen micro-organisms. The most important are: proteins, nucleic acids and non-protein nitrogen consisting of amino acids, peptides, amides, amines, volatile amines, ammonium salts, nitrates, nitrites, urea and at times biuret intentionally put into the diet. The non-protein-nitrogen in many natural feedstuffs ranges from 4 to 5% of total nitrogen in some seeds and 60-75% of total nitrogen in unwilted silages. (Church, 1975c). Allison (1970) indicated that about 5-10% of the total nitrogen may be bound with lignin in the cell wall and is largely indigestible. Yu and Thomas (1976) estimated that about 7% of total nitrogen is present in the acid-detergent insoluble nitrogen in normal forages. The diversity of nitrogenous compounds presented to rumen microbes results in considerable variation in the nitrogen metabolism in the reticulo-rumen.

Nitrogen metabolism in the ruminant is described by the diagram of Houpt (1970) reproduced as Figure 1.

Degradation of Proteins

Many researchers (Allison and Peel, 1971; Mathison and Milligan, 1971; Nolan and Leng, 1972; Umuna *et al.*, 1975) have indicated that rumen micro-organisms prefer *de novo* synthesis of proteins. Dietary proteins, except globulins in young animals, may be degraded before

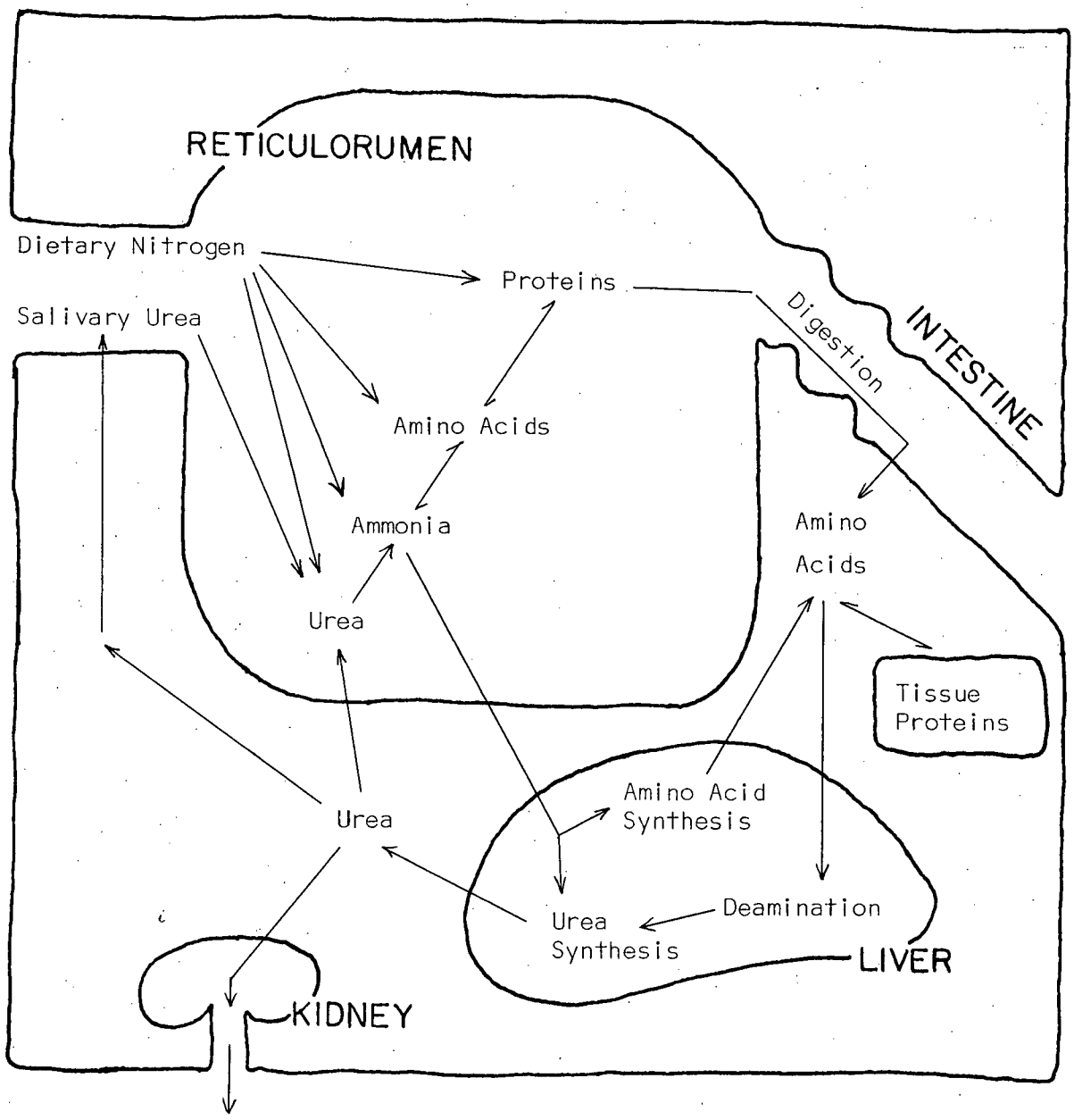

Fig. 1. Schematic representation of the protein regeneration cycle in ruminants. (Adapted from Houpt, 1970)

being utilized by the microbes. The end-products of protein degradation in the rumen are carbon dioxide, hydrogen sulphide acetic, propionic and butyric acids, higher branched-chain fatty acids containing six carbon atoms and ammonia (0-130 mg%, as NH_3-N) (Church, 1975c). Some intermediary products including free amino acids (0.1-1.5 mg%), diffusible peptides (0.2-1.0 mg%), nucleotide - N (1.5-40 mg%), and protein-N(100-400 mg%) have been shown (el-Shazly, 1952a and b; Annison, 1956; Ellis and Pfander, 1965; Allison, 1970; Smith and McAllan, 1970; Allison and Peel, 1971). Microbial nitrogen as a proportion of the total nitrogen in the rumen is quite variable - about 63-81% (Weller et al., 1958; Blackburn and Hobson, 1960). Blackburn and Hobson (1960) estimated that about 47-77% of the nitrogen in the rumen was contained in protozoa and bacteria, while 54-74% was present in the rumen fluid.

The proportion of dietary protein escaping degradation in the rumen is affected by many factors which affect retention times in the reticulo-rumen. These include specific gravity, particle size of diet, and high water consumption resulting from high salt intake (Chalupa, 1975). Solubility of the protein also affects dietary protein degradation in the rumen (Hemsley et al., 1970; Chalupa, 1975). Chalupa (1975) also indicated that feeding high levels of soluble carbohydrates, which decreases rumen pH, decreased degradation of protein in the rumen. However, Tagari et al. (1964) reported greater degradation of protein in the rumen by substituting readily soluble carbohydrates for roughages. Chalupa (1975) reported that about 40-80% of dietary protein may be degraded in the rumen. Satter and Roffler (1975) gave the following as the percentage of protein

escaping degradation for various feedstuffs fed in basal rations: barley 10%, cottonseed meal and peanut meal 20%, sunflower meal 25%, soybean meal 45%, dried grass and white fish meal 50%, and Peruvian fish meal 70%. Ferguson (1975) indicated that 61% of soybean meal, 56% of zein, and 9% of casein proteins escaped degradation. Nolan and Leng (1972) reported that about 59% of the protein entering the rumen was degraded there; while 29% of the degraded protein was utilized as amino acids, 71% was further degraded to ammonia. Nolan (1975) reported that about 30% of dietary protein intake is generally degraded to ammonia with dried and processed forage diets. He estimated that about 70% of the dietary protein either passes intact from the rumen or is assimilated by rumen micro-organisms in the form of compounds other than ammonia, such as peptides, amino acids or nucleic acid bases. Pilgrim et al. (1969) estimated that 23-27% of dietary protein in alfalfa hay was converted to ammonia as compared to 17% from alfalfa pellets.

The rate of deamination of amino acids varies with the amino acids in question. Isaacs and Owens (1971) reported that aspartic and glutamic acids, and arginine were degraded in the rumen to the extent of about 90%; valine, leucine, isoleucine, methionine, alanine and glycine appeared to be relatively stable toward microbial action. The phenolic amino acids were degraded to about 50%.

Some of the proteolytic and ammonia producing bacteria have been listed previously under rumen microbiology. Blackburn and Hobson (1960) indicated that proteolytic activity was maximum between pH 6 and 7 and is not dependent on diet. Blackburn (1968) also suggested that proteases

obtained from B. amylophilus had a broad area of activity between pH 5.5-9.5. Isaac and Owens (1971) reported that extra-cellular enzymes other than amino peptidase could possibly help in hydrolyzing proteins in the rumen. Allison (1970) indicated that although proteolytic enzymes may be mainly cell-bound, some gram positive cocci such as Clostridium sp., Eubacterium sp., and Lachnospira multiparus produce extra-cellular enzymes. Allison (1970) also reported that in B. amylophilus the protease is a constitutive enzyme. Abou Akkada and Blackburn (1963) reported that proteases in some proteolytic microbes possessed both exo- and endo-peptidase activities.

Proteolytic activity of rumen protozoa is not well understood. Abou Akkada and Howard (1962) reported rumen protozoa to hydrolyze casein to peptides and amino acids, as principal end-products. Ammonia was formed as a result of the hydrolysis of the amide groups in the casein but not from de-amination of amino acids. Warner (1956) thought that ammonia was the end-product of nitrogen metabolism in mixed suspensions of protozoa. Purser and Moir (1966) observed high concentrations of ammonia in the rumen of sheep containing protozoa compared to defaunated animals. Blackburn (1965) noted that though many proteolytic enzymes have been extracted from rumen protozoa, it is difficult to claim with certainty that they were produced by the protozoa and not by bacteria found in them. Ciliates are noted to take up only small quantities of ^{15}N-labelled amino acids and may therefore depend greatly on rumen bacteria for amino acids and should most probably have proteolytic enzymes (Allison, 1970).

The concentration of ammonia in the rumen fluid is affected by time of feeding and type of diet (Church, 1975c). Ammonia-nitrogen concentration is highest at 90-130 minutes after feeding (Church, 1975c). The addition of starch to roughage basal diets containing either casein or urea resulted in increased efficiency of ammonia utilization and consequently lowered rumen ammonia levels (Barej et al., 1970). In contrast, Tagari et al. (1964) and Schwartz and Gilchrist (1975) indicated that protein breakdown in the rumen could be enhanced and ammonia levels increased when high levels of starch are fed since the most proteolytic species, B. amylophilus, S. ruminantium and M. elsdenii could increase in numbers ten fold. Satter and Roffler (1975) reported that maintenance of ruminal ammonia-nitrogen levels in excess of 5mg/100 ml rumen fluid does not improve microbial protein synthesis. They calculated that rumen ammonia-nitrogen levels in dairy cattle fed normal dairy diets may vary from 1-5mg/100ml depending on the energy content of the diet with the highest levels associated with lowest energy content diet.

Studies on the degradation of proteins in the rumen are complicated by secretion of urea into the rumen via saliva, and of ammonia through the rumen epithelium, absorption of ammonia and other nitrogenous compounds by the rumen epithelium and recycling of microbial protein within the rumen (Church, 1975c). Microbial protein is readily hydrolyzed and deaminated. Microbial protein recycling and microbial protein synthesis from urea and/or ammonia and free amino acids may be relatively more important than degradation of ingested protein to be used for microbial protein synthesis (Church, 1975c). Nolan and Leng (1972), estimated

that about 4.3gm/N might be recycled per day within the rumen of sheep. They also suggested that about 30% of ammonia continually being incorporated into ruminal microbial protein may have been recycled through the amino acid and ammonia pools. Chalupa (1975), reported that about 30% of bacterial protein is degraded in the rumen. Hume (1970), was also of the opinion that desquamation of rumen epithelium though small, could be a source of nitrogen to rumen micro-organisms.

Urea transfer into the rumen ammonia pool from blood (dependent on rumen ammonia concentration) is one source of nitrogen to rumen microbes (Church, 1975c). Nolan (1975) reported that about 1-2g of urea was transferred daily in sheep fed lucerne chaff diets. Houpt (1970) reported that on low protein diets about 92% of endogenous urea enters the alimentary canal and about 84% is converted to complex nitrogenous compounds. Hemler and Bartley (1971) were of the opinion that more urea enters the rumen from blood than from saliva. Hogan (1975) however thought that more urea entered the rumen from saliva than from plasma. Hogan et al. (1969) and Hogan (1975) estimated urea entering the rumen of sheep to be about 2-5g N per day and thought almost all came from saliva since about 2-5g, N/day could be present in saliva secreted (about 10 litres containing 28mg N/100 ml on a roughage diet). Allen and Miller (1976) reported urea entry into rumen to be an active process rather than by simple diffusion. Houpt (1970) considers that the major portion of the urea entering the rumen from blood is converted to ammonia by rumen bacterial urease within the cornified layers before entering the rumen.

Urea from saliva, blood and in the diet is hydrolyzed by ureolytic enzymes probably produced by several bacteria (Allison, 1970). The enzymes from the mixed bacteria were stimulated by Mn, Mg, Ca, Sr, Ba, but inhibited by Na, K and Co. An enzyme from a single bacteria strain was not stimulated by all the divalent ions.

Ammonia produced in the rumen could be used for (1) microbial amino acid synthesis or (2) could be absorbed into the blood stream from the reticulo-rumen and omasum or (3) could pass into the abomasum and consequently the duodenum, since there is virtually no absorption of ammonia from the abomasum. Part of the ammonia absorbed from the forestomach could be lost through urine after conversion to urea or the urea could be recycled. Part of the ammonia could also be used for the synthesis of amino acids in the liver (Houpt, 1970; Hembry et al., 1975). The absorption of ammonia from the rumen is dependent on the rate of microbial protein synthesis which in turn is dependent on the rate of ammonia formation and energy made available to the microbes (Hembry et al., 1975). Hemler and Bartley (1971) and Hogan (1961) also reported that ammonia absorption from the rumen depends on the concentration gradient at pH 6.5. It was negligible at pH 4.5. Estimates of ammonia utilized for any of the three functions have been variable. Nolan and Leng (1972) showed that about 80% of the microbial nitrogen was derived from ammonia and only about 20% of microbial nitrogen came directly from amino acids. Mathison and Milligan (1971) estimated that 50-65% of bacterial nitrogen and 31-55% of protozoal nitrogen were derived from rumen ammonia. They also indicated that 17-54% of the ammonia derived from dietary protein (about 60-92% of dietary protein transferred to ammonia by their

estimation) of chopped hay or barley plus chopped hay, was absorbed from the rumen. Nolan (1975) reported that bacterial nitrogen derived from ammonia was about 30-80% and protozoal nitrogen derived from ammonia 25-64%. Hogan and Weston (1967) reported that in sheep fed high protein diets (C.P. 19.8%) up to 31% of the nitrogen disappeared between the ingested feed and duodenal digestion. Pilgrim et al. (1969) estimated that 57-66% of the ammonia-nitrogen was absorbed from the rumen or utilized for microbial protein synthesis and the remainder passed on into the omasum.

Protein anabolism by rumen bacteria

Rumen microbes are able to synthesize both essential and non-essential amino acids. Sauer et al. (1975) reported that rumen microbes readily utilized ^{14}C-labelled-HCO_3 and acetate for the synthesis of the carbon skeletons of amino acids and subsequently the amino acids. In contrast, ^{14}C-labelled propionate was utilized for isoleucine biosynthesis but labelled ^{14}C from propionate failed to appear in other amino acids to any significant extent. Forward tricarboxylic acid cycle reactions only proceeded to keto- or 2-oxo- glutarate. Acetate was carboxylated to pyruvate which was then carboxylated to oxaloacetate. Oxaloacetate then equilibrated with fumarate and thereby carbon atoms 1 and 4 as well as 2 and 3 became randomized. Most of the 2-oxo precursors of amino acids appeared to be formed via ferrodoxin dependent reductive carboxylation. Of the amino acid precursors investigated,

only 3-hydroxypyruvate, the precursor of serine, appeared to be synthesized via an oxidative step, (i.e. 3-phosphoglyceric acid to 3-phosphohydroxypyruvic acid). Rumen microbes reutilized benzene rings in the biosynthesis of phenylalanine and tyrosine. De novo synthesis of the benzene ring was of minor importance. Kristensen (1974) was also of the opinion that reductive carboxylation of phenylacetate and indole-3-acetic acid to form phenylalanine and tryptophan respectively was of greater importance than de novo synthesis of the carbon skeletons.

Reductive carboxylation of preformed isobutyrate to form valine was reported by Sauer et al. (1975) to be of minor importance to de novo synthesis of the carbon skeleton from acetate. There was however a considerable degree of reductive carboxylation of preformed isovaleric acid to form leucine as compared to de novo synthesis of the carbon skeleton. Allison et al. (1962), Allison and Bryant (1963), and Allison et al. (1966) suggested that biosynthesis of the isopropyl moiety was inadequate because a considerable quantity of materials containing this group is present in cellular lipid where isopentadecanoic acid is a major fatty acid, after finding branched-chain fatty acids as requirements for R. flavefaciens strain C94.

Effect of formaldehyde treatment on the digestion of proteins in the rumen

The digestion of dietary proteins in the rumen by rumen micro-organisms could either be beneficial or wasteful to the host (Phillipson, 1972). The extent of microbial degradation is dependent on protein

solubility. One way to reduce protein solubility and then digestion in the rumen is to treat the feedstuff with formaldehyde (Ferguson et al., 1967; Ferguson, 1975).

Barry (1976a) described the reaction of formaldehyde with proteins by three equations:

$$R - XH + HCHO \rightleftharpoons R - X - CH_2OH \quad (1)$$

$$R - X - CH_2OH + R^1 - \overset{\alpha}{NH_2} \longrightarrow R - X - CH_2 - \overset{\alpha}{NH} - R^1 + H_2O \quad (2)$$

(Methlylene compound)

$$R - XH + R^1 - \overset{\alpha}{NH} - CH_2OH \longrightarrow R - X - CH_2 - \overset{\alpha}{NH} - R^1 + H_2O \quad (3)$$

The first reaction is said to be rapid and occurs at neutral pH and room temperature. -XH can be a terminal amino group, the primary amide groups of asparagine and glutamine and the epsilon amino and guanidyl groups of lysine, and arginine respectively. The phenol group of tyrosine and phenyl group from phenylalanine, the indole group of tryptophan and the imadazole group of histidine may take part in the reactions under conditions other than neutral pH and room temperature, such as high temperature. After the formation of the methylol compounds, condensation reactions then take place slowly over time, with the formation of methylene cross-linkages between protein chains (Equations 2 and 3). These methylene cross-linkages are stable in the near-neutral pH of the rumen but the H^+ ions in the abomasum break down the linkage with the

release of formaldehyde (Rattray and Joyce, 1970).

Formaldehyde is not harmful to rumen microbes at the low concentrations used to treat feeds. Mills et al. (1972) using ^{14}C-labelled formaldehyde reported that ruminants effectively metabolized formaldehyde and there was no accumulation of it in the carcass or milk. Sixty to eighty percent (60-80%) of the formaldehyde was metabolized to carbon dioxide and methane, 11-27% was voided in the faeces and 5-6% excreted in the urine. Small activities of ^{14}C from labelled formaldehyde were detected in milk and body tissues but not as formaldehyde. The pathways whereby formaldehyde is converted to methane are obscure, but three mechanisms have been proposed by Mills et al. (1972).

(a) Formaldehyde is converted to formate and it is subsequently metabolized to carbon dioxide and methane.

(b) Formaldehyde is successively reduced to methanol and methane.

(c) There is an acyloin condensation of formaldehyde with ribose-5-phosphate to form allulose-6-phosphate which is further metabolized via the glycolytic sequences to produce methane and carbon dioxide.

Dinius et al. (1974) also reported that treatment of protein per se with formaldehyde did not interfere with rumen microbial activity.

Feedstuffs that have been treated with formaldehyde can be classified into oil seed meals, fish meal, casein, dried forages, silages, hays and cereal grains. The response with formaldehyde treatment of proteins has been variable. Treatment of casein has been reported to consistently yield positive responses of nitrogen balance, growth rate and wool growth (Little and Mitchell, 1967; Schelling and Hatfield, 1968; Reis and Tunks, 1969; Faichney, 1971; Faichney and Weston, 1971; Phillipson, 1972; Sharma and Ingalls, 1974). Treatment of oil seed meals such as peanut, soybean and rapeseed meals at times gives positive response and at times no response or at times negative response. Schmidt et al. (1971) and Schmidt et al. (1974) obtained negative responses while Peter et al. (1970), Peter et al. (1971), Nimrick et al. (1972) and Amos et al. (1974) obtained positive responses of nitrogen balance growth rate and wool growth with treatment of soybean meal. Sharma and Ingalls (1974) and Sharma et al. (1972) did not get a response with the treatment of rapeseed meal.

Faichney and Davies (1972) and Faichney and Davies (1973) reported that the growth rate of calves improved when formaldehyde treated groundnut (peanut) meal was used in diets containing either 12% or 13.4% crude protein. However, formaldehyde treatment did not improve gains when the diets contained either 15% or 20.5% crude protein. Faichney (1972) however reported that with higher crude protein diets (C.P. 20%) in which 50% of the crude protein was from treated or untreated peanut meal, treatment increased the proportion of protein digested in the small intestine, compared with a diet of lower crude protein content (13% C.P.).

Langlands (1971b) and Saville et al. (1971) observed no response with treatment of cottonseed meal. Rattray and Joyce (1970) reported responses with treatment of linseed meal but not meat meal.

Many workers have treated crops with formaldehyde before ensiling (Barry and Fennessy, 1972; Brown and Valentine, 1972; Barker et al., 1973; Valentine and Brown, 1973; Barry, 1975; Valentine and Radcliffe, 1975; Barry, 1976b; Binnie and Barry, 1976). Some workers have reported positive responses such as increased feed intake, feed conversion efficiency, gain in weight and production of milk, butterfat, milk protein and SNF with formaldehyde treatment of silages (Valentine and Brown, 1973; Barry, 1975; Valentine and Radcliffe, 1975; Binnie and Barry, 1976).

Barry (1973a) reported that formaldehyde treatment of rye-grass clover hay significantly reduced nitrogen, energy and organic matter digestibilities. Barry (1973b) reported reduction in liveweight losses when formaldehyde treated rye-grass-clover hay was fed at maintenance and half-maintenance levels to sheep. Amos et al. (1976b) observed no significant differences in nitrogen-balance when formaldehyde treated grass hay or untreated hay were fed at 600g DM to 18kg sheep. When 775g DM were fed daily, there were significant improvements in the flow of essential and non-essential amino acids, when treated hay was fed compared to the untreated. Langlands (1973a and 1973b) and Entwistle (1973) reported improvement in nitrogen utilization with treatment of wheat.

There has been limited work reported on formaldehyde treatment of artificially dried forages. Dinius et al. (1975) using diets (16% C.P.) containing 75% alfalfa meal on a DM basis showed that formaldehyde treatment of the alfalfa at 1% and 2% levels significantly reduced energy, dry matter, crude protein, acid-detergent fibre digestibilities and nitrogen retention.

However, Hemsley et al. (1970) reported that 1% formaldehyde treatment of dried forage containing about 25% C.P. improved wool growth by 15%. Digestibility of fibre was not affected but nitrogen digestibility was reduced. Beever et al. (1976) reported decreased nitrogen digestibility with formaldehyde treatment of dried forage but cellulose digestion was improved over untreated.

Possible reasons for the varible responses to formaldehyde treatment of different types of proteins

Treatment of casein with formaldehyde has been associated with positive responses of nitrogen retention, milk production, wool growth or growth rate because this source of protein is of higher quality than microbial protein. The host therefore benefits from protection against microbial degradation (Reis and Tunks, 1969; Langlands, 1971a; Amos et al., 1974; Phillipson, 1972). Treatment also reduces digestion of proteins in the rumen which also reduces losses of nitrogen in the form of ammonia. Ammonia levels in rumen fluid have been reduced by formaldehyde treatment (Hemsley et al., 1970; Hogan and Weston, 1970; Sharma et al., 1972; Sharma and Ingalls, 1973; Bhargava and Ranjhan, 1974;

Sharma and Nicholson, 1975b). Other workers however did not observe reduced rumen ammonia-nitrogen levels with treatment (Dinius et al., 1975; Sharma and Nicholson, 1975a). Sharma et al. (1972) reported reduced plasma urea-nitrogen levels with treatment of rapeseed meal but in the subsequent work (Sharma and Ingalls, 1974) there was no effect on urea-nitrogen levels in the blood by treatment. Barry and Fennessy (1973) reported that before feeding, rumen ammonia levels were higher in sheep being fed formaldehyde treated silages compared to those being fed untreated silages. Sharma and Nicholson (1975b) observed decreased rumen ammonia nitrogen levels with formaldehyde treatment of faba bean meal one hour after feeding but not four hours after feeding.

Hemsley et al. (1970) postulated as one reason for positive responses to formaldehyde treatment, that a greater amount of protein was digested in the intestine. They also postulated that in their experiment the plant proteins might have been more digestible than bacterial protein, in the intestines. According to these workers, the digestion of the plant proteins in the intestines might have increased available energy from amino acids to the animal. Barry (1973a) also reported increased digestion of nitrogen in the small intestine with formaldehyde treatment.

Langlands (1973a, 1973b) reported improvement in the utilization of protein of treated wheat. He attributed this improvement to the greater amount of nitrogen escaping fermentation in the rumen.

Faichney and Weston (1971) reported that the increased amount of protein digested in the intestine as a result of formaldehyde treatment stimulated the secretion of insulin. The insulin increased the entry rate of amino acids into the cells. It also has an effect on the secretion of growth hormone. This hormonal aspect has not been fully investigated.

Ferguson (1975) (without giving data) also postulated that treatment with formaldehyde might increase feed intake and thus improve performance. Davies and Faichney (1973) however reported formaldehyde treatment of barley to decrease feed intake and performance of steers.

With silages positive responses of milk and SNF production have been attributed to the prevention of breakdown of protein during ensiling and in the rumen (Brown and Valentine, 1972; Valentine and Brown, 1973; Valentine and Radcliffe, 1975).

In experiments where no responses or negative responses had been obtained, various reasons have been given. Schmidt et al. (1974) thought that overtreatment of soybean meal with formaldehyde resulted in the negative response. Sharkey et al. (1972) observed no response with treated hay. They attributed this to losses of formaldehyde during the hay making process and thus the protein was not adequately protected. Langlands (1971b) observed no response with treatment of cottonseed meal. He attributed this to extreme depression in the digestibility of nitrogen and organic matter as a result of both heat applied during extraction of the oil and the application of the formalin. Saville et al. (1971) attributed no response to treatment of cottonseed meal to

under- or over-protection of the meal and depressed rumen pH as a result of feeding of grain which might have broken the methylene bridges. Sharma and Ingalls (1974) however had pH values of about 5.5 for formaldehyde treated casein and 5.7 for formaldehyde treated rapeseed meal and still had adequate protection of the proteins. Sharma et al. (1974) indicated that diets containing about 15% CP for steers had protein levels in excess of the amounts required and this was the cause of no response to the rapeseed meal treatment. They suggested crude protein levels of about 11-12%. It should however be noted that in the same paper, they had a response to treatment of casein in diets with similar crude protein levels as the rapeseed meal diets.

Rattray and Joyce (1970) observed a response in nitrogen retention but not wool growth or growth rate with treatment of linseed meal. Their experimental period of five weeks, according to them, may have been too short for a response of growth rate to be demonstrated. They also reported a negative response with treatment of meat meal using any of the three parameters. They suggested that a level of 2.5% formaldehyde application overprotected the protein in the meat-meal.

Digestion and absorption of nitrogen in the small intestine

The main proteolytic enzyme in the abomasum of ruminants is pepsin. Rennin is also present in the young pre-ruminant. The abomasum secretes hydrochloric acid which kills rumen micro-organisms (Maynard

and Loosli, 1969). Maximal proteolysis by abomasal contents was shown to occur between pH 2 and 3 (Hill, 1961; Phillipson, 1975) but Church (1975a) claimed it occurred at pH 2.1. Digestion of proteins occurs mostly in the intestines, after the reticulo-rumen.

Materials entering the small intestine of ruminants include endogenous nitrogen secreted into the abomasum and small intestine, microbial nitrogen, non-protein-nitrogen resulting from microbial fermentation in the reticulo-rumen and nitrogenous components of dietary or endogenous origin that have escaped ruminal fermentation (Armstrong and Hutton, 1975).

There is no quantitative information on the endogenous secretion into the small intestine (Armstrong and Hutton, 1975). Approximately 29% of the ammonia-nitrogen in duodenal flow is derived from ammonia in rumen fluid. It is hypothesized that the other 71% is derived from urea that passes into the abomasum with gastric secretions (Nolan, 1975). Other non-protein-nitrogen might be nucleic acids since the digesta from the rumen has up to 20% of microbial nitrogen in the form of RNA and DNA (Nolan, 1975). Nolan (1975) reported that when 21g of nitrogen was fed to sheep 7.1g, 11.0g and 1.2g of the nitrogen arriving at the duodenum was from dietary protein, microbial protein and ammonia respectively.

The protein entering the small intestine is hydrolyzed by proteases in pancreatic juice and peptidases from intestinal secretions. Gray and Cooper (1971) reported that pancreatic juice contains endopeptidases (attacking centrally located peptide bonds) and exopeptidase (cleaving only terminal bonds of proteins or peptides). The endopeptidases are:

(a) Trypsin - this attacks proteins at locations of the basic amino acids yielding arginine and lysine terminal peptides.

(b) Chymotrypsin - this acts interiorly at aromatic amino acid sites to produce C-terminal phenylalanine, tyrosine, and tryptophan peptides.

(c) Pancreopeptidase E (elastase) - attacks aliphatic (non-polar) amino acid-containing portion of protein to produce aliphatic carbon terminal peptides.

The exopeptidases are:

Carboxypeptidase A:

Attacks peptides resulting from actions of chymotrypsin and pancreopeptidase, yielding neutral amino acids and small peptides.

Carboxypeptidase B:

Attacks peptides resulting from action of trypsin to give basic amino acids and small peptides.

These enzymes are secreted as zymogens. Activation of a small amount of trypsinogen is by enterokinase. Then the trypsin produced activates the rest of the trypsinogen to trypsin. Trypsin then activates all the other pancreatic proteases (Gray and Cooper, 1971). Pancreatic secretion is under hormonal and nervous control. The nervous part is through the vagus nerve which can be stimulated by distension of the abomasum. This is the initial flow known as the cephalic phase. The more rapid flow is under the control of hormones. Pancreozymin is a

hormone which has an effect on pancreatic flow and its effect is similar to the vagal reflex. The hormone which induces the more rapid flow is secretin. The most powerful stimulus for the release of secretin is acid ingesta present in the duodenum. Other factors which affect secretin release are peptone, soaps and amino acids (Hill, 1975).

The acid conditions from the abomasum extend into the upper part of the small intestine due in part to the copious secretion of acid by the abomasum and partly to the weakly alkaline nature of the bile and pancreatic secretions of the ruminant. The slow rise in pH in the proximal part of the small intestine may extend the activity of the abomasal pepsin but delay that of the pancreatic proteases. The activation of pancreatic zymogens require a pH above 5 (Armstrong and Hutton, 1975). Ben - Ghedalia et al. (1974) working with sheep reported that the section of the intestines betwen 1 and 3 metres and 3 and 7 metres from the pylorus were sites of great proteolysis but poor for absorption. They reported that the section 7 to 15 metres distant from the pylorus showed the greatest absorption of digestive products of nitrogen. The lower section of the intestine from 15 to 25 metres distant from the pylorus had low net absorption. In a subsequent paper, Ben-Ghedalia et al. (1976) reported that if protein was administered at the lower section (15 to 25 metres distant from the pylorus) there was a considerable degree of digestion and absorption of proteins. The earlier work indicating low net absorption was due to the fact that most proteins had been digested and products absorbed in the upper sections of the small intestine if proteins were supplied at maintenance levels. The lower section therefore did not have digestive and absorptive limitations.

Peptidases are present in trace amounts in pancreatic or intestinal secretions (Gray and Cooper, 1971). About 10-20% of intestinal dipeptidase activity is known to occur in the microvillar membrane (brush-border). Intracellular peptidases account for 80% of the mucosal peptidase activity. There are about 4 to 8 peptidases which are hydrolases.

The major end-products absorbed after protein digestion are free amino acids and small peptides, with the latter predominating (Matthews, 1972; Armstrong and Hutton, 1975). Gray and Cooper (1971) suggested that since glycine peptides were more readily absorbed than glycine, it indicated that the small peptides probably entered the cells at rates comparable to the amino acid, presumably at the same entry sites. If oligopeptides are absorbed and the entry rate is the same as the free amino acid entry rate, more amino acids are present in the cell on further hydrolysis by intracellular peptidases than when the free amino acids are absorbed. Some of the peptides are also digested at the brush-border by the brush-border enzymes before entering the cell.

Oxidative metabolism is linked up with sodium movement out of the cell in the transport of amino acids into the cells. A ternary complex of the amino acid, Na^+ and a membrane compound, presumably a protein, is formed thereby permitting entry and release beyond the outer cell barrier, (Gray and Cooper, 1971; Matthews, 1972). Since the oxidative metabolism is not linked directly with the amino acid entry, the system is not active transport but rather termed secondary active transport (Gray and Cooper, 1971). There are some amino acids, the dependence

of whose transport on sodium is not fully known (e.g. the dicarboxylic amino acids). Williams (1969) reported that there were considerable variations in absorption between and within species. The proportions of amino acids presented to the liver were not necessarily the same in different subjects of the same species when the proportions of available amino acids for absorption from the small intestines were the same. He also grouped amino acids into six (6) categories according to rate of absorption in sheep. In order of decreasing rate these are:

(a) Isoleucine, arginine, methionine, valine

(b) Leucine, lysine, phenylalanine

(c) Aspartic acid, serine, tyrosine, alanine

(d) Alanine, proline, threonine

(e) Proline, threonine, glutamic acid, histidine

(f) Glycine

Some amino acids have an effect on the transport of other amino acids (Gray and Cooper, 1971; Matthews, 1972). For instance leucine and methionine may inhibit the transport of other amino acids (Matthews, 1972). Leucine augments the transport of lysine and arginine but the reason for this is unknown (Gray and Cooper, 1971).

The interaction of one kind of amino acid with another may be allosteric - due to attachment of an amino acid to one carrier site inducing configurational changes in another adjacent carrier - or even due to competition for energy supply. They may not necessarily be sharing the same carrier (Matthews, 1972). Hexoses (D-glucose and D-galactose)

reduce absorption of amino acids. This is perhaps because they share the same carrier mechanism or compete for the limited source of energy (Gray and Cooper, 1971).

Digestion of protein in the large intestine

Undigested and unabsorbed residues of nitrogenous compounds leaving the small intestines enter the caecum and the large intestine. These are mainly feed residues, undigested rumen micro-organisms and endogenous materials. In addition, substantial quantities of urea-nitrogen enter the caecum from the blood. The total input of nitrogen into the caecum and the large intestine may range between 4 and 15g per day for sheep. The micro-organisms present in the caecum and colon carry out proteolytic activities. In fact, proteolytic activity appears to be greater in the contents of the large intestine and caecum than in the contents of the reticulo-rumen. The presence of iso-butyric and iso-valeric acids in the caecum in proportions higher than those occuring in the rumen indicates there is extensive breakdown of protein in this region (Nolan, 1975).

Absorption of nitrogen from the hindgut is probably in the form of ammonia, about 43% of which is used for the synthesis of non-essential amino acid in the liver (Nolan, 1975). Since the microbes present are not killed by acids and later subjected to proteases as occurs with rumen microbes, they are perhaps of little nutritive value, in terms of nitrogen, to the host (Nolan, 1975).

Carbohydrate metabolism in the rumen

The metabolism of carbohydrates by rumen micro-organisms is illustrated by the pathways indicated in Figures 2 and 3. The end-products of carbohydrate metabolism in the rumen are generally volatile fatty acids, methane, hydrogen gas, and carbon dioxide (Hobson, 1971). Ethanol has been produced by rumen microbes in in vitro cultures but in in vivo only traces have been detected. It is possible that in in vivo, ethanol is produced but may be absorbed through the rumen wall, since it has the ability to absorb ethanol if infused in large quantities. It may also be metabolized to acetic acid and methane. This has been observed when small quantities of alcohol are introduced into the rumen at a time. It could also be metabolized with acetate to produce butyrate and higher volatile fatty acids (Hobson, 1971).

In the pathways shown by Leng (1970), methane is produced from formic acid but Hungate et al. (1970) and Hungate (1967) indicated that this pathway may not be quantitatively important. Those workers indicated that methane is produced from the reduction of carbon dioxide with hydrogen. Miller and Wolin (1973) working with R. albus reported that even formic acid itself is produced from the reduction of carbon dioxide but not from formate-producing pyruvate lyase reaction. They reported that very small amounts of carbon dioxide were produced from formic acid breakdown and this occurs only during the growth of this organism. The pathway is not yet understood.

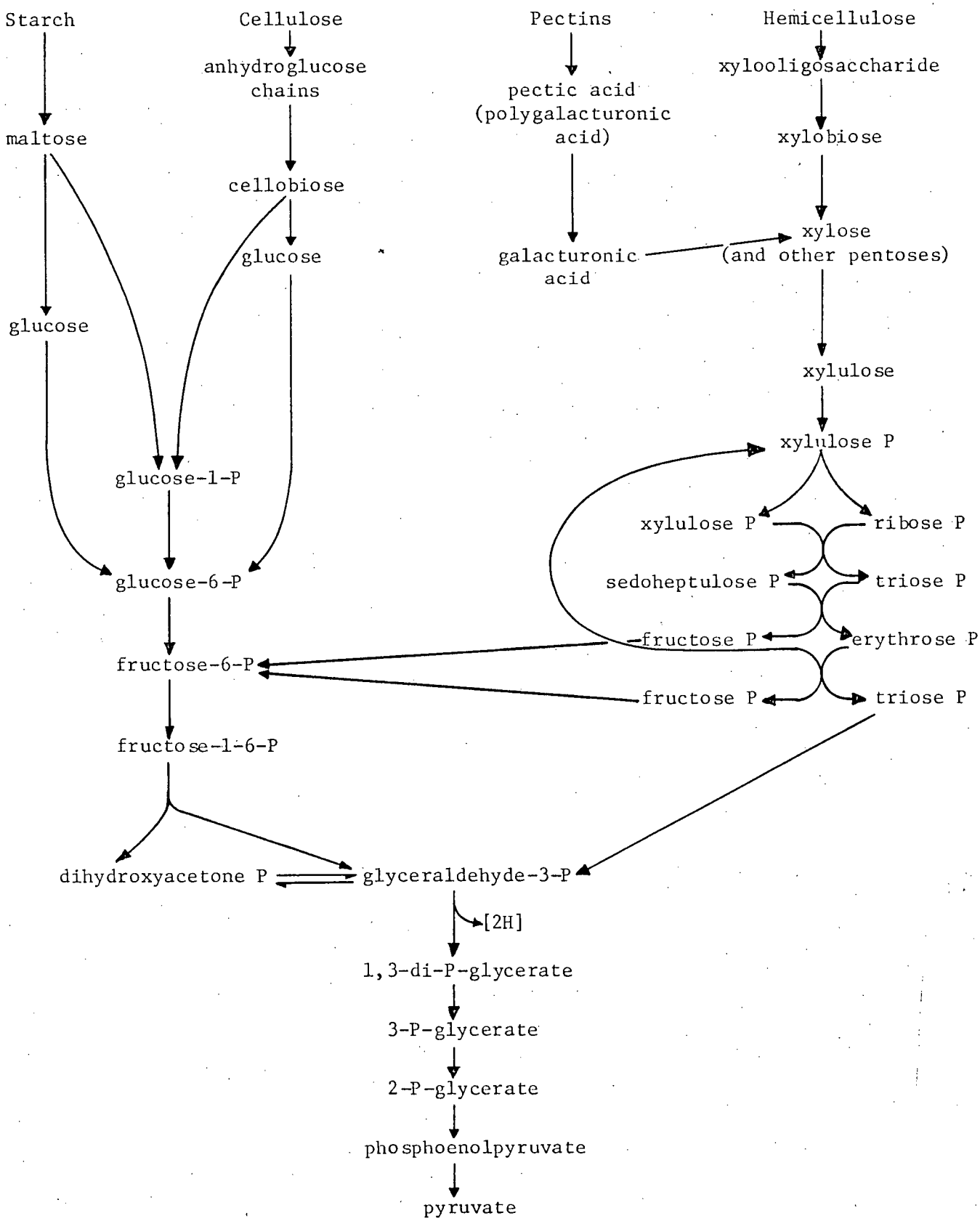

Fig. 2. An outline of the pathways of fermentation of the major carbohydrate constituents of plants to 3C units in the rumen. (Adapted from Leng, 1970)

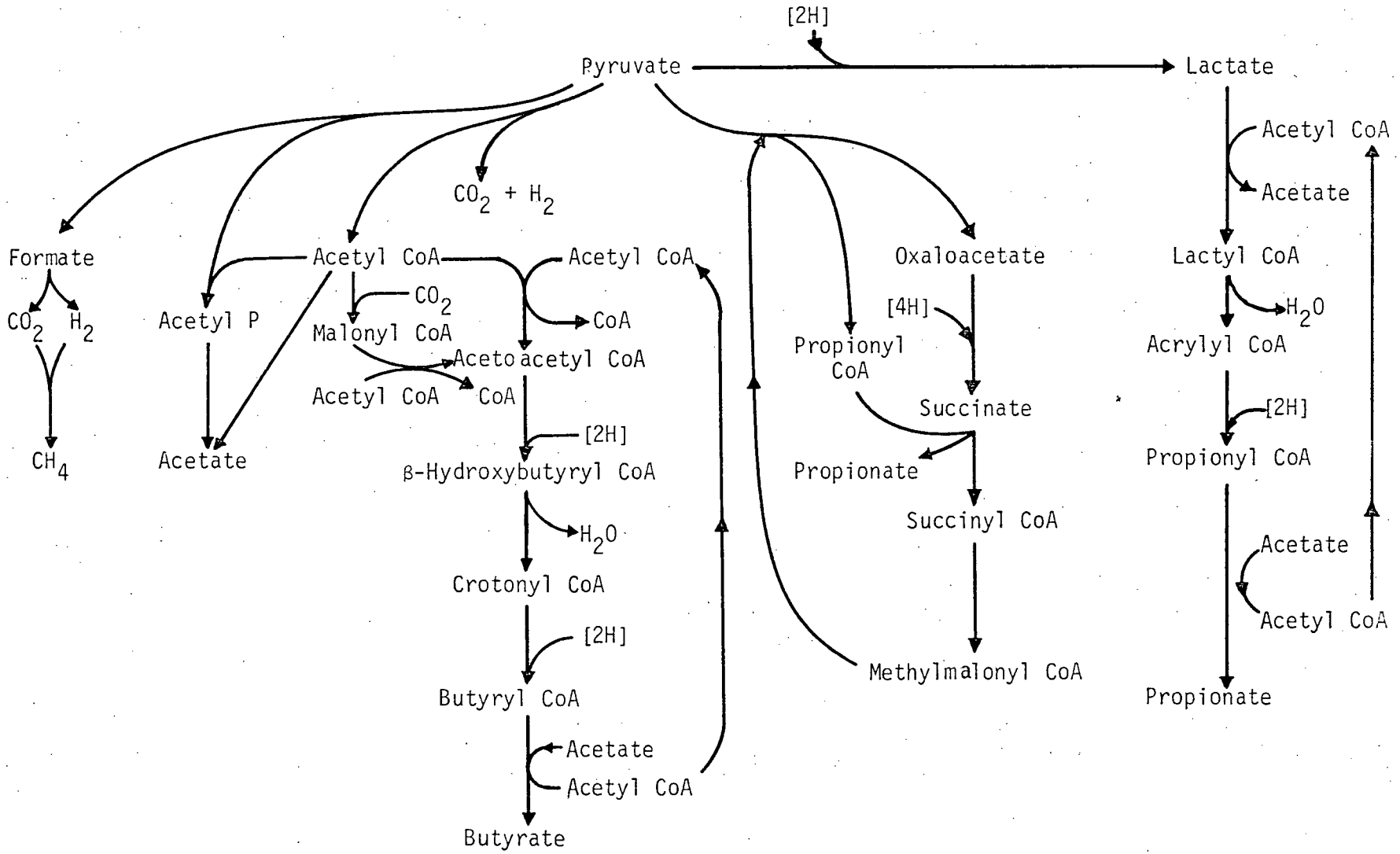

Fig. 3. An outline of the pathways of degradation of 3C units in the rumen. (Adapted from Leng, 1970).

The major end-products of carbohydrate metabolism in the rumen are perhaps the short chain volatile fatty acids, acetic, propionic and butyric. The concentration of total short chain fatty acids has been observed to increase with increasing levels of feeding, reflecting the amount of feed in the rumen (Hodgson et al., 1976). The average proportions of the major volatile fatty acids when forages are fed are: acetic 65%, propionic 20%, and butyric 9%, (Leng and Brett, 1966). There are a number of factors which affect the proportions of the three volatile fatty acids. The changes in the proportions of the various fatty acids are mostly brought about by changes in rumen pH which in turn affects microbial populations. Some of these factors are discussed below.

Roughage: concentrate ratio or a concentrate versus a roughage diet. The most important aspect of this is the content of readily fermentable carbohydrate. Ørskov (1975), Nicholson and Sutton (1969), Wilke and Merwe (1976) indicated that high levels of concentrate in the diet can result in increased propionic acid proportion and decrease in acetic acid proportion. Ørskov (1975) indicated that high levels of fermentable carbohydrate in young forages can also result in high propionic acid and low butyric acid levels. Whitelaw et al. (1970) and Nicholson and Sutton (1969) reported that when sheep fed barley are given diets below full feeding the butyric acid portion is increased with a corresponding decrease in the propionic acid fraction. Hodgson et al. (1976) and Ørskov (1975) also reported higher proportions of propionic acid at the expense of acetic acid, on high concentrate diets.

The particle size of roughage can also affect fermentation patterns. Grinding of the feed decreases the size of particles and this results in the exposure of a greater surface area, increases the proportion of propionic acid and alters rumen pH (Ørskov, 1975). Feed processing such as pelleting of cereal grains may increase the proportion of propionic acid compared to the feeding of unprocessed whole grains (Ørskov, 1975).

Feeding frequency also affects the proportions of the volatile fatty acids. High frequency of feeding of high concentrate diets abolishes the great fluctuation in rumen pH and increases butyric acid proportions because of the survival of protozoa (Ørskov, 1975).

The type of carbohydrate also affects end-products of fermentation. Glucose or sucrose results in higher butyric acid while high proportions of starch results in propionic acid type of fermentation (Ørskov, 1975).

Buffers added to the rumen can result in changes in fermentation pattern. Harrison et al. (1976) by infusing four litres of artificial saliva a day altered propionate fermentation to acetate fermentation. Alhassan et al. (1969) and Krabil et al. (1969) by feeding sodium sulphite increased the proportion of propionic acid. Hobson (1972) reported that the addition of one acid may suppress the production of that particular acid - this is referred to as fermentation product suppression.

The other volatile fatty acids normally reported are isovaleric, isobutyric and valeric acids. Isovaleric and isobutyric acids normally result from deamination of leucine and valine respectively (el-Shazly, 1952a and 1952b). Lactic acid may accumulate in the rumen under abnormal conditions of lactic acidosis (Dunlop, 1972).

Effects of formaldehyde treatment on carbohydrate metabolism and volatile fatty acid production in the rumen

The effects of formaldehyde treatment of feedstuffs on volatile fatty acid production have been variable. Sharma and Ingalls (1973), Beever et al. (1976), and Beever et al. (1977), reported no differences in volatile fatty acid production or proportions of the individual fatty acids. Sharma et al. (1972) reported decreased volatile fatty acid production with treatment of rapeseed meal. Langlands (1973b) in one experiment observed no differences in total volatile fatty acid production and proportions of the major fatty acids. He reported total volatile fatty acid production to be reduced significantly in another experiment reported in the same paper (Langlands, 1973b).

Barry (1973a) reported volatile fatty acid concentration to decrease with formaldehyde treatment of hay. The levels of iso- and n-valeric acid were reduced by formaldehyde treatment of silages (Barry and Fennesy, 1973). In this experiment, they reported volatile fatty acid levels to be higher in sheep fed treated silage about one hour before feeding. Barry (1972) reported similar prefeeding conditions with treatment of casein. These two workers thought that the high levels of volatile fatty acid and ammonia in the rumen before feeding in sheep fed treated silage indicated greater stability of conditions in the rumen for fermentation. Barry (1976c) reported variable results with volatile fatty acid production in three series of experiments. In experiment 2, total volatile fatty acid, iso- and n-valeric acids were reduced but

not isobutyric. In experiment 3, formalin treatment did not affect total volatile fatty acid production.

Dinius et al. (1975) reported reduced apparent digestibility coefficients of acid-detergent fibre, neutral detergent fibre and hemicellulose with treatment of dried alfalfa. There was no difference in rumen pH, five hours after feeding. Beever et al. (1976), however observed enhanced digestibility of cellulose with treatment of dried grass. There was a depression in digestibility of cellulose in the rumen but subsequent increased digestion of cellulose in the caecum resulted in higher cellulose digestibility with formalin treatment of dried grass.

OBJECTIVES

These studies reported in this thesis were undertaken with the following objectives:

1) To determine by *in vitro* studies the optimum level of formaldehyde treatment of grass-legume forage protein to obtain protection from rumen microbial degradation without subsequent decrease in enzymatic digestion.

2) To assess the effects of formaldehyde treatment of the forage protein in terms of nitrogen and fibre utilization *in vivo*.

3) To determine if dietary supplementation with sulphur and/or branched chain fatty acids is necessary when a portion of the dietary protein is protected from microbial degradation.

MATERIALS AND METHODS

Introduction

The experimental work was conducted in four phases. The first phase employed *in vitro* procedures to determine the optimum level of formaldehyde treatment of dehydrated and hammermilled grass-clover forage. This was followed by *in vivo* experiments in which apparent digestibilities of dry matter, organic matter, nitrogen, acid-detergent fibre and cellulose were determined as well as feed intake, nitrogen and sulphur balances. At the end of the above determinations the animals were slaughtered for the purpose of measuring rumen pH, rumen dry matter content, and the rumen levels of ammonia-nitrogen and volatile fatty acids. Abomasal pH and abomasal digesta contents of acid-detergent fibre, total nitrogen and RNA-N were also measured. The fourth phase of the study involved the use of a sheep fitted with a duodenal re-entrant cannula for measuring digesta flow.

EXPERIMENT I

Treatment of the forage with formaldehyde

The dehydrated and hammermilled rye-grass-ladino clover forage was treated with 10% formaldehyde stock solution (formalin) in a small upright mixer similar to the method used by Schmidt *et al.* (1974). The formalin was sprayed on the forage, using an aerosol spray gun, as it was being turned around in the mixer. Ten-kilogram (10 kg) batches of the

forage were treated at a time. The treatment resulted in formaldehyde sprayed on the forage, on an air dry basis, of 0.0%, 0.8%, 1.0% and 1.2% with the same quantities of fluid added (12 ml/100 g). The forage was sealed in polythene bags for one week after the treatment as described by Saville et al. (1971).

In vitro incubation

1g samples (DM basis) of the treated grass-clover were incubated in rumen fluid plus artificial saliva, according to the method of Troelsen (1969). After 48 hours of incubation, the mixture was centrifuged at 1500 x g and filtered through ashless filter paper. The supernatant was used for determining ammonia-nitrogen using the autoanalyzer (Technicon model EDP 910 120-45-5). The residue was then washed with 50 ml of deionized water and filtered again. Rumen fluid plus artificial saliva served as blank. The nitrogen content of the residue was determined by the macro-Kjeldahl method (AOAC, 1970). Nitrogen digestibility was calculated by the method of Barry (1972). There were three replications for each treatment and blank.

Three replicates of each treatment and blank were also taken through the two-stage digestion procedure. After the rumen inoculum plus buffer digestion, each replicate was centrifuged and the supernatant was used for ammonia-nitrogen determination as described above. The residue was then digested with acid-pepsin for 48 hours. The acid-pepsin digested residue was then centrifuged, filtered and washed as described for the first stage incubation. The nitrogen content of the residue was determined

by the macro-Kjeldahl method (AOAC, 1970). The digestibility of nitrogen was then calculated.

Treatment of grass-clover forage for in vivo trials

The grass-clover forage was treated with 1% level of formaldehyde in the same manner as described for the in vitro trials above. The treated forage was then used in rations for animal feeding trials.

EXPERIMENT II

Nitrogen, carbohydrate and sulphur metabolism and feed intake studies

The diets (Table 1) contained grass-clover forage, cassava, barley and a mineral premix. Sodium sulphate was added to diets 4 and 5. The diets were pelleted (9mm) after mixing in a horizontal mixer. Isovaleric and isobutyric acids were sprayed on diets 3 and 5 at the rate of 3.0g/kg diet for isovaleric and 2.3g/kg diet for isobutyric immediately before feeding. After measuring the required volumes of the acids for Ikg diet into a measuring cylinder, it was diluted to 10ml and sprayed using an aerosol spray gun.

The diets contained approximately 14% crude protein as reported by Bryant (1973) to meet the requirement of confined lambs weighing 29kg up to slaughter.

Table 1. Composition of Rations.

Ingredient (% DM)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Cassava	38	38	38	37.33	37.33
Barley	11	11	11	11	11
Untreated grass	50	--	--	--	--
Treated grass	--	50	50	50	50
Sodium sulphate	--	--	--	0.67	0.67
Mineral premix*	1	1	1	1	1

* The composition of the sheep mineral premix, given by the manufacturers was as follows: Ca 20%, P 19%, Mn 0.15%, Zn 0.60%, Fe 0.20%, I 0.01%, Co 0.008%, Cu 0.015%; the maximum levels of Fluorine was 0.2% while the minimum levels of Vitamins A and D_3 were 551,150 Iu/kg and 110,230 Iu/kg respectively.

Twenty-five young ram lambs of the Dorset breed were used for the metabolism trials, at South campus University of British Columbia sheep unit. Five animals were randomly assigned to each diet. There was a pre-experimental period of twenty days and a collection period of seven days.

The animals were kept in slatted floor individual pens and fed individually (five animals at a time one on each diet) during seventeen days of the pre-experimental period of twenty days. After seventeen days in the individual pens, they were transferred to the cages, allowed a three-day adjustment period, before the seven-day collection period.

The pre-experimental period of seventeen days was also used for feed intake assays. During the feed intake assay, there was a ten-day pre-conditioning period and a seven-day measurement of feed intake, as recommended by Heaney et al. (1968). During the ten-day pre-conditioning period, the animals were brought to maximum feed intake. They were fed ad libitum and residues weighed back. If they left less than 10% of feed offered, the feed allowance was increased by 25%. The minimum level of feeding during the seven-day feed intake assay period was the same as the maximum level of feed intake during the 10-day pre-conditioning period. The amount of feed leftover was weighed daily. If the animals left less than 10% of the feed offered, the feed allowance was increased by 25%.

During the metabolism trials, the animals were fed ad libitum based on the level determined during the feed intake assay period. The animals were fed twice daily at 8:30 a.m. and 3:00 p.m., throughout the twenty-seven-day period.

Total amount of faeces voided were collected and weighed daily during the seven-day metabolism trial. Samples of faeces were dried in a forced draft oven at 80°C for 24 hr similar to the procedure of Hume et al. (1970).

Urine was collected over 100 ml of 6N HCl in plastic-containers with narrow necks into which were fitted the rubber tubes draining the urine from the urine compartment of the metabolism cage. This limited exposure of the urine to the atmosphere. Samples of urine were taken daily after measuring the volume. The samples were kept in the freezer compartment of a refrigerator. The addition of 100 ml of 6N HCl reduced pH of urine to between 2 and 3. This pH level (between 2 and 3) was recommended by Martin (1966) to reduce to insignificant levels the ammonia lost from the urine.

At the end of the trials, the urine samples were thawed and filtered through layers of cheese cloth as was carried out by Bryant (1973). The filtrate was used for nitrogen and sulphur analyses.

The animals were weighed on the 1st, 10th, 17th and 27th days of the trial.

EXPERIMENT III

Rumen and abomasal digesta metabolites studies

The animals used in the previous experiment were slaughtered for these studies. The animals were fed at about 2:00 a.m. on the day of slaughter. They were then transported to the slaughter house and slaughtered between 7:00 a.m. - 8:00 a.m. The gastro-intestinal tract

was removed intact immediately after slaughter placed in buckets containing ice and transported directly to the laboratory for the various measurements. The pH of the rumen fluid was determined immediately after straining rumen digesta. The ammonia-nitrogen levels were determined using the autoanalyzer used in experiment I. The rumen fluid for rumen ammonia-nitrogen determination was acidified as described by Chalmers et al. (1954). Samples of rumen fluid were collected as described by Alhassan et al. (1969) and the measurements of total and individual volatile fatty acids were carried out as described by Ross and Kitts (1971). The total rumen contents were weighed. Samples of the rumen content were freeze-dried for storage. Samples of the freeze-dried material were oven dried (105°C for 24 hours) for dry matter determination. This was similar to the method of MacRae and Ulyatt (1974).

Abomasal fluid was also strained for pH measurement. The contents of the abomasum were freeze dried. Samples of the freeze-dried abomasal contents were used for ash, acid-detergent fibre, cellulose, total nitrogen and RNA-N analyses as described under chemical analysis. Samples of the freeze-dried materials were further dried at 105°C for 24 hours to determine dry matter content of the abomasum (MacRae and Ulyatt, 1974).

EXPERIMENT IV

Duodenal digesta flow studies

A ram fitted with a duodenal re-entrant cannula was used for this experiment. The re-entrant cannula was fitted in a surgical operation as described by Brown et al.(1968). This animal was used for studies of duodenal flow 6 weeks after the operation. The animal was fed each of the diets in turn for ten days. On the tenth day, all the materials flowing from the abomasum into the duodenum were collected over a 24-hour period.

The animal was fed 1,500g (air dry basis) of each diet in two equal portions twice daily at 8:30 a.m. and 3:00 p.m.

Collection and sampling of digesta

The piece of tube joining the two ends of the cannula was removed during the day of collection. Separate lengths of rubber tubings were attached to each end. The tubing attached to the exit cannula was arranged so that the digesta were delivered into a glass conical flask (2,000 ml capacity) placed in a bucket. The top of the flask was sealed with laboratory parafilm. The flask was maintained at ambient temperature.

The tubing attached to the return cannula was fastened to the crate, about 40-50 cm above the cannula, allowing return of digesta to the duodenum. After every one and a half hours (1½ hr), the flask was replaced. The volume of the collected digesta was measured and the weight recorded. A ten percent (10%) sample was taken after vigorous shaking. The sample taken was immediately placed in a capped plastic container and kept in the freezer section of a refrigerator. Following the collection of the sample the remainder of the digesta was returned

manually into the intestine through the return cannula, after adding donor sample collected previously to make up for the quantity taken for analyses. The digesta was warmed in a water bath (39°C) and returned in small quantities over a period of one and a half hours. This method of sample collection was similar to that of Thompson and Lamming (1972). The amount of feed left after every one and a half hours was weighed.

The frozen duodenal samples were thawed in a refrigerator before further analysis. Weighed samples were freeze-dried and analyzed for nitrogen, acid-detergent fibre, RNA-N, ash and TCA-N. A sample of the freeze-dried material was further dried in the oven for 24 hours, for dry matter determination, as described by MacRae and Ulyatt (1974).

Chemical analyses

The chemical analyses of all samples, unless otherwise described, were carried out as outlined below.

(a) Nitrogen: Nitrogen contents of feedstuffs, rations, faeces, digesta and urine were determined by macro-Kjeldahl method of the AOAC (1970).

(b) Ash: Ash contents of the rations, faeces and digesta were determined by the AOAC (1970) method.

(c) Moisture: Moisture content of the rations and faeces were determined using the method of AOAC (1970). Moisture content of rumen, abomasal and duodenal digesta samples was determined in two stages: freeze-drying followed by oven-drying as described by MacRae and Ulyatt (1974).

(d) Acid-detergent fibre and cellulose: Acid-detergent fibre and cellulose contents of rations, faeces and digesta were determined according to the method of Van Soest and Wine (1968) as modified by Waldern (1971).

(e) Acid-detergent fibre insoluble nitrogen: Acid-detergent fibre insoluble nitrogen content of the grass-clover forage was determined using the method of Yu and Veira (1977).

(f) Sulphur: Ashing of urine, faeces, ration and feedstuff samples for sulphur determinations, was done according to the method of Bird and Fountain (1970). For the feedstuff, ration, and faecal samples, 2g dry matter were weighed into an ashing crucible. This was mixed thoroughly with 2.5 g of mixture of sodium bicarbonate-silver oxide (25:1) as an oxidizing agent. The mixture was ashed at 550°C for five (5) hours in a muffle furnace. With the urine samples, 1 ml-samples were pipetted into the crucibles, then dried at 55°C in an oven. The dried urine sample was then ashed.

The ash in each case was dissolved in 20 ml 6N HCl, after cooling, following the method of Johnson et al. (1970). The solution was then diluted to 1000 ml with distilled water. A 10 ml aliquot was combined with 10 ml of gelatin barium chloride solution containing 0.3g, $BaCl_2$. The gelatin barium chloride solution was prepared following the method of Tabatabi (1974). The suspension of the ash solution and gelatin-barium-chloride solution was shaken for five minutes and allowed to stand for one hour. The turbidity of the sulphate precipitated was determined using spectrophotometer (Spectronic 20 at wavelength of 500 mμ) following the method of Johnson et al. (1970).

The extractable sulphate-sulphur in the grass-clover forage was determined using the procedure outlined in the bulletin of the Ministry of Agriculture, Fisheries, and Food, London, Technical Bulletin 27 (1973). The weighed sample was shaken with 0.12N hydrochloric acid and activated charcoal. After shaking for 30 min, it was filtered through Whatman No.2 filter paper and the filtrate used for sulphate-sulphur analysis.

Standards of sulphate-sulphur were prepared according to the procedure outlined in the technical bulletin referred to above. The only modification was that the sulphate was dissolved in 0.12N HCl.

(g) RNA: Ribonucleic acid contents of the abomasal and duodenal digesta were determined using the method of Guinn (1966). The homogenized samples were treated with ethanol-NaCl (4:1 ratio of 95% ethanol and 10% NaCl), 95% ethanol, and ethanol-10% NaCl to remove chlorophyll and other pigments. The nucleic acids were then extracted from the moist samples with 10% NaCl at 100°C. The tubes containing the samples were stoppered. This method was used by Ling and Buttery (1975), for extracting nucleic acids from rumen bacteria and duodenal digesta.

RNA extracted from the samples was determined with spectrophotometer (Unicam SP800B), U.V., wavelength 260 mμ. Standard solutions of RNA were prepared and a standard curve obtained as described by Akinwande (1973). The nitrogen content of the RNA extracted from the duodenal digesta was assumed to be of the same magnitude as the torula yeast RNA used for preparing the standards. This assumption was also

made by Smith and McAllan (1970). The microbial protein content of the digesta was calculated by assuming RNA-N: total N in rumen microbes to be 0.075 as was reported by Smith (1975). Sutton et al. (1975) made a similar assumption.

(h) NPN: The non-protein-nitrogen fractions of the grass-legume forage, abomasal and duodenal samples were determined using the method of Goshtasbpour-Parsi et al. (1977). One gram (1 g) sample of the freeze-dried duodenal or abomasal digesta or grass-legume forage was mixed with 10 ml deionized water. Ten millilitres (10 ml) of 20% TCA solution was then added. It was shaken for ten minutes mechanically, heated to 90^{o}C in a water bath, and further shaken in the hot water bath for 10 minutes. The tube was then centrifuged at 15,000 xg. The supernatant plus second washing of the residue, was analyzed for nitrogen by the macro-Kjeldahl method (AOAC, 1970). This was the non-protein-nitrogen fraction.

Experimental designs and statistical analysis

Results from the trials were subjected to statistical analysis. In the in vitro digestibility determinations, a completely randomized design (CRD) was used. The F-test was applied and significant differences between means were determined using Tukey's W values (Steel and Torrie, 1960).

Randomized block designs were used in the other trials. The block in this case was the group or batch. The F-test was applied and significant differences between means were determined using Tukey's W values as above (Steel and Torrie, 1960).

No statistical analysis was applied to data collected from the studies using the cannulated animal.

RESULTS

In Vitro digestion trials

The results of the nitrogen digestibility trials at the first stage *in vitro* digestion of the rye-grass-clover with different levels of formaldehyde treatment are shown in Table 2. The average values for nitrogen digestibility for the treatments were 31.88%, 15.72%, 6.87%, and 5.69% for 0%, 0.8%, 1.0%, and 1.2% levels of formaldehyde treatment respectively. There were significant ($p < 0.05$) differences between the treatments at 0% level formaldehyde and the rest of the treatments(0.8%, 1.0%, and 1.2% levels of formaldehyde). Treatment at 0.8% level of formaldehyde was significantly ($p < 0.05$) different from 1.0% and 1.2% levels of formaldehyde. Differences between 1% and 1.2% levels of formaldehyde treatments were not significant ($p > 0.05$).

The results of nitrogen digestibility at the end of the second stage of *in vitro* digestion (combined microbial and acid-pepsin stages) of the forage at the four levels of formaldehyde treatment are shown in Table 2. The average values for nitrogen digestibility for the treatments are 80.95%, 79.76%, 75.85% and 71.01% for the 0.0%, 0.8%, 1.0% and 1.2% levels of formaldehyde respectively. Treatment at 1.2% significantly ($p < 0.05$) reduced nitrogen digestibility compared with treatments at 0.0% or 0.8%. All other differences were not significant ($p > 0.05$).

The mean differences between the second stage (combined microbial and acid-pepsin) and first stages of *in vitro* nitrogen digestibility are 49.07, 64.04, 68.98 and 65.32 percentage units for 0.0%, 0.8%, 1.0%, and 1.2% levels of formaldehyde treatment respectively.

Table 2. *In Vitro* Digestion Trials.

Parameter	Formaldehyde Treatment 0.0%	0.8%	1.0%	1.2%	No. of Replicates	S.E.
1st Stage N digestion %	31.88a	15.72b	6.87c	5.69c	3	± 1.22
2nd Stage N digestion %	80.95a	79.76a	75.85ab	71.01b	3	± 1.41
NH_3-N production (ppm)	228.79a	78.58b	65.27b	30.04c	6	± 7.60
Calculated N digestion from NH_3-N%	30.26	10.39	8.63	3.97		

Means on the same line bearing different letters (a, b, c, d) differ significantly ($p < 0.05$).

Formaldehyde treatment significantly ($p < 0.05$) reduced *in vitro* ammonia-nitrogen production during the microbial stage of incubation (Table 2). The mean values for *in vitro* ammonia-nitrogen production at 0.0%, 0.8%, 1.0% and 1.2% levels of formaldehyde treatment are 228.79 ppm, 78.58 ppm, 65.27 ppm and 30.04 ppm respectively. Ammonia-nitrogen productions at 0.8% and 1.0% were not significantly ($p > 0.05$) different. The differences between any other pair were significant ($p < 0.05$).

Nitrogen digestibilities calculated using the ammonia-nitrogen production figures are shown in Table 2.

Chemical composition of diets and ingredients

The chemical composition of the feed ingredients and the diets fed to the animals for the feed intake and metabolism trials are shown in Table 3.

Daily feed intake, daily urine output, metabolic body sizes of animals (kg) at the beginning of the metabolism study period, and the daily gain in weight during the pre-metabolism assay period

There were no significant ($p > 0.05$) differences between diets with respect to daily feed intake (g D.M.) per unit of metabolic body size both during the pre-metabolism and the metabolism assay periods (Table 4). Daily nitrogen intake ($g/Wkg^{0.75}$), urine output per unit of metabolic body size per day and the growth rates of the animals over seventeen

Table 3. Chemical Composition of Diets and Ingredients (D.M. basis).

Diet/Ingredient	Chemical Fraction % (D.M. Basis)							
	N	S	ADF	Cell.	Ash	Ext.S	ADF-IN	NPN
Diet 1	2.29	0.17	16.87	13.03	7.47	---	---	---
Diet 2	2.24	0.18	16.98	13.28	7.76	---	---	---
Diet 3	2.25	0.18	16.83	12.95	7.53	---	---	---
Diet 4	2.28	0.27	17.12	13.19	8.11	---	---	---
Diet 5	2.29	0.27	17.33	13.38	8.48	---	---	---
Grass-legume forage	3.78	0.25	---	---	---	0.07	0.20	0.66
Cassava	0.42	0.05	---	---	---	---	---	---
Barley	1.77	0.12	---	---	---	---	---	---

day period were also not significantly ($p > 0.05$) different (Table 4). The metabolic body sizes of the animals at the beginning of the metabolism assay period were not significantly ($p > 0.05$) different (Table 4). However, the animals used in the first group were significantly ($p < 0.05$) heavier than animals used in the third group. The mean metabolic body sizes of animals used in the five groups were 14.37kg, 14.11kg, 12.74kg, 13.13kg, and 14.19kg for group one, two, three, four and five respectively.

Apparent digestibility coefficients of dry matter, organic matter, nitrogen, acid-detergent fibre and cellulose

The apparent digestibility coefficients of dry matter, (D.M.), organic matter (O.M.), nitrogen (N), acid-detergent fibre (A.D.F.) and cellulose are presented in Table 5. The apparent digestibility coefficients of dry matter, and organic matter were not significantly ($p > 0.05$) different between dietary treatments. The apparent digestibility coefficient of nitrogen was significantly ($p < 0.05$) higher for diet one than for diets two, four and five. The difference between diets one and three was not significant ($p > 0.05$). The difference between diets three and four was significant ($p < 0.05$), with diet three having the greater value.

The apparent digestibility coefficients of acid-detergent fibre and cellulose were higher for all the diets containing the treated forage than for diet one ($p < 0.05$).

Table 4. Daily Feed Intake, Daily Urine Output, Metabolic Body Sizes of Animals at the Beginning of the Metabolism Study Period and the Daily Gain in Weight During the Pre-metabolism Assay Period.

Daily Intake ($g/Wkg^{0.75}$)	Diet containing untreated forage	Diets containing formaldehyde treated forage + VFAS	+ SO_4†	+ VFAS + SO_4		
	1	2	3	4	5	S.E.
D.M. during pre-metabolism assay period	91.33 *	100.62	101.93	98.02	96.47	± 4.70
D.M. during the metabolism study period	90.17	96.65	104.29	90.88	94.25	± 4.52
N during the metabolism study period	2.07	2.18	2.38	2.08	2.18	± 0.10
Growth rate during 1st 17 days (g/day)	154.76	170.77	170.77	160.10	149.42	±21.31
Metabolic body size at the beginning of metabolism studies (kg)	14.42	13.17	14.02	13.43	13.50	± 0.36
Urine output ($mls/day/Wkg^{0.75}$)	80.32	77.02	61.44	79.81	78.86	±12.29

* Each value represents the mean of five determinations.

† Sulphur was added as Na_2SO_4

Table 5. Apparent Digestibility Coefficients of Some Chemical Fractions (%).

	Diet containing untreated forage	Diets containing formaldehyde treated forage				
			+ VFAS	+ SO_4†	+ VFAS + SO_4	
Apparent Dig.Coeff.	1	2	3	4	5	S.E.
D.M. %	64.78*	65.69	65.82	64.80	63.71	± 1.02
O.M. %	65.35	65.91	66.01	65.17	63.83	± 0.95
N %	54.13a	47.06bc	51.25ac	44.90bd	47.24bc	± 1.17
A.D.F. %	32.57a	36.97b	36.91b	36.45b	36.59b	± 0.89
Cellulose %	42.95a	49.10b	49.04b	49.33b	48.76b	± 1.33

* Each value represents the mean of five determinations.

† Sulphur was added as Na_2SO_4

Means on the same line bearing different letters (a, b, c, d) are significantly different ($p < 0.05$).

Nitrogen metabolism

Statistical analysis of the nitrogen metabolism study indicated a significant ($p < 0.05$) period effect for nitrogen excretion per unit of metabolic size. The animals used in the second group excreted a significantly ($p < 0.05$) greater amount of nitrogen ($g/day/Wkg^{0.75}$) in the urine than animals used in groups four and five. The values were 0.595, 0.618, 0.543, 0.461 and 0.448 ($g/day/Wkg^{0.75}$) for groups one, two, three, four and five respectively.

The parameters associated with nitrogen metabolism - are presented in Table 6. The daily nitrogen excreted in urine per unit of metabolic body size was significantly ($p < 0.05$) higher for diet one than for the rest of the diets. Urinary excretion of nitrogen as a percentage of intake was significantly ($p < 0.05$) greater for diet one than for diets two, three and four but there was no significant ($p > 0.05$) difference between diets one and five. The percentage of digested nitrogen excreted in urine was significantly ($p < 0.05$) higher for diet one than for the rest of the diets. Nitrogen retained as a percentage of intake was significantly ($p < 0.05$) increased by formaldehyde treatment of the forage. On diet three the animals also retained a significantly ($p < 0.05$) greater amount of nitrogen as a percentage of intake than the animals on diet five.

Nitrogen retained as a percentage of digested was significantly ($p < 0.05$) improved by formaldehyde treatment of the forage portion of the diets. Daily nitrogen balance ($g/day/Wkg^{0.75}$) was significantly ($p < 0.05$) improved for animals on diets two and three compared to animals on diet one. Nitrogen balance (g/day) was significantly ($p < 0.05$) different between animals on diet one and diet three. Nitrogen intake (g/day) was not significantly ($p > 0.05$) affected by treatments.

Table 6. Nitrogen Metabolism.

	Diet containing untreated forage	Diets containing formaldehyde treated forage				
			+ VFAS	+ SO_4†	+ VFAS + SO_4	
	1	2	3	4	5	S.E.
Nitrogen intake (g/day)	29.81*	28.64	33.44	27.80	29.51	± 1.65
Daily N intake ($g/Wkg^{0.75}$)	2.07	2.18	2.38	2.08	2.18	± 0.10
App.dig.coeff. of N %	54.13a	47.06bc	51.25ac	44.90bd	47.24bc	± 1.17
Daily N excreted in urine ($g/Wkg^{0.75}$)	0.805a	0.461b	0.467b	0.417b	0.518b	± 0.03
N excreted in urine, % of intake	39.07a	21.07b	19.97b	19.86b	23.94ab	± 3.69
N excreted in urine, % of digested	72.10a	44.65b	39.11b	44.25b	50.39b	± 3.42
N retained, % of intake	15.06a	26.00bc	30.24bc	25.04bc	23.30bd	± 1.48
N retained, % of digested	27.90a	55.39b	58.93b	55.75b	49.61b	± 3.41
Nitrogen balance (g/day)	4.49a	7.43ab	10.19b	6.99ab	6.86ab	± 0.86
Nitrogen balance ($g/day/Wkg^{0.75}$)	0.313a	0.567b	0.726b	0.525ab	0.508ab	± 0.05

* Each value represents the mean of five determinations.
† Sulphur was added as Na_2SO_4
Means on the same line bearing different letters (a, b, c, d) differ significantly ($p < 0.05$).

Sulphur metabolism

The parameters associated with sulphur metabolism are presented in Table 7.

Animals on diets four and five consumed significantly ($p < 0.05$) greater amounts of sulphur per day than animals on diets one and two but not more than animals on diet three. Animals fed the sulphur supplemented diets consumed significantly ($p < 0.05$) a greater amount of sulphur per day per unit of metabolic body size than animals on the other diets.

Urinary excretion of sulphur as a percentage of intake was significantly ($p < 0.05$) greater for animals on diets four and five than for animals on diets two and three. Faecal and urinary sulphur losses expressed as a percentage of intake was significantly ($p < 0.05$) greater for animals on diets one and five than animals on diets two and three. Animals on diet four lost significantly ($p < 0.05$) a greater percentage of consumed sulphur in urine and faeces than animals on diet two but not animals on diet three. The animals fed the sulphur supplemented diets excreted a significantly ($p < 0.05$) greater amount of sulphur in the urine per day than the animals fed the other diets ($g/Wkg^{0.75}$).

Animals on diets two, three and four retained significantly ($p < 0.05$) a greater amount of sulphur (g) per day than animals on diet one. Sulphur balance per day per unit of metabolic body size was significantly ($p < 0.05$) greater for animals on diets two, three and four than for animals on diet one.

Table 7. Sulphur Metabolism.

	Diet containing untreated forage	Diets containing formaldehyde treated forage				
			+ VFAS	+ SO_4†	+ VFAS + SO_4	
	1	2	3	4	5	S.E.
Daily sulphur intake (g)	2.26a*	2.20a	2.64ab	3.30b	3.39b	± 0.18
Daily sulphur intake ($g/Wkg^{0.75}$)	0.157a	0.168a	0.188a	0.247b	0.251b	± 0.01
Sulphur excreted in urine, % of intake	31.38a	14.17b	17.41b	41.43a	42.23a	± 2.76
Sulphur excreted in urine daily ($g/Wkg^{0.75}$)	0.050a	0.024a	0.034a	0.102b	0.105b	± 0.007
Sulphur balance (g/day)	0.459a	0.802b	0.843b	0.752b	0.672ab	± 0.064
Sulphur balance per day ($g/Wkg^{0.75}$)	0.032a	0.061b	0.061b	0.056b	0.050ab	± 0.005
Ratio of retained sulphur to retained nitrogen (1:X)	10.13	9.78	12.26	9.73	10.36	± 1.26
Sulphur lost in urine and faeces, % of intake	79.67a	63.69b	67.57bc	76.89ac	80.28ad	± 2.37

* Each value represents the mean of five determinations.
† Sulphur was added as Na_2SO_4
Means on the same line bearing different letters (a, b, c, d) differ significantly ($p < 0.05$).

The ratio of sulphur retained to nitrogen retained was not significantly ($p > 0.05$) different for any of the diets.

Rumen parameters (Data collected from slaughtered animals)

The rumen pH, rumen ammonia-nitrogen concentration (ppm), dry matter content of the rumen at the time of slaughter of the animals (g), total volatile fatty acid concentration (μ-mole/ml) and molar proporations of acetic, propionic, butyric, isobutyric, isovaleric and valeric acids are presented in Table 8. There were no significant ($p > 0.05$) differences between any of the treatments with respect to rumen pH, rumen dry matter content at the time of slaughter of the animals, and total volatile fatty acid concentrations. There were no significant ($p > 0.05$) differences between treatments with respect to acetic, propionic, and butyric acid proportions. There were however significant ($p < 0.05$) differences between the animals used in the second and fourth groups with respect to acetic acid and propionic acid concentration. The average values of acetic acid proportions for the five groups (blocks) of animals were 40.73%, 46.69%, 41.98%, 37.61%, and 42.81% for first, second, third, fourth and fifth groups respectively. The average values of propionic acid proportions for the five groups (blocks) of animals were 31.24%, 22.66%, 26.49%, 35.26% and 29.35% for the first, second, third, fourth and fifth groups respectively.

The proportions of isovaleric acid and isobutyric acid were significantly ($p < 0.05$) lower for diets two and four compared with diet three. The other treatments were not significantly ($p > 0.05$) different.

Table 8. Rumen parameters.

	Diet containing untreated forage	Diets containing formaldehyde treated forage				
		+ VFAS	+ SO_4†	+ VFAS +	SO_4	
	1	2	3	4	5	S.E.
Rumen pH	5.50*	5.11	5.25	5.22	5.26	0.16
Rumen NH_3-N (ppm)	21.14a	14.36b	14.30b	12.90b	13.54b	1.54
Dry matter in rumen (g)	574.93	643.73	447.89	449.04	478.15	109.43
Total VFA conc. (μ-mole/ml)	166.12	186.03	165.44	166.81	150.06	18.17
Molar proportions of VFA:						
Acetic %	42.04	45.24	39.03	42.74	40.77	1.57
Propionic %	29.82	27.91	28.39	31.91	26.97	2.13
n-Butyric %	22.26	23.02	24.70	21.17	25.20	2.62
Isobutyric %	1.54a	0.71ab	2.11ac	0.66a	1.73a	0.27
Isovaleric %	2.53a	0.60ab	2.58ac	0.39a	2.18a	0.44
n-Valeric %	1.82a	3.30b	3.19b	3.34b	3.15b	0.28

* Each value represents the mean of five determinations.
† Sulphur was added as Na_2SO_4
Means on the same line bearing different letters (a, b, c, d) differ significantly ($p < 0.05$).

Formaldehyde treatment resulted in significantly ($p < 0.05$) higher levels of n-valeric acid.

Abomasal parameters (Data collected from slaughtered animals)

Abomasal pH, percent nitrogen, acid-detergent fibre, cellulose, non-protein nitrogen, ribonucleic-acid-nitrogen, and microbial-nitrogen contents of the abomasal digesta are presented in Table 9. The ratios of % RNA-N: % total abomasal digesta-N and % microbial-N: % total abomasal digesta-N are also shown in Table 9.

Formaldehyde treatment of the forage significantly ($p < 0.05$) reduced the non-protein-nitrogen of the abomsasl digesta. The ratios of % RNA-N: % total abomasal digesta N and % microbial-N: % total abomasal digesta N were greater ($p < 0.05$) for digesta from animals fed diet one than for digesta from animals fed diets two, four and five but not for digesta from animals fed diet three. All the other parameters measured were not signficantly ($p > 0.05$) affected by diet.

Chemical composition of diets used for duodenal flow rate measurements

The chemical composition of the batch of diets used for duodenal flow rate measurements is given in Table 10.

Table 9. Abomasal parameters.

	Diet containing untreated forage	Diets containing formaldehyde treated forage				
			+ VFAS	+ SO_4†	+ VFAS + SO_4	
	1	2	3	4	5	S.E.
Abomasal pH	3.84*	3.94	3.88	3.83	3.70	0.22
Abomasal digesta (D.M. basis):						
N%	2.27	3.01	2.80	3.16	3.06	0.23
ADF %	17.31	18.28	17.40	17.77	17.29	0.40
Cellulose %	12.27	13.47	12.84	12.96	12.73	0.35
NPN %	0.864a	0.676b	0.643b	0.650b	0.656b	0.043
RNA-N %	0.132	0.108	0.120	0.102	0.101	0.012
% RNA-N: % Total N ratio (x:1)	0.059a	0.036b	0.044ab	0.034b	0.033b	0.005
% Microbial N	1.76	1.44	1.60	1.36	1.35	0.16
% Microbial N: % Total N ratio (x:1)	0.786a	0.480b	0.583ab	0.451b	0.439b	0.068

* Each value represents the mean of five determinations.
† Sulphur was added as Na_2SO_4
Means on the same line bearing different letters (a, b) differ significantly ($p < 0.05$).

Table 10. Chemical composition of diets used for duodenal flow rate measurements.

Chemical Fraction	Diet containing Untreated forage	Diets containing formaldehyde treated forage			
			+ VFAS	+ SO_4†	+ VFAS + SO_4
	1	2	3	4	5
D.M. %	86.71	86.95	86.95	86.95	86.95
N % (D.M. basis)	2.21	2.24	2.24	2.20	2.20
ADF % (D.M. basis)	16.80	17.10	17.10	16.92	16.92
Cellulose % (D.M. basis)	12.64	13.01	13.01	13.01	13.01
Ash % (D.M. basis)	8.12	8.16	8.16	8.20	8.20

† Sulphur was added as Na_2SO_4

Feed intake during the duodenal flow measurements

The daily total feed intake, the feed intake during first, second, third and fourth six-hour periods are also given in Table 11. Feed intake during the first, second, third and fourth six-hour periods as a percentage of total feed intake is also given in Table 11. The mean feed intake as a percentage of total for all of the diets during the first, second, third and fourth six-hour periods and first and second twelve-hour periods were 40.72%, 17.01%, 22.52%, 20.20%, 57.28% and 42.72% respectively.

Duodenal flow parameters

The daily digesta flow (ml and g), the average hourly digesta flow (ml) during the first, second, third and fourth six-hour periods and first and second twelve-hour periods are given in Table 12. The percentage of total daily flow during the first, second, third and fourth six-hour periods and also during the first and second twelve-hour periods are also shown in Table 12.

The mean flow as a percentage of total for all of the diets during the first, second, third and fourth six-hour periods and first and second twelve-hour periods were: 25.20%, 24.96%, 24.37%, 24.35%, 50.13% and 49.87% respectively.

Table 11. Feed intake during the duodenal flow measurements:

	Diet containing Untreated forage	Diets containing formaldehyde treated forage			
			+ VFAS	+ SO_4† + VFAS	+ SO_4
	1	2	3	4	5
Feed Intake (g): (D.M. basis)					
Daily total	1300.65	1304.25	1304.25	1304.25	1304.25
Intake 1st 6 hrs	515.06	429.53	436.49	646.91	596.48
Intake 2nd 6 hrs	86.71	198.25	365.19	173.90	285.20
Intake 3rd 6 hrs	477.77	285.20	189.55	309.54	206.20
Intake 4th 6 hrs	221.11	391.28	313.02	173.96	217.38
% Intake of total:					
1st 6 hrs	39.60	32.93	33.47	49.60	45.73
2nd 6 hrs	6.67	15.20	28.00	13.33	21.87
3rd 6 hrs	36.73	21.87	14.53	23.73	15.73
4th 6 hrs	17.00	30.00	24.00	13.33	16.67

† Sulphur was added as Na_2SO_4

Table 12. Duodenal flow parameters.

	Diet containing untreated forage	Diets containing formaldehyde treated forage			
		+ VFAS	+ SO_4†	+ VFAS + SO_4	
Duodenal digesta flow	1	2	3	4	5
Total (ml)	15533	16760	17019	16615	16340
Total (g)	15576	16790	17053	16720	16338
Hourly (ml)	647.2	698.3	709.1	692.3	680.8
1st 6 hrs (ml)	3150	4075	5104	4225	4245
2nd 6 hrs (ml)	4235	3910	4630	4000	3745
3rd 6 hrs (ml)	4090	4810	3630	4370	4075
4th 6 hrs (ml)	4078	3965	3655	4020	4275
1st 12 hrs (ml)	7385	7985	9734	8225	7990
2nd 12 hrs (ml)	8168	8775	7285	8390	8350
Flow % of total:					
1st 6 hrs	20.28	24.31	29.99	25.43	25.98
2nd 6 hrs	27.26	23.33	27.20	24.07	22.92
3rd 6 hrs	26.33	28.70	21.33	26.30	24.94
4th 6 hrs	26.25	23.66	21.48	24.20	26.16
1st 12 hrs	47.54	47.64	57.19	49.50	48.78
2nd 12 hrs	52.58	52.36	42.81	50.50	51.10

† Sulphur was added as Na_2SO_4

Some chemical fractions of duodenal digesta, total daily intake and daily digesta flow through the duodenum of these fractions

The daily dry matter, organic matter, acid-detergent-fibre and cellulose intakes (g) are given in Table 13. The dry-matter, organic matter, acid-detergent-fibre and cellulose composition (%) of the duodenal digesta is shown in Table 13. The total daily flow through the duodenum (g) of dry matter, organic matter, acid-detergent fibre and cellulose are also shown in Table 13.

Daily nitrogen intake, nitrogen components of duodenal digesta and the daily flow of these components through the duodenum

The total daily nitrogen intake (g) on each of the diets is shown in Table 14. The nitrogen, non-protein-nitrogen, ribonucleic acid-nitrogen and microbial-nitrogen composition of the duodenal digesta is shown in Table 14. Table 14 also contains the % RNA-N: % total N and % microbial-N: % total N ratios, total daily RNA-N: total daily N and total daily microbial N: total daily N ratios. The total daily nitrogen, non-protein-nitrogen, true protein nitrogen, ribonucleic-acid-nitrogen and microbial nitrogen flowing through the duodenum are also given in Table 14.

Apparent digestibility coefficients of some chemical fractions in the pre-duodenum portion of digestive tract (all compartments of stomach) and the change in quantity of nitrogen entering the duodenum daily compared with intake

The apparent digestibility coefficients (%) in the preduodenum of the digestive tract (all compartments of stomach) of dry matter, organic matter, acid-detergent-fibre and cellulose are shown in Table 15. The

Table 13. Some chemical fractions of duodenal digesta, total daily intake and daily digesta flow through the duodenum of these fractions.

	Diet containing untreated forage	Diets containing formaldehyde treated forage			
		+ VFAS	+ SO_4†	+ VFAS +	SO_4
	1	2	3	4	5
Total daily intake(g):					
D.M.	1300.65	1304.25	1304.25	1304.25	1304.25
O.M.	1195.04	1197.82	1197.82	1197.30	1197.30
A.D.F.	218.51	223.03	223.03	220.68	220.68
Cellulose	164.40	169.68	169.68	169.68	169.68
Duodenal digesta: (Chemical fraction)					
D.M. %	5.68	5.97	5.96	5.95	5.99
O.M. % (D.M. basis)	86.16	87.24	86.98	88.44	87.96
A.D.F.% (D.M. basis)	17.10	18.25	17.65	18.10	17.80
Cellulose % (D.M. basis)	12.03	13.10	12.80	13.12	12.95
Total Daily Flow (g):					
D.M.	883.41	1000.57	1014.33	988.59	978.77
O.M.	761.15	872.90	882.27	874.31	860.92
A.D.F.	151.06	182.60	179.03	178.94	174.22
Cellulose	106.27	131.07	129.83	129.70	126.75

† Sulphur was added as Na_2SO_4

Table 14. Daily nitrogen intake, nitrogen components of duodenal digesta and the daily flow of these components through the duodenum.

	Diet containing untreated forage	Diets containing formaldehyde treated forage			
		+ VFAS	+ SO_4†	+ VFAS + SO_4	
	1	2	3	4	5
Total daily nitrogen intake (g)	28.74	29.22	29.22	28.69	28.69
Duodenal digesta:(D.M.basis)					
N %	2.40	3.10	2.96	3.15	2.98
NPN %	0.842	0.614	0.701	0.662	0.681
RNA-N %	0.145	0.111	0.122	0.094	0.093
Microbial-N %	1.93	1.48	1.63	1.25	1.24
% RNA-N:% Total N ratio	0.060	0.036	0.041	0.030	0.031
% Microbial-N:% Total N	0.804	0.477	0.551	0.397	0.416
Total daily flow (g):					
N	21.20	31.02	30.02	31.14	29.16
NPN	7.44	6.10	7.10	6.52	6.66
True protein N	13.76	24.92	22.92	24.62	22.50
RNA-N	1.28	1.11	1.24	0.929	0.910
Microbial-N	17.05	14.81	16.53	12.36	12.14
Total daily RNA-N: Total daily					
N	0.060	0.036	0.041	0.030	0.031
Total daily Microbial-N: Total dialy N	0.804	0.477	0.551	0.397	0.416

† Sulphur was added as Na_2SO_4

Table 15. Apparent digestibility coefficient of some chemical fractions in the pre-duodenum portion of digestive tract (all compartments of stomach) and the change in quantity of nitrogen entering the duodenum daily compared with intake.

	Diet containing untreated forage	Diets containing formaldehyde treated forage			
		+ VFAS	+ SO_4†	+ VFAS + SO_4	
	1	2	3	4	5
% App. Dig. Coeff. of:					
D.M.	32.08	23.28	22.23	24.20	24.96
O.M.	36.31	27.12	26.34	26.98	28.09
A.D.F.	30.87	18.12	19.72	18.92	21.05
Cellulose	35.35	22.75	23.48	23.56	25.30
Change in quantity of N entering duodenum daily compared with intake (g)	-7.54	+1.88	+0.80	+2.45	+0.47

† Sulphur was added as Na_2SO_4

change in quantity of nitrogen entering duodenum daily compared with intake is also given in Table 15.

DISCUSSION

In vitro digestibility trials

Formaldehyde treatment up to a level of 1.0% decreased *in vitro* nitrogen digestibility during the microbial stage (Table 2). The values in this experiment (31.88%, 15.72%, 6.87% and 5.69% for 0.0%,0.8%,1.0% and 1.2% level of formaldehyde treatment respectively) were lower than the values reported by Barry (1973a) and Barry (1976c). In the earlier study by Barry (1973a), he reported nitrogen digestibility at the microbial stage of digestion to be less than 30% for the treated and about 42% for the untreated, using grass-legume hay. The figures he reported in the later experiment were 59.5% and 39.9% for the untreated and the treated grass-legume hay respectively (Barry, 1976c). There are a number of factors which possibly contributed to the rather lower figures obtained in this trial compared to those of Barry (1973a and 1976c). One possible reason for the low figures obtained in the present study is that the heat applied during the drying of the forage reduced solubility of protein. However, it was unlikely that the heat applied caused great heat-damage to the forage as the acid-detergent fibre insoluble nitrogen level was less than 7% of total nitrogen, which was the maximum level reported by Yu and Thomas (1976) for undamaged forages. If heat-damage had occurred, digestibility of nitrogen at the second stage of incubation (combined microbial and acid-pepsin) would also have been reduced, but this did not happen. In fact, the digestibility of nitrogen at the second stage of incubation reported later on in this

discussion compared favourably with those reported by Barry (1976c). His figures ranged from 77.2% to 82.5% for the treated material and the figure for untreated was 84.8%. Another possible reason for the lower figures in the present trial for the first stage incubation compared to those of Barry (1973a and 1976c) was that tannins in the forage might have protected the proteins. It was not likely that the tannins in the forage greatly protected the proteins as, if that had occurred, digestibility of nitrogen at the second stage of incubation would have been reduced greatly. This is because Ferguson (1975) and McLeod (1974) reported that forage tannins which are of the condensed type, protect proteins permanently and the bonds are not broken in acidic medium. The hydrolyzable types of tannins, which form reversible bonding with proteins are present only in seeds. A third possible reason for the lower figures of first stage in vitro incubation nitrogen digestibility compared with figures of Barry (1973a and 1976c) is that a great amount of soluble nitrogen was converted to microbial protein-nitrogen and thus was not measured. Annison et al. (1954) reported that the extent of accumulation of degraded products of amino-acids in the rumen could be reduced when readily fermentable carbohydrates were present in great amounts. The rumen micro-organisms grew very fast and utilized nitrogen from the breakdown of proteins. In the present trial, although the readily fermentable carbohydrate level of the forage was not measured, it was most likely high as the forage was immature, as indicated by the high nitrogen level.

There was a compensatory stepwise increase in the digestibility of nitrogen at the second stage of incubation with the formaldehyde treated samples up to 1% level of treatment. The nitrogen digestibility figures for the second stage in vitro trial (combined microbial and acid-pepsin) were 80.95%, 79.76%, 75.85%, and 71.01% for 0.0%, 0.8%, 1.0%, and 1.2% levels of formaldehyde treatment respectively. The differences between the overall (combined microbial and acid-pepsin) and the first stage incubation nitrogen digestibility figures were 49.07, 64.04, 68.98, and 65.32 percentage units for 0.0%, 0.8%, 1.0%, and 1.2% levels of formaldehyde treatment respectively. Barry (1976c) also reported a compensatory increase in nitrogen digestibility at the second stage of incubation with formaldehyde treatment. The compensatory increase in nitrogen digestibility for the treated samples perhaps occurred because all the digestible nitrogen which was not in solution at the first stage of incubation, was digested by the acid-pepsin after the acidity broke down the bonds between the formaldehyde and the protein molecules.

Ammonia-nitrogen production was significantly reduced with increasing levels of formaldehyde treatment up to the level of 1.2% whereas nitrogen digestibility decreased only up to the 1.0% level. The ammonia-nitrogen levels per gram of dry matter incubated, as shown in Table 2, were 228.79 ppm, 78.58 ppm, 65.27 ppm, and 30.04 ppm for 0.0%, 0.8%, 1.0% and 1.2% level of formaldehyde treatment respectively. The reason for the further decrease in ammonia-nitrogen production beyond the 1.0% level of formaldehyde treatment is not clear. Nitrogen digestibility

figures (at the first stage of incubation) obtained from calculations based on the means of the ammonia-nitrogen figures were 30.26%, 10.39%, 8.63% and 3.97% for 0.0%, 0.8%, 1.0% and 1.2% levels of formaldehyde treatment respectively. These figures were slightly lower, except for the 1.0% level formaldehyde treatment, than those obtained from the actual measurements of digestibility. The reason for the slight increase in the figure for the 1.0% level of formaldehyde application compared to the actual measured value is not obvious. Barry (1976c) also reported lower figures of nitrogen digestibility at the first stage of incubation using ammonia-nitrogen figures (28% and 17% for untreated and formaldehyde treated hay respectively) compared with figures obtained from actual measurements (59.5% and 39.9% for untreated and formaldehyde treated hay respectively). Sharkey et al. (1972) reported that the nitrogen digestibility figures obtained from actual measurements includes all soluble nitrogen compounds released during fermentation while ammonia-nitrogen takes into account only nitrogen fermented to ammonia. It should also be noted that dipeptides, amino acids and nucleic acids could be utilized by rumen microbes without first being broken down to ammonia (Nolan, 1975). These compounds would not be measured if they are in solution when only ammonia-nitrogen level is measured and therefore the actual level of utilization of protein in the feed by microbes would not be correctly assessed by measuring only the ammonia-nitrogen level.
It seems therefore that ammonia-nitrogen production may not be the best indicator of the optimum level of formaldehyde treatment. It also gives no indication of the effect of formaldehyde treatment on the digestion

of nitrogen by enzymes in the intestine. Barry (1976a) reported that nitrogen digestibility measurement using both microbial and acid-pepsin stages of incubation was a more reliable method of determining optimum levels of formaldehyde treatment than ammonia-nitrogen levels.

Feed Intake

The daily dry matter intake per unit of metabolic bodysize both during the pre-metabolism and metabolism study periods was not affected significantly ($p > 0.05$) by treatments (Table 4). The values for the pre-metabolism assay period were 91.33, 100.62, 101.93, 98.02 and 96.47 g/ $Wkg^{0.75}$ per day for diets one, two, three, four and five respectively; the values for the metabolism assay period were 90.17, 96.65, 104.29, 90.88 and 94.25 $g/Wkg^{0.75}$ daily for diets one, two, three, four and five respectively. Feed intake is an important factor affecting the nutritive value of diets of ruminants (Crampton and Harris, 1969). Any differences in the performances of the animals on the different diets in this experiment could therefore not be attributed to variations in feed intake. Kempton et al. (1977) and Ferguson (1975) reported that response to formaldehyde treatment of diets was mediated mainly through increased feed intake compared to untreated diets. Davies and Faichney (1973) reported decreased feed intake with formaldehyde treatment of barley rations. It is noteworthy that the addition of the pure volatile fatty acids to some of the diets containing the formaldehyde treated forage (diets three and five) did not depress feed intake. Hume (1970) postulated that the addition of the pure volatile fatty acids to diets

could decrease palatibility and therefore in his studies sodium salts of these acids were used. However, Hume (1970) did not indicate that the hypothesis that the pure volatile fatty acids reduced palatibility was tested.

Animals were fed ad libitum in this experiment as was done by Hemsley and Moir (1963) and Adeleye (1972). Some workers have restricted feed intake during metabolism studies (Barry, 1973b, Barry and Andrews, 1973; and Amos et al., 1976b). The argument for restricting feed intake is to reduce variability in intake of dry matter and nitrogen, especially nitrogen, which could affect nitrogen balance. One reason for feeding these animals ad libitum in the present trial was that if restricted feeding was carried out, results from such experiments could not be applied, without limitations, to practical farm conditions where animals are fed ad libitum. Nimrick et al. (1972) noted that when animals are fed ad libitum, performance may not be the same as when restriction of feed intake is practised. With ad libitum feeding low rumen pH occurs, especially when high levels of readily fermentable carbohydrate are fed. Protein solubility is reduced and its utilization is improved when ad libitum feeding is carried out compared to restricted feeding, when protein breakdown in the rumen may be great thus reducing its utilization. Eadie and Mann (1970) observed that the types of bacteria in the rumen may also be different when high carbohydrate diets are fed ad libitum as compared to restricted feeding. Amos et al. (1976b) restricted feed intake and observed no difference in nitrogen-balance between formaldehyde treated and untreated coastal bermudagrass hay.

When feed intake was increased from 600g dry matter to 775g dry matter, the essential and non-essential amino acids arriving at the duodenum were increased significantly for the formaldehyde treated material.

Nitrogen intake per day per unit of metabolic body size (2.07, 2.18, 2.38, 2.08 and 2.18 g for diets one, two, three, four and five respectively, Tables 4 and 6) was also not affected by treatment. This is understandable since nitrogen levels in the diets (Table 3), were almost identical (2.29%, 2.24%, 2.25%, 2.28% and 2.29% for diets one, two, three, four and five respectively) and dry matter intake, as discussed above, was not affected. With nitrogen intake being almost identical on all diets, dietary sources of nitrogen, for microbial activities in the rumen, could be limited for the diets containing the formaldehyde treated forage. The non-protein-nitrogen from the grass-legume forage and the nitrogen from the barley and cassava totalled about 0.681g for every 100g of the rations. There could be a supply of nitrogen to the rumen micro-organisms with the diets containing the formaldehyde treated forage through recycling. It seemed that nitrogen was limiting in the rumens of the animals fed the diets containing formaldehyde forage as rumen ammonia-nitrogen, and microbial protein content of abomasal digesta were reduced (these results are discussed later). The concentration of valeric acid in the rumens of animals fed the diets containing the formaldehyde treated forage was lower compared to the control. This indicated a limitation to rumen microbial growth most likely by nitrogen. It is therefore surprising that feed intake was

not depressed for the diets containing the formaldehyde treated forage as a result of the limitation of available nitrogen for microbial growth.

Apparent digestibility coefficients of nitrogen, acid-detergent fibre, cellulose, dry matter and organic matter

Apparent digestibility coefficients of nitrogen

The values of apparent digestibility coefficients of nitrogen obtained in this experiment were generally lower than the values reported by Beever et al. (1976). The values for this experiment were 54.13, 47.06, 51.25, 44.90 and 47.24% for diets one, two, three, four and five respectively, while figures reported by Beever et al. (1976) were 69.8% for untreated and 61.8% for treated rye grass. The values obtained in this experiment were however similar to those reported by Dinius et al. (1975) and Barry (1973a). Dinius et al. (1975) reported apparent nitrogen digestibility coefficients of 63.1, 41.1 and 29.8% for 0.0, 1.0 and 2.0% formaldehyde treatment of alfalfa meal. Barry (1973a) reported values of 55.2, 50.6, and 51.1% for 0.0, 2.4 and 4.8% levels of application of formaldehyde to rye-grass-clover during the process of hay making. The rather low value obtained for the untreated diet (diet one) in this experiment compared with the results of Beever et al. (1976) and Dinius et al. (1975) could possibly be attributed to a number of factors reducing digestion in the rumen:

(a) pelleting of the ration. Pelleting of rations has been reported to increase protection of proteins as a result of heat generated during the pelleting process (Kempton et al., 1977).

(b) High levels of soluble carbohydrates. Cassava and barley constituted about 50% of the ration. High levels of soluble carbohydrate in diets have been reported to decrease rumen pH and thus reduce solubility of protein in the rumen fluid (Ørskov, 1975; Kempton et al., 1977). However, Tagari et al. (1964) reported increased nitrogen digestibility in the rumen with the addition of soluble carbohydrates to hay diets. It is interesting to note that the most proteolytic microbes in the rumen are the amylolytic ones (Hungate, 1967; Church, 1975b). It is therefore surprising that lowered digestion of nitrogen occurs in the rumen when high levels of soluble carbohydrate are fed, which should promote the growth of the amylolytic types of rumen microbes. Perhaps nitrogen digestibility of the diets containing the formaldehyde treated forage (except diet three) was reduced as a result of less digestion of nitrogen taking place in the rumen. Although the heat treatment of the grass-clover forage, the pelleting of the rations, and the high levels of soluble carbohydrates could have caused reduction in the digestibility of nitrogen in the rumen as discussed above for the control untreated diet, formaldehyde application further reduced nitrogen digestibility in the rumen. Dinius et al. (1974) reported that heating of protein had additive effects to formaldehyde treatment. The lowered levels of ammonia-nitrogen in the rumens of the animals fed the diets containing the treated forage compared with the diets containing the untreated forage supports the contention that there was generally reduced digestion of nitrogen in the rumen.

The reason for the rather high degree of digestion of nitrogen for diet three compared to the other diets containing the formaldehyde treated forage is not clear. The ammonia-nitrogen levels in the rumens of the animals fed diet three were still lower than the levels in the rumens of animals fed diet one although nitrogen digestibility was not significantly ($p > 0.05$) different. It was possible that the addition of volatile fatty acids to diet three promoted the growth of some microbes which could utilize nitrogen efficiently in the rumen.

Hemsley et al. (1970) reported that forage protein which by-passed the rumen was more digestible than bacterial protein. In this experiment, a greater amount of protein from the forage by-passed the rumen for the diets containing the formaldehyde treated forage as indicated by the duodenal flow data (Table 14). This protein by-passing the rumen was still not digested to a greater extent in the intestine than the protein in diet one. Hill (1975) and Horn and Huber (1975) observed that the most powerful stimulus for the release of secretin, which also has an effect on the secretion of pancreatic enzymes, is acid ingesta in the duodenum. In this experiment abomasal pH was not different for any of the dietary treatments. It was thought that with treatment of the forage portion of the diets with formaldehyde, abomasal pH would be high as hydrogen ions would be required to break the bond between the protein and formaldehyde as indicated by Barry (1976a). It seems from this trial that there was a possible compensatory increase in the production of hydrochloric acid in the abomasum with formaldehyde

treatment of the forage portion of the diets since abomasal pH values were not affected (Table 9).

The time after feeding when pH measurements were carried out was perhaps not optimum to demonstrate differences. Knight et al. (1972) observed that the greatest fall in abomasal pH occurred in the first hour after feeding. In the present trial, the animals were slaughtered about four to six hours (4-6 hrs) after feeding. The results of the experiments of Knight et al. (1972) indicated a rise in abomasal pH after the first hour after feeding with pH levels reaching pre-prandial conditions about six hours (6 hrs) after feeding. Hill (1975) reported that other factors which also affect the release of secretin are peptones, soaps and amino acids arriving at the duodenum. Since these parameters were not measured, it is not possible to comment on their effects on the digestibility of nitrogen for the diets containing the formaldehyde treated forage.

Abomasal non-protein-nitrogen levels determined with slaughtered animals were lower with the diets containing the treated forage (Table 9). The total daily flow of non-protein-nitrogen through duodenum measured with the cannulated sheep tended to be somewhat lower for the diets containing the treated forage than for the diet containing the untreated forage (7.44, 6.10, 7.10, 6.52 and 6.66 g/day for diets one, two, three, four and five respectively).

The addition of sulphate alone to one of the diets containing formaldehyde treated forage (diet four) somehow reduced nitrogen digestibility. It is not clear how the sulphate adversely affected nitrogen digestibility. Umuna and Woods (1975) reported reduction in nitrogen digestibility when urea in diets was coated with sulphur.

The apparent digestibility coefficients of nitrogen obtained in the *in vivo* trials were generally lower than the nitrogen digestibility obtained in the *in vitro* trials (combined microbial and acid-pepsin stages). Barry (1976c) reported *in vitro* nitrogen digestibility (both microbial and acid-pepsin stages of digestion) to be about 72-84% while in the *in vivo* trials, values obtained ranged from 52% to 68%. The discrepancy between the *in vitro* and the *in vivo* nitrogen digestibility in the present trial could perhaps be explained as follows: the *in vitro* measurement was true digestibility of the grass-legume forage while the *in vivo* was apparent, not taking into account endogenous secretions of nitrogen. It must also be noted that during the *in vitro* measurement, only the grass-legume forage was used, while with the *in vivo* cassava and barley formed part of the ration.

Apparent digestibility coefficients of ADF and Cellulose

There was a significant ($p < 0.05$) increase in the *in vivo* digestibility of acid-detergent fibre and cellulose with formaldehyde treatment of the forage (Table 5, ADF values: 32.57, 36.97, 36.91, 36.45 and 36.59% for diets one, two, three, four and five respectively; cellulose: 42.95, 49.10, 49.04, 49.33 and 48.76% for diets one, two, three, four and five respectively). Dinius *et al.* (1975) reported a significant

reduction in the digestibility of acid-detergent fibre (38.8%, 33.8%, and 26.4% for 0.0%, 1.0%, and 2.0% levels of formaldehyde treatment respectively). Beever et al. (1976) reported higher values of cellulose digestibility for formaldehyde treated high temperature (900°C) dried grass forage (83.9% vs 89.5% for the untreated and treated respectively).

The levels of acid-detergent fibre and cellulose in the abomasal digesta samples (Table 9) were not significantly ($p > 0.05$) different. The digestibility of acid-detergent-fibre in the stomach (pre-duodenum) from the animal fitted with re-entrant cannula (Table 15) was markedly greater for diet one than for the rest (30.87%, 18.12%, 19.72%, 18.92% and 21.05% for diets one, two, three, four and five respectively). The same trend was observed for cellulose (35.35%, 22.75%, 23.48%, 23.56% and 25.30% for diets one, two, three, four and five respectively). It could be argued that with the diets containing the formaldehyde treated forage most of the digestion of acid-detergent-fibre and cellulose took place outside the reticulo-rumen. A great quantity of nitrogen might have reached the caecum and the colon as there was a reduction in the digestion of nitrogen in the reticulo-rumen when the diets containing the formaldehyde treated forage were fed. The micro-organisms in the caecum and colon having access to a great amount of nitrogen might have had greater activities than those in animals fed the diet containing the untreated forage, with less nitrogen reaching the caecum and colon. Nolan (1975) reviewing literature on digestion in the hindgut reported that proteolytic activity appears to be greater in the contents of the large intestine than in the contents of the rumen. He also observed

that a great deal of fermentation of structural carbohydrates occurs in the hindgut especially with sheep fed diets containing high levels of cereals. The sources of nitrogen in the hindgut for such fermentation activities of the micro-organisms are: nitrogeneous compounds arriving from the small intestine in the form of feed residues, undigested rumen micro-organisms and endogenous secretions; and entry of urea-nitrogen from the blood.

In the case of diet three, though nitrogen digestibility was not significantly ($p > 0.05$) lower than diet one fibre digestibility was significantly ($p < 0.05$) higher. The microbes in the hindguts of animals fed diet three were probably supplied with a greater amount of nitrogen from the small intestine than for diet one. It must be noted that the concentration of nitrogen in the abomasal digesta samples was not affected by dietary treatment (Table 9) and the nitrogen concentration of the duodenal digesta was not markedly different. It was still possible that the animals fed the diets containing the formaldehyde treated forage had a greater amount of nitrogen arriving in the duodenum as flow of dry matter could be different. From the data obtained with the animal fitted with re-entrant cannula, the daily flow of nitrogen was markedly higher for the diets containing the formaldehyde treated forage than for the diets containing untreated material (Table 14, 21.20g, 31.02g, 30.02g, 31.14g and 29.16g for diets one, two, three, four and five respectively). Nitrogen arriving in the duodenum was greater for diet three compared with diet one. Enough nitrogen was

probably left for microbial activity in the hindgut even if a greater portion of it was digested and absorbed in the small intestine with diet three compared to diet one. With a greater amount of nitrogen arriving in the hindgut for diet three than for diet one, microbial activity would be greater and therefore digestion of fibre in the hind gut would be greater for diet three than for diet one.

Beever et al. (1976) observed a greater amount of digestion of fibre in the hindgut for the treated compared to the untreated diet. In their study, 95% of the digestion of fibre occurred in the reticulo-rumen for the untreated diet and 70% for the formaldehyde treated high temperature dried grass (overall digestibility of fibre being 83.9%, and 89.5% for untreated and treated respectively). In the present study, the sites for the digestion of fibre were different for the diet containing the untreated forage and the diets containing the formaldehyde treated forage. Nitrogen was perhaps the main limiting factor for microbial degradation of fibre in the rumen when the diets containing the formaldehyde treated forage were fed (rumen ammonia-nitrogen levels were lower for diets two, three, four and five than for diet one, Table 8). A greater proportion of fibre was digested in the hindgut where nitrogen might not have limited microbial activities when the diets containing the formaldehyde treated forage were fed (the apparent digestibility coefficients of A.D.F. and cellulose in the four compartments of the stomach were observed to be higher for diet one than for the others using the sheep fitted with the duodenal re-entrant cannula, Table 15). However, when the diet containing the untreated forage was fed, nitrogen was most likely not a limiting factor in the rumen but was most probably

limiting in the hindgut. There were high levels of readily fermentable carbohydrate present in the rumen. Therefore, the digestion of fibre could not be carried out to the maximum extent.

Apparent digestibility coefficients of dry matter and organic matter

The apparent digestibility coefficients of dry matter (64.78%, 65.69%, 65.82%, 64.80%, and 63.71% for diets one, two, three, four and five respectively) and organic matter (65.35%, 65.91%, 66.01%, 65.17% and 63.83% for diets one, two, three, four, and five respectively) were not affected significantly ($p > 0.05$) by treatments. The values obtained were similar to those reported by other workers (Amos et al., 1976b; Sharma and Nicholson, 1975b). Amos et al. (1976b) reported dry matter digestibility figures of 64.4%, 63.4%, 61.3%, and 56.3% for 0.0%, 0.5%, 1.0% and 1.5% levels of formaldehyde treatment of coastal bermuda grass respectively. Sharma and Nicholson (1975b) reported dry matter digestibilities of 65.4% and 63.9%, for rations containing untreated and formaldehyde treated faba bean meal respectively. They also reported dry matter digestibilities of 53.5% and 55.2% for rations containing untreated and treated rapeseed meal respectively (Sharma and Nicholson, 1975a). In some of the reports cited above (Sharma and Nicholson, 1975a, 1975b; Barry 1976c) dry matter digestibilities were not significantly affected by formaldehyde treatment as was the case in the present experiment. Sharma and Nicholson (1975a and 1975b) did not observe a significant reduction in nitrogen digestibilities in the two trials, with formaldehyde treatments of faba bean and rapeseed meal. Barry (1976c) reported no significant reduction in dry matter digestibility of diets treated with formaldehyde although there was a significant

reduction in nitrogen digestibility. Dinius et al. (1975) however reported a significant reduction in dry matter, nitrogen and fibre digestibilities with formaldehyde treatment.

In the present experiment, although nitrogen digestibility was significantly reduced (except for diet three) by treatment of the forage portion of the diets with formaldehyde, dry matter and organic matter digestibilities were not affected because of an increase in the digestion of some other fractions, especially fibre.

For diet three, nitrogen digestibility was not significantly less than for diet one and fibre digestibility was significantly ($p < 0.05$) greater than for diet one. It was expected that dry matter and organic matter digestibilities would have been significantly different for the two diets. However, there were slight but non-significant differences between diets one and three with respect to nitrogen and dry matter or organic matter digestibilities. The nitrogen digestibility was slightly higher for diet one than for diet three (54.13% for diet one and 51.25% for diet three); dry matter digestibility was slightly higher for diet three than for diet one (64.78% for diet one, and 65.82% for diet three); organic matter digestibility was also slightly higher for diet three than for diet one (65.35% for diet one and 66.01% for diet three).

Rumen Parameters

Rumen pH

The pH value of the rumen fluid ranged from 5.11 to 5.50 for all the diets. Rumen pH was not significantly ($p > 0.05$) affected by

the treatments. Dinius et al. (1975) also observed no differences in rumen pH with treatment of alfalfa meal with formaldehyde. The pH values obtained in the present trial were similar to the values reported by Sharma and Ingalls (1974). They reported average pH values of about 5.6. They also reported no differences in pH with formaldehyde treatment of diets. In the present experiment, the samples for pH measurements were taken 4-6 hours after feeding. Hodgson et al. (1976) reported that as volatile fatty acid concentration increased, the rumen pH decreased. In the present experiment, as the pH was measured at the time when concentration of volatile fatty acids was expected to be highest it is not surprising that the pH values were relatively low. Saville et al. (1971) postulated that if rumen pH values were low, it was possible that the bonds between formaldehyde and proteins could be broken. Sharma and Ingalls (1974) reported low pH values around 5.6 but still no breakdown in the bonding occurred. There is also a cyclical variation in rumen pH and the values obtained in this experiment could have been at the lowest for the day. The animals also did not refuse feed and there were no signs of rumenitis.

Rumen ammonia-nitrogen

Rumen ammonia-nitrogen levels (Table 8) were signifcantly ($p < 0.05$) reduced by formaldehyde treatment of the forage portion of the diets (21.14, 14.36, 14.30, 12.90 and 13.54 ppm for diets one, two, three, four and five respectively). Reports by some workers indicated reduced rumen ammonia-nitrogen levels with treatment of diets with formaldehyde (Sharma and Nicholson, 1975b; Bhargava and Ranjhan, 1974;

Sharma and Ingalls, 1973; Sharma et al., 1972; Hogan and Weston, 1970; Hemsley et al., 1970). Other workers however observed no decrease in rumen ammonia nitrogen levels with formaldehyde treatment of diets (Dinius et al., 1975; Sharma and Nicholson, 1975a).

Ammonia present in the rumen could be from a variety of sources: recycled urea-nitrogen either from blood or saliva; from the breakdown of protein or amino acids, and reduction of nitrates; from the breakdown of microbial protein during recycling of nitrogen within the rumen (Houpt, 1970).

Ammonia-nitrogen could also be lost through the ruminal wall. Such losses have been reported to be dependent on pH with maximum losses at pH 6.5, and negligible at pH 4.5 (Hemler and Bartley, 1971; Hogan, 1961). Ammonia-nitrogen could be passed on to the abomasum and subsequently to the intestine as there is little, absorption of ammonia-nitrogen from the abomasum (Houpt, 1970; Hembry et al., 1975). In the present experiment, rumen pH values were not significantly different, and therefore losses of ammonia-nitrogen through the ruminal wall are unlikely to have contributed to the variations in rumen ammonia-nitrogen with formaldehyde treatment. If anything, losses of nitrogen in the rumen were possibly greater for diet one than for the other four diets. This is because the quantity of nitrogen leaving the abomasum was greater for all the four diets containing the treated forage compared to the control, using the animal fitted with re-entrant cannula (Table 14). The greater amounts of nitrogen arriving at the duodenum with the diets containing the formaldehyde treated forage compared with diet one were unlikely to be due to differences in the losses of ammonia in the abomasum as such losses

are reported to be negligible (Houpt, 1970; Hembry et al., 1975). Nitrogen recycling into the rumen is reported to be highest when ammonia-nitrogen levels in the rumen are low (Church, 1975c, Houpt, 1970). It is therefore also unlikely that the higher ammonia-nitrogen levels in the rumens of animals fed diet one compared to the rest, was due to greater recycling of nitrogen into the rumens from saliva and blood urea-nitrogen. One possible reason for the higher levels of rumen ammonia-nitrogen in the rumens of animals fed diet one compared with the rest was a greater degree of recycling of microbial nitrogen within the rumen. Perhaps the most probable explanation for the higher levels of ammonia-nitrogen in the rumens of animals fed diet one compared with the rest is a greater degree of digestion of nitrogen in the rumens of the animals fed this diet. This is supported by the lower quantity of nitrogen arriving in the duodenum when diet one was fed to the sheep fitted with the re-entrant cannula, compared to the other diets. The quantity of non-protein-nitrogen arriving at the duodenum was also slightly greater for diet one than for the rest. The concentration of non-protein-nitrogen in the abomasal digesta samples from the slaughtered animals was also significantly ($p < 0.05$) higher for diet one than for the rest (Table 9).

Generally, the levels of ammonia in the rumens for all the diets were low compared with figures reported by Barry (1973a) and Sharma and Nicholson (1975a and 1975b). The ammonia-nitrogen levels reported by Sharma and Nicholson (1975a) were 8.99mg/100ml and 5.47mg/100ml for diets containing untreated and formaldehyde treated rapeseed meal respectively.

These measurements were carried out on samples collected one hour after feeding. These workers (Sharma and Nicholson, 1975b) reported rumen ammonia-nitrogen levels of 24.25mg/100ml and 13.66mg/100ml for diets containing untreated and formaldehyde treated faba bean meal respectively. These measurements were also carried out on samples collected one hour after feeding. Measurements carried out on samples collected four hours after feeding in the same experiment gave results of 15.40mg/100ml and 6.96mg/100ml for diets containing untreated and formaldehyde treated faba bean diets. Barry (1973a) taking measurements on samples collected four hours after feeding, reported figures of 27.0mg/100ml for untreated and 14.8mg/100ml for formaldehyde treated lucerne hay.

Dinius et al. (1975) however reported low ammonia-nitrogen levels of 3 to 7 mg/100ml. They took samples for ammonia-nitrogen measurements just before feeding and at one hour intervals for eight hours and combined the results to arrive at the values reported above.

The rather low levels of ammonia-nitrogen observed in the present experiment could perhaps be attributed to the time of sampling. Samples were taken four to six hours after feeding. Rumen ammonia-nitrogen level is reported to reach peak levels about 90 to 130 minutes after feeding (Church, 1975c). Hembry et al. (1975) reported rumen ammonia levels of 12 to 30 ppm six hours after feeding and 16 to 46 ppm four hours after feeding diets containing urea, soybean meal and casein.

Rumen total volatile fatty acid concentration

There were no significant ($p > 0.05$) differences between treatments with respect to the production of volatile fatty acids in the rumen (166.12, 186.03, 165.44, 166.81 and 150.06 μ-moles/ml for diets one, two, three, four and five respectively). Several workers have also reported no significantly differences in the production of volatile fatty acids in the rumen with formaldehyde treatment. (Beever et al., 1976; Beever et al., 1977; Sharma and Ingalls, 1973). Other workers however have observed decreased concentrations of volatile fatty acids with treated diets (Sharma et al., 1972; Barry, 1973a). Microbial protein-nitrogen in the abomasal digesta samples (measured by the ratio of % microbial - N : % total digesta N) was decreased significantly with all the diets containing the formaldehyde treated forage, except for diet three (0.768, 0.480, 0.583, 0.451, and 0.439 for diets one, two, three, four and five respectively). The ratios for duodenal digesta, using the sheep fitted with re-entrant cannula (0.804, 0.477, 0.551, 0.397, and 0.416 for diets one, two, three, four and five respectively) and the total amounts of microbial protein arriving at the duodenum per day (17.05, 14.81, 16.53, 12.36, and 12.14g for diets one, two, three, four and five respectively) were also reduced by treatment of the forage portion of the diets with formaldehyde. No statistical analysis was performed however with the duodenal digesta as only one animal was used but it still indicated the trend shown by the abomasal digesta samples.

Smith et al. (1968) reported that the proportion of ribonucleic-acid nitrogen to total nitrogen in duodenal digesta paralleled the proportion of ribonucleic acid-nitrogen to total nitrogen in rumen fluid

except that the levels in the duodenal digesta were consistently lower because of the addition of endogenous secretions of nitrogen in the abomasum. It is therefore in line to extrapolate measurements of microbial protein-nitrogen in duodenal digesta to what happened in the rumen.

From the data reported above for the present trial, it could therefore be concluded that microbial protein synthesis in the rumen was reduced by formaldehyde treatment of the forage portion of the diet, except for diet three. However, volatile fatty acid production was not affected. Beever et al. (1977) reported that rumen microbial populations were capable of adapting their metabolic pathways to make maximum use of carbohydrates while microbial protein synthesis may be depressed. This phenomenon was termed by these workers as uncoupled fermentation. It is probable that this occurred in the present study. Hodgson et al. (1976) reported that the amount of dry matter in the rumen could affect the concentration of short chain fatty acids in the rumen. The quantities of dry matter in the rumens of the sheep (Table 8) were not significantly ($p > 0.05$) different with dietary treatment (574.93, 643.73, 447.89, 449.04, and 478.15g for diets one, two, three, four, and five respectively). Therefore, possibly this parameter had no effect on the production of volatile fatty acids in the rumen. Dinius et al. (1975) reported that time of sampling had some effects on the concentration of volatile fatty acids in the rumen. In their study, the volatile fatty acid concentration at two, three, and four hours after feeding was significantly reduced by formaldehyde treatment of the lucerne portion of the diet but at six hours there were no significant differences between the formaldehyde

treated and the untreated. Sharma et al. (1972) and Barry (1973a) who observed differences in concentration of volatile fatty acids in the rumen due to formaldehyde treatment took their samples three to four hours after feeding. Beever et al. (1976), and Beever et al. (1977) who reported no significant reduction in total volatile fatty acid production in the rumen measured its production over twenty four hours. Sharma and Ingalls (1973) who also reported no significant reduction in the production of total volatile fatty acid, took their samples three to four hours after feeding. Therefore, it is possible that the equality of production of volatile fatty acids reported in the present study was not due to the time of sampling.

The levels of volatile fatty acids in the rumens were high compared with figures reported by Dinius et al. (1975) and Nicholson and Sutton (1969). Dinius et al. (1975) reported figures (at four hours after feeding) of about 100, 86, and 50 μ-moles/ml for diets containing lucerne meal treated with formaldehyde at levels of 0, 1 and 2% respectively. The corresponding figures for six hours after feeding were about 80, 72, and 60 μ-moles/ml respectively. In the case of their experiments the level of lucerne in the diets was about 75% while in the present experiment the level of forage was only about 50% with the remainder of the rations made up of readily fermentable carbohydrates. This high level of readily fermentable carbohydrate might have produced the high level of total volatile fatty acid observed in the present experiment. Nicholson and Sutton (1969) reported total volatile fatty acid levels of about 82.1 to 97.0 μ-moles/litre when they fed diets containing 20% hay and 80% concentrate to sheep. The levels of readily soluble carbohydrate were high in their diets. They however, composited

samples taken at three, six, nine, and eleven hours after feeding for the volatile fatty acid measurements. Therefore, the high levels of volatile fatty acid observed in the present experiment compared to the levels reported by Nicholson and Sutton (1969) could most likely be due to the time of sampling.

Acetic, propionic, and butyric acid proportions in the rumen fluid

The molar proportions of acetic, propionic and butyric acids in the rumen fluid were not affected by dietary treatment (Table 8). The second group of animals however had significantly ($p < 0.05$) higher molar proportions of acetic acid than the fourth group, while the fourth group had significantly ($p < 0.05$) higher molar proportions of propionic acid than the second group (Results, page 68). Differences in the molar proportions of acetic and propionic acids in the rumens of the second and fourth groups (blocks) of animals could have occurred because of individual animal differences. Slyter et al. (1970) reported variations in types of rumen microbes with full feeding, even with identical twins. These variations in populations of types of microbes could also affect proportions of the various short-chain fatty acids. In the present experiment the proportion of soluble carbohydrates in the diets fed to the different groups (blocks) of animals was not different. Therefore, the higher levels of propionic acid and the lower levels of acetic acid in the rumens of sheep in the fourth group compared to those in the second group could not be due to different levels of soluble carbohydrates.

Wilke and Merwe (1976) reported that high levels of concentrate in the diet could result in high molar proportions of propionic acid at the expense of the acetic acid proportion. The average proportions of the various fatty acids for all the diets (acetic 41.96%, propionic 29.00%, n-butyric 23.27%, isobutyric 1.35%, isovaleric 1.66%, and n-valeric 2.96%) were similar (except for acetic and butyric) to the values reported by Sutton (1969). He reported that when a cow was fed a diet containing flaked maize (5kg per day) and hay (1.0kg per day) the proportions of the various acids were: acetic 54.8%, propionic 25.1%, butyric 14.0%, isobutyric 1.4%, isovaleric 2.3%, and valeric 2.4%. Ørskov et al. (1970) reported the following levels of the various acids in the rumen of sheep fed chopped dried grass: acetic 68.6%, propionic 20.2%, butyric 6.0%, isobutyric 2.5%, isovaleric 2.0%, and valeric 0.8%. In the experiment of Ørskov et al. (1970) the sheep were slaughtered one hour after feeding and then samples taken. The same workers in the same paper, reported the following levels of various acids when barley was fed (animals slaughtered one hour after feeding and samples taken): acetic 53.6%, propionic 20.6%, butyric 16.2%, isobutyric 1.7%, isovaleric 1.4%, n-valeric 5.4%, and caproic 1.1%. Nicholson and Sutton (1969) and Whitelaw et al. (1970) reported that when sheep on barley diets are fed below full feeding the proportion of butyric acid is increased with a corresponding decrease in the propionic acid fraction. In the present experiment, both butyric acid and propionic acid proportions were very high. There was rather a decrease in acetic acid proportion which is usually reported above 50% even when animals are fed diets containing

great amounts of readily fermentable carbohydrate (Sutton, 1969; and Ørskov et al., 1970). The animals were fed ad libitum. The lowered acetic acid level was therefore probably due to the high level of soluble carbohydrates in the rations.

Isovaleric and Isobutyric acid proportions in the rumens

The molar proportions of isovaleric and isobutyric acids were higher for diet three than for diets two and four ($p < 0.05$). The isovaleric acid proportions were 2.53, 0.60, 2.58, 0.39 and 2.18% for diets one, two, three, four and five respectively. The isobutyric acid proportions were 1.54, 0.71, 2.11, 0.66 and 1.73% respectively for diets one, two, three, four and five. It was expected that with formaldehyde treatment of the forage portion of the diets molar proportions of isobutyric and isovaleric acids would be reduced. These acids are derived from branched-chain amino acids (el-Shazly, 1952a and 1952b). Allison and Bryant (1963) reported that the mechanism for the synthesis of the isopropyl moiety in the branched-chain fatty acids was inadequate in some micro-organisms. Langlands (1973a) reported reduction (not significant) in the levels of isobutyric and isovaleric acids following formaldehyde treatment of wheat. Faichney and White (1977a) reported significant reductions of isobutyric and isovaleric acid levels with formaldehyde treatment of concentrate diets. Barry and Fennessy (1973) reported reduced levels of isovaleric and valeric acids combined with formaldehyde treated silages. Barry (1976c) reported

reduced levels of isovaleric and valeric acid combined, but not isobutyric acid, with treatment of diets with formaldehyde. Hobson (1971) reported that the addition of one acid may suppress its production. This phenomenon was not observed by Hume (1970) who reported increased levels of branched-chain fatty acids in the rumens of animals fed diets supplemented with the sodium salts of these acids. The present results seem to conform with those of Hume (1970). It is surprising that the levels of isovaleric and isobutyric acids were not significantly higher for diet five which was supplemented with volatile fatty acids than for diets two and four. It is to be noted that three animals on diet two, and one on diet four had levels of isobutyric and isovaleric acids which could not be measured. Other sources of protein, which could supply branched-chain amino acids to be deaminated to branched-chain fatty acids are

(a) recycled microbial protein,

(b) desquamated rumen epithelium, and

(c) protein present in saliva.

For diet three, it is difficult to speculate which of the three sources mentioned above contributed to the higher levels of the branched-chain fatty acids in addition to what was supplemented and what came from the breakdown of dietary protein in the rumen. The high levels for diet five, though not significantly higher than diets two and four, could be due mainly to the supplemented quantities. It is interesting to note that, unlike diet three, the digestibility of nitrogen was not

significantly different from either diet two or diet four {diet three had significantly ($p < 0.05$) higher nitrogen digestibility than diet four}. The amounts of microbial protein nitrogen arriving at the duodenum were not markedly different with diet five compared with diets two and four. The high levels of the branched-chain fatty acids in the rumens of animals fed diet one, although not significantly different, compared with diets two and four, could be attributed to greater breakdown of dietary protein in the rumens.

Valeric acid proportions

The valeric acid levels in the rumens of animals fed the diets containing formaldehyde treated forage were significantly ($p < 0.05$) higher than the levels in the rumens of animals fed diet one (1.82, 3.30, 3.19, 3.34 and 3.15% for diets one, two, three, four and five respectively). Cline et al. (1958) reported that with a decrease in available nitrogen in the rumen, valeric acid levels increased. In the present trial, microbial protein synthesis, measured by % RNA-N: % total abomasal digesta N or % microbial-N: % total abomasal digesta N ratio, was decreased with the other four diets compared to diet one (Table 9), although not significantly for diet three.

The amount of microbial nitrogen arriving at the duodenum daily was markedly less for the formaldehyde treated diets compared to diet one using the sheep fitted with re-entrant cannula. It is likely that there was a limitation to microbial protein synthesis by nitrogen and hence the higher levels of valeric acid with diets two, three, four, and five compared with diet one.

Barry (1973a and 1976c) and Barry and Fennessy (1973) however reported lowered levels of isovaleric and n-valeric acids combined. Since the two acids were combined in these reports, it is difficult to say which of these acids actually contributed to the lowered levels with formaldehyde treatment. Langlands (1973a) however reported a reduction in the levels of n-valeric acid, with formaldehyde treatment of diets. However, Faichney and White (1977a) reported an increase in the level of n-valeric acid with formaldehyde treatment of concentrate diets. The values of n-valeric acid for the untreated and the treated diets (4.10% and 4.98% for untreated and formaldehyde treated diets respectively) were however not significant.

The increased level of valeric acid in the case of animals fed diet three, compared with diet one could be attributed to less digestion of protein in the rumen for the former diet, although overall digestibility was not significantly different for the two diets. The apparent digestibility coefficient of nitrogen for diet one was slightly higher than that of diet three.

Abomasal and duodenal digesta parameters

Abomasal pH

The pH values of the abomasal fluid were generally higher than what have been reported by some workers (Knight et al., 1972; Wheeler and Noller, 1977). The values obtained in this experiment were 3.84, 3.94, 3.88, 3.83 and 3.70 for diets one, two, three, four and five respectively. Wheeler and Noller (1977) reported abomasal pH

values of 2.74 ± 0.185 for sheep fed diets containing about 80% corn. Knight et al. (1972) reported that there was a considerable cyclical variation in abomasal pH. The lowest abomasal pH, about 2.05, occurred about one hour after feeding. The prefeeding pH level was approximately 2.90. There was a gradual increase in the abomasal pH from one hour after feeding, with levels six to eight hours after feeding, approaching the pre-feeding levels. The rather high pH levels observed in this experiment cannot be explained fully.

The samples for pH measurements were taken about four to six hours after feeding and this timing effect could have contributed to the high values. It is interesting to note that Lee (1977) also reported high abomasal pH values for sheep fed diets of either wheat or lucerne (3.20 ± 0.20 for wheat diets and 3.49 ± 0.33 for lucerne diets). Lee (1977) did not indicate the length of time after feeding when the animals were slaughtered for the measurements.

Abomasal digesta N%, duodenal digesta N%, and total daily flow of nitrogen from the abomasum into the duodenum

The abomasal nitrogen concentration (N%) was not significantly ($p > 0.05$) affected by formaldehyde treatment of the forage portion of the diets. The values, on a dry matter basis, were 2.27, 3.01, 2.80, 3.16 and 3.06% for diets one, two, three, four and five respectively. Sharma and Nicholson (1975a) also observed no significant increase in the concentration of nitrogen in abomasal digesta with diets containing formaldehyde treated rapeseed meal (3.13% for untreated and 3.20% for

formaldehyde treated). However, the total daily amount of nitrogen flowing through the duodenum was significantly higher for the diet containing formaldehyde treated rapeseed meal than for the diet containing the untreated rapeseed meal. The nitrogen content of duodenal digesta, on a dry matter basis, in the present experiment was 2.40, 3.10, 2.96, 3.15 and 2.98% for diets one, two, three, four and five respectively. Since only one animal was used it is not possible to say the differences between the diets containing the formaldehyde treated forages and the diet containing the untreated forage were significant. They were most likely not significant as they were similar to the abomasal digesta nitrogen concentration reported above. The total quantities of nitrogen arriving at the duodenum daily were 21.20, 31.02, 30.02, 31.14, and 29.16g for diets one, two, three, four and five respectively (Table 14). The figures above indicate that the formaldehyde treatment of the forage portion of the diet greatly increased the daily flow of nitrogen from the abomasum to the duodenum, whereas the concentration of nitrogen in the duodenal digesta was not markedly affected. The increase in the quantity of nitrogen flowing through the duodenum daily with formaldehyde treatment of the forage portion of the diet was possibly because of the increase in the daily flow of dry matter with formaldehyde treatment of the forage. Sharma and Nicholson (1975a) who reported no significant increase in the nitrogen concentration of abomasal digesta but significant increase in the total daily flow of nitrogen through the duodenum with formaldehyde treatment of the rapeseed meal portion of diet, also observed an increase in the flow of dry matter through the duodenum with formaldehyde treatment.

The daily quantities of nitrogen flowing through the duodenum on all the diets containing the formaldehyde treated forage were greater than the total quantities of nitrogen consumed daily (-7.54g, +1.88g, +0.8g, +2.45g, and +0.47g for diets one, two, three, four and five respectively). The increase in the amount of nitrogen arriving daily at the duodenum compared to the total daily intake could be due to

(a) increased recycling of urea-nitrogen into the four compartments of the stomach,

(b) reduction in the amount of loss of nitrogen through the ruminal wall.

Beever et al. (1976) and Hemsley et al. (1970) reported a greater amount of nitrogen flowing through the duodenum compared with intake with diets containing formaldehyde treated forages. Hemsley et al. (1970) reported lower quantities of nitrogen flowing through the duodenum per day than consumed with untreated forage diet. Faichney and White (1977b) reported daily net gain in nitrogen in the four compartments of the stomach when four concentrate diets, both untreated and formaldehyde treated were fed (except for one untreated diet where there was loss). The daily net gain of nitrogen was greater for the treated diets than for the untreated. Sharma and Nicholson (1975a) reported greater quantities of nitrogen flowing through the duodenum daily than consumed on diets containing both formaldehyde treated and untreated rapeseed meal. The same workers (Sharma and Nicholson,1975b) however reported lower quantities of nitrogen flowing through the duodenum than consumed daily for diets

containing untreated and formaldehyde treated faba beans (net gain in nitrogen daily: -6g and -3g for diets containing untreated and formaldehyde treated faba beans respectively).

Abomasal and duodenal digesta non-protein nitrogen levels

The abomasal digesta concentration of non-protein-nitrogen was significantly ($p < 0.05$) higher for animals fed diet one than for animals fed the other diets. The values, on a dry matter basis, were 0.864, 0.676, 0.643, 0.650 and 0.656% for diets one, two, three, four and five respectively. Sharma and Nicholson (1975a) reported abomasal digesta non-protein-nitrogen concentrations of 0.61% and 0.64% on a dry matter basis for diets containing untreated and formaldehyde treated rapeseed meal respectively. These were not significantly different.

The non-protein-nitrogen in the abomasal digesta could come from dietary sources or from intermediary products of protein breakdown in the reticulo-rumen. Since, in the present study, the ingredient composition of all the diets was the same, the most likely source for the increase in abomasal digesta non-protein-nitrogen with the diets containing the untreated forage was intermediary products of the breakdown of protein in the reticulo-rumen.

The concentration of non-protein-nitrogen in the duodenal digesta (Table 14) was also greater with the diet containing untreated forage compared with the other four diets (0.842, 0.614, 0.701, 0.662 and 0.681 on a dry matter basis, for diets one, two, three, four and five respectively).

The total quantities of non-protein-nitrogen flowing through the duodenum per day was also slightly greater with the diet containing untreated forage compared with the others (7.44, 6.10, 7.10, 6.52 and 6.66g for diets one, two, three, four and five in that order). The total quantities of true-protein-nitrogen arriving at the duodenum daily was however markedly greater with the diets containing the formaldehyde treated forage than for the diet containing the untreated forage (13.76, 24.92, 22.92, 24.62 and 22.50g for diets one, two, three, four, and five respectively). Some workers reported significantly lower quantities of non-ammonia-nitrogen flowing through the duodenum daily for untreated diets compared with formaldehyde treated diets (Williams and Smith, 1976; Hemsley et al., 1970; Faichney and White, 1977a). Sharma and Nicholson (1975a) reported significantly greater quantities of true-protein-nitrogen arriving at the duodenum with a diet containing formaldehyde treated rapeseed meal compared with a diet containing untreated rapeseed meal.

Abomasal and duodenal digesta RNA-N, microbial N, %RNA-N: % total digesta N, % microbial N: % total digesta N

The abomasal digesta concentration of ribonucleic acid-nitrogen and therefore microbial protein-nitrogen were not affected significantly ($p > 0.05$) by treatment (Table 9).

The ratios of % ribonucleic acid-nitrogen to % total abomasal digesta nitrogen and % microbial protein-nitrogen to % total abomasal digesta nitrogen were significantly ($p < 0.05$) higher for diet one than

for diets two, four, and five. Ling and Buttery (1975) used the ratio of microbial nitrogen to total digesta nitrogen to measure the contribution of microbial protein-N to total duodenal digesta N. The mean values reported by those workers were 0.58, 1.01, and 0.63 for diets containing fish meal, urea and soybean meal respectively. Their values for diets containing fish meal and soybean were similar to the values obtained for the untreated diet in the present experiment. (0.786, 0.480, 0.583, 0.451 and 0.439 for diets one, two, three, four and five respectively). McAllan and Smith (1974) also reported microbial nitrogen to total digesta non-ammonia-nitrogen ratios to be 0.60, 0.58, 0.79, and 0.59 for diets containing flaked maize, crushed oats, rolled barley and flaked maize plus urea respectively. For diets containing dairy cubes, the ratio was reported to be 0.78.

The results of the present experiment indicate that the contribution of microbial protein-nitrogen to the nitrogen arriving at the abomasum (and subsequently the duodenum) could not be measured by the concentration of microbial protein-nitrogen (microbial-protein-nitrogen %) as this was not affected by treatment but the ratio of % microbial protein nitrogen to % total nitrogen was affected. With the duodenal digesta, the ratio obtained using % microbial protein nitrogen: % total digesta nitrogen was the same as total microbial protein nitrogen: total digesta nitrogen (Table 14). Therefore, it seems valid to use % microbial protein nitrogen: % total nitrogen ratio to calculate the microbial protein nitrogen contribution to total N arriving at the duodenum. The calculations for the abomasal samples are therefore valid.

In the experiments of McAllan and Smith (1974) RNA-N was determined and converted to microbial nitrogen by dividing the RNA-N by 0.075 and multiplying the product by 100. The 0.075 figure was supposed to be the ratio of RNA-N to total N in microbial protein (Smith, 1975). Sutton et al. (1975) also reported a ratio of 0.076:1. In the experiment of Ling and Buttery (1975) the ratio used was 0.095. Kropp et al. (1977) reported that the percentage of RNA-N in rumen bacterial protein was 10% or a ratio of 0.10. Allison (1970) reviewing literature on the composition of bacterial nitrogen reported that nucleic acids accounted for 14-19% of total microbial nitrogen with most of it coming from RNA since DNA accounts for only 2.2-4.1%. In experiments where the ratio has been determined, usually bacteria samples are used and the ratio may not apply to mixed rumen microbes containing protozoa. It is therefore suggested here that in experiments where the ratio is not determined on mixed rumen microbes containing protozoa, and where figures for absolute microbial protein synthesis are not required but only comparisons between treatments are to be made, it may be better to use a ratio of RNA-N to total digesta nitrogen. In this experiment this ratio, though lower than that of microbial protein-N: total N, parallelled it (Tables 9 and 14).

The ratios (RNA-N: total N or microbial protein N: total N obtained from duodenal digesta were similar to those of the abomasal digesta. Diets one and three had the highest quantities of microbial protein nitrogen arriving at the duodenum. While with diet one it can

be said that there was a great deal of dietary protein converted to microbial protein with some losses of nitrogen, with diet three there was also a great deal of conversion of dietary protein to microbial protein but with little or no losses of dietary protein. It is not clear why the addition of the volatile fatty acids alone had those effects.

Abomasal ADF%, cellulose%, duodenal ADF%, cellulose%, and quantities of ADF and cellulose arriving at the duodenum

There were no differences between the treatments with respect to concentration of cellulose and acid detergent fibre in the abomasal digesta (Table 9). Beever et al. (1976) reported cellulose concentrations of 13.62%, and 19.67% on an organic matter basis, for untreated and treated diets. Beever et al. (1977) reported duodenal digesta concentrations of 10.43%, 10.78%, and 11.97% for control, formaldehyde treated silage and formaldehyde treated dried silage (on OM basis). In the two experiments of (Beever, et al., 1976; Beever, et al., 1977) the quantities of cellulose arriving at the duodenum were higher for the treated than the untreated materials. In the first experiment it could be argued that the concentration was also higher for the treated than the untreated. In the second experiment the levels were almost identical. The differences in the quantities arriving at the duodenum were affected in this case by increased flow of organic matter through the duodenum. In the present trial, it is therefore possible that even though the abomasal digesta concentrations of acid-detergent fibre and cellulose

were similar on all the diets, the quantities escaping digestion in the four compartments of the stomach may have been greater for the diets containing the formaldehyde treated forage than for the diet containing the untreated forage. In fact, the quantities of acid-detergent fibre and cellulose arriving at the duodenum of the sheep fitted with the re-entrant cannula were higher for the diets containing the treated forage than the diet containing the untreated forage (Table 13). The concentrations of cellulose and acid-detergent fibre in the duodenal digesta were not markedly different between treatments (Table 13).

The apparent digestibility coefficients of these fractions in the four compartments of the stomach were higher for the diet containing the untreated forage than for the diets containing the formaldehyde treated forage (Table 15).

Duodenal digesta flow

The experiment of MacRae and Wilson (1978) demonstrated that there were no differences between intact sheep and sheep fitted with duodenal re-entrant cannulae with respect to feed intake, dry matter digestibility and nitrogen balance. It is therefore assumed that the results obtained from the animal fitted with the re-entrant cannula in this experiment could apply to intact sheep.

Oldham and Ling (1977) and Leibholz and Hartman (1972) reported that the duodenal digesta flow measured over twenty four hours without a marker correction, gave valid estimates of duodenal flow through the duodenum. They further claimed that correcting flow for 100% recovery of a marker was of doubtful value for reducing variability in parameters measured. Therefore, in the present trial no such marker corrections were made. The duodenal digesta flow rate (ml/hr), total duodenal digesta flow (ml) per 24 hrs, total duodenal digesta dry matter flow per 24 hr (g) and total duodenal digesta organic matter flow per 24 hrs (g), (Tables 12 and 13) were greater for the diets containing the formaldehyde treated forage than for diet one. This was expected as there was greater digestion of dry matter or organic matter in the four compartments of the stomach when diet one was fed compared to the other diets (% D.M. digestion in the four compartments of the stomach: 32.08, 23.28, 22.23, 24.20 and 24.96 for diets one, two, three, four and five respectively; % O.M. digestion in the four compartments of the stomach: 36.31, 27.12, 26.34, 26.98, and 28.09 for diets one, two, three, four and five respectively).

The dry matter and organic matter concentrations of the duodenal digesta were not markedly different for treatments. Sharma and Nicholson (1975a) reported dry matter digestibilities of 33.6% for diets containing untreated rapeseed and 22.0% for the diets containing formaldehyde treated rapeseed meal in the four compartments of the stomach. The values of this experiment therefore are similar to the values of Sharma and Nicholson (1975a).

The average hourly digesta flow rates were 647.20, 698.33, 709.135, 692.29 and 680.83 ml for diets one, two, three, four and five respectively. Phillips and Dyck (1964) reported values of 672, 739, 651 and 622 ml/hr for daily dry matter intakes of 900, 850, 800, and 900g respectively. Duodenal digesta flow rates of 720 to 773 ml/hr and 861 ml/hr were reported by Van't Klooster et al. (1969) for some of their experiments. Thompson and Lamming (1972) however reported digesta flow rates of 267.65, 299.84 and 290.64 ml/hr for diets containing 30% long, chopped and ground barley straw respectively. Feed intake was about 900g/day (D.M.) and ground maize accounted for 54.7% of the rations.

Phillips and Dyck (1964) reported that there was a diurnal cyclical pattern of flow. The highest flow occurred at feeding with the lowest occuring 6-12 hrs after feeding. In their experiments, the animals were fed once a day and all the feed was consumed within a short time after feeding. This pattern was not observed in the present experiment. Leibholz and Hartman (1972) also did not observe a consistent diurnal cyclical pattern of flow. In this trial, with diet one there was a rise at 12 hrs and slight fall in flow rate at 18 hrs and almost a levelling off effect up to 24 hrs. With diets two, four and five, there were slight decreases in the flow rate at 12 hrs and rises at 18 hrs and falls at 24 hrs. With diet three, there was a continuous fall throughout the twenty-four hour period. The flow rates did not follow feed intake patterns either. With all the diets, there were decreases in feed intake at 12 hrs. With diets three and five, the decline in feed intake continued up to the 18 hr period and from there, there was an increase.

With diet two, there was a rise in feed intake from 12 hrs up to twenty four hours. There were increases in feed intake at 18 hrs and declines up to twenty four hours with diets one and four (Table 11). Perhaps there were no cyclical patterns of flow in this experiment because feed intake was spread almost over the twenty-four hour period. Leibholz and Hartman (1972) fed their animals hourly from automatic continuous feeders.

Perhaps one of the reasons for the use of very small numbers of animals for duodenal flow measurements where automation does not exist, is the tendiousness of collecting data over very long periods of time. Some workers have therefore reduced the time period over which measurements are made (Harris and Phillipson, 1962). Harris and Phillipson (1962) observed that the accumulated recovery in duodenal contents, collected for six separate 12-hr periods from each of four sheep was only 86-90% of the expected value. When their observed values were corrected for 100% recovery of Cr_2O_3 the flow rates approximated expected values. In the present trial the average percentage flow for each 6-hr period of the total flow for the five diets approximated 25% (Table 12) and for a 12-hr period, approximated 50% (Table 12) although there were considerable variations for each diet. It seems therefore that in the present trial, if five separate measurements had been taken for each diet for 6-hr or 12-hr period, the average values could have been extrapolated to a 24-hr collection period, although no marker correction was made. It must be pointed out here however that, since only one animal was used the effects of animal variations cannot be accounted for.

One other problem with duodenal digesta flow rate measurement is feed intake. Feed intake is usually restricted in most experiments to levels below ad libitum intake. This is because there is a diurnal variation in feed intake with animals fed ad libitum and considerable diurnal variations in flow rate could occur. Therefore, if sampling is done once over a 24-hr period when there is variation in feed intake, figures obtained might not be easily interpreted. With automation, if the period of measurement was extended, say to seven days, as is done with digestibility trials, then animals could be fed ad libitum. In the present experiment feed intake was restricted as measurement was carried out once over a 24-hr period for each diet. The level of feeding was fixed at average daily ad libitum intake for diet two at the beginning of the trial. Only one diet was used to fix the intake level as results from feed intake assays earlier indicated there were no significant ($p \geq 0.05$) differences between the diets (Table 4). It is possible however that at the actual time of measurement of flow rate for each diet, the level of feed intake determined was not actually the ad libitum intake as the animal continued growing throughout the experimental period.

Sulphur metabolism

Sulphur intake, if expressed as grams intake per unit of metabolic body size per day, was significantly higher for diets four and five than for diets one, two, and three ($p < 0.05$). It was not surprising that

animals fed diets four and five consumed greater amounts of sulphur per day compared to the others (g) as sulphur concentration in those diets was higher and feed intake was not significantly different for all the diets (Tables 3 and 4). When sulphur intake was expressed in absolute terms (g/day) the animals on diets four and five consumed more sulphur per day ($p < 0.05$) than the animals on diets one and two but not animals on diet three. It must be noted that dry matter intake (g/day) of animals on diet three tended to be greater than intake of animals on diets four and five. Converting the sulphur intakes to metabolic body size basis, removed, to some extent, the effects of the intake, and the slightly larger size of animals on diet three compared to diets four and five.

The amounts of sulphur excreted in the urine per day expressed as grams per unit of metabolic body size were greater for diets four and five than for the other diets (Table 7, $p < 0.05$). The higher excretion of sulphur in the urine by animals on the two sulphur-supplemented diets, compared to the others could partly be due to the higher intakes. The sulphur added was most likely not utilized greatly by the rumen micro-organisms for the synthesis of sulphur-containing amino acids. The low utilization of the added sulphur was possibly not due to non-adaptation of the rumen microbes to the supplement. Bird and Moir (1971) postulated that micro-organisms might adapt to sulphur supplementation within twenty four to twenty seven hours while Bird (1972b) claimed that a period of nine days was required. The preliminary period in the present trial

before the start of the metabolism studies was twenty days. Kahlon et al. (1975a) reported that in in vitro culture systems, the availability of sulphur from sodium sulphate for microbial protein synthesis was 55.4%. These workers also observed that a sulphur concentration of 21.5 μg/ml of rumen innoculum was not adequate to meet the needs of rumen microbes in an in vitro system while concentrations of 86.7 μg/ml and 130 μg/ml were apparently inhibitory to microbial protein synthesis. A sulphur concentration of 43.3 ug/ml was reported by them to result in the greatest microbial protein synthesis. Hume and Bird (1970) reported that there were no differences in microbial protein synthesis due to source of sulphur. Johnson et al. (1970) however reported that losses of sulphur to rumen microbes due to its excretion in the faeces were 20.39%, 21.77%, and 63.32% for methionine, sodium sulphate and elemental sulphur,supplementory sources respectively. The low utilization of the added sulphate in the present study was not likely due to its low availability for microbial protein synthesis. The total amount of sulphur lost in the urine and faeces expressed as a percentage of intake was not significantly higher ($p > 0.05$) for diets four and five than for diet one and not higher for diet four than for diet three; (79.67%, 63.70%, 67.57%, 76.89%, 80.28% for diets one, two, three, four, and five). There was better sulphur utilization only for diet two compared to diets four and five ($p < 0.05$). It could be inferred from data on the contribution of microbial protein-nitrogen to total abomasal or duodenal digesta nitrogen (Tables 9 and 14) that the added sulphate did not inhibit microbial protein synthesis to a great extent

and therefore its low utilization could not be attributed to that. The added sulphate supplied about 0.15% sulphur which was below the 0.2% level Bird (1972b) suggested was the maximum level for supplementation. Above this level according to Bird (1972b) hydrogen sulphide toxicity could occur especially if energy or nitrogen was limiting. The hydrogen sulphide results from the reduction of sulphate to sulphide. Sulphate is converted to sulphide before it is incorporated into sulphur containing amino acids by the rumen microbes (Bird, 1971; Saeur et al., 1975; Dodgson and Rose, 1966).

Sulphur supplementation was not required in the present trial. Sulphur was most likely not limiting in the diets containing formaldehyde treated forage without sulphate supplementation. There were high levels of extractable sulphate-sulphur in the grass-legume forage (Table 3). Beaton et al. (1968) and Martin (1972) reported that sulphur in sulphur containing amino acids accounts for about ninety percent of the total sulphur in plants. Sulphate-sulphur from this estimate, would account for a maximum of ten-percent of total sulphur in plants, not taking into account sulphur present in organic compounds other than amino acids. The level of extractable sulphate-sulphur in the grass-legume forage (about 29%) was higher than the figure of Beaton et al. (1968) and Martin (1972). Sulphate-sulphur levels in plants could increase with fertilizer application (Bray and Hemsley, 1969). Jones and Quagliato (1973) applying fertilizer sulphur to some tropical forages reported marked increases in sulphate-sulphur compared to total sulphur content.

With formaldehyde treatment even if all the organic sulphur from the grass-legume forage was not available, the total amounts of sulphur from the sulphate-sulphur in the grass-legume forage, and the cassava and barley would be about 0.069%. The total amount of nitrogen from the non-protein nitrogen of the grass-legume forage (assuming all the protein nitrogen was protected) the nitrogen in the cassava and barley would be 0.68%. The ratio of sulphur to nitrogen would be about 1:10 which is the same as the optimum reported by Kennedy et al. (1975), for low quality forage. They took into account the fact that recycled nitrogen into the rumen was greater than recycled sulphur, when arriving at the above ratio. Whanger and Matrone (1966) reported that with sulphur deficiency in the rumen, there was an accumulation of lactic acid. The accumulation of lactic acid was due to the non-functioning of the acrylate pathway for conversion of lactic acid to propionic acid (Whanger and Matrone, 1967). These workers, previously reported reduced levels of propionic acid, butyric and higher fatty acids in rumens of animals fed sulphur-deficient diets compared to animals fed diets containing adequate levels of sulphur (Whanger and Matrone, 1965). In the present trial propionic and butyric acid levels were not lower for the diets containing the formaldehyde treated forage without sulphur supplementation than the diets containing the formaldehyde treated forage with sulphur supplementation.

The percentages of sulphur intake excreted in the urine were higher for diets four and five than for diets two and three ($p < 0.05$)

but not for diet one ($p > 0.05$). The percentage of sulphur intake excreted in the urine was higher for diet one than was for diets two and three ($p < 0.05$). The reasons for the higher percentages of excretion of sulphur in the urine for diets four and five compared to diets two and three are similar to those given already above. There was a greater percentage of sulphur excreted in urine with diet one compared to diets two and three although the amounts excreted per unit of metabolic body size were similar because when calculated on a percentage basis, differences in intake are not taken into account.

Sulphur balance, expressed either as grams per unit of metabolic body size or in absolute terms was better for diets two, three and four than for diet one ($p < 0.05$). Sulphur retention was closely linked with nitrogen retention. Since animals on diets two and three had greater (significant) retention of nitrogen than animals on diet one, it was not surprising that sulphur retention was better on these two diets than for diet one. With diet four the animals retained more nitrogen (approaching 5% significance) than animals on diet one. The sulphur-nitrogen ratio also tended to be better, though not signiificant, for animals on diet four than animals on diet one.

The sulphur to nitrogen ratios were not affected by treatments (10.13, 9.78, 12.26, 9.73, and 10.36 for diets one, two, three, four and five respectively). Bird (1972a) reported a somewhat higher ratio of about 13.5 ± 0.58. It is not clear why the ratios were lower in this experiment except that perhaps sulphur utilization might have been better

than the experiments of Bird (1972a). It must also be noted that there is considerable variation in the reported ratios as Bird (1973) reported the ratio in sheep tissues to be 15 and Kahlon et al. (1975b) reported ratios of 1:8.14 to 1:27.16 of retained sulphur to retained nitrogen in sheep.

Nitrogen Metabolism

The daily nitrogen intake, expressed in absolute terms (29.81, 28.64, 33.44, 27.80 and 29.51g for diets one, two, three, four and five respectively) or expressed as intake/day/unit of metabolic body size (2.07, 2.18, 2.38, 2.08 and 2.18g, for diets one, two, three, four and five respectively) were not affected by treatments ($p > 0.05$, Table 6). This is perhaps due to the fact that dry matter intake per unit of metabolic body size (Table 4), the concentration of nitrogen in the diets (Table 3), and the metabolic body sizes of the animals (Table 4) were not significantly different ($p > 0.05$).

The nitrogen balance expressed as g/day (4.49, 7.43, 10.19, 6.99, and 6.86g for diets one, two, three, four and five respectively) was only greater for diet three than for diet one ($p < 0.05$). Nitrogen balance expressed as grams per day per unit of metabolic body size (0.313, 0.567, 0.726, 0.522, and 0.508g for diets one, two, three, four and five respectively) was however greater for diets two and three than for diet one ($p < 0.05$). There was an improvement in nitrogen balance for diet two when expressed as retained nitrogen per unit of metabolic body size perhaps because of the slightly larger weight of animals on

diet one than on diet two (Table 4). Animals on diet three were however about the same weight as animals on diet one and retained a significantly greater amount of nitrogen in each case and hence size per se was not a factor contributing to the greater retention of nitrogen by animals on diet three than animals on diet one. The greater retention of nitrogen of animals on diets two and three was due mainly to the greater urinary losses of nitrogen by animals on diet one than the animals on diets two and three. Microbial degradation of protein in animals fed diet one was greater than animals on diets two and three as ammonia concentration in the rumens of animals fed diet one was greater than the ammonia concentration in the rumens of animals fed diets two and three (Table 8). The synthesis of microbial protein from the dietary sources was also greater for diet one than for diet two (Tables 9 and 14). With the sheep fitted with re-entrant cannula there were losses of nitrogen in the four compartments of the stomach for diet one while there were net gains for diets two and three (Table 15). Ammonia could be lost through the ruminal wall. When such ammonia arrives in the liver, it may be converted to urea which may be lost in the urine as described by Houpt (1970). With diet three, although nitrogen digestibility was not decreased significantly ($p > 0.05$) compared to diet one, there was no net loss of nitrogen in the four compartments of the stomach. This is an indication of more efficient utilization of nitrogen by the rumen microbes in the rumens of animals fed diet three compared to animals fed diet one. In fact, microbial protein N contribution to duodenal digesta N was not significantly ($p > 0.05$) lower for diet three than for diet one (Table 9).

Within the diets containing the formaldehyde treated forage sulphur supplementation tended to reduce nitrogen retention (diets 4 and 5 vs diet 2 and 3). The cause of this effect is not clear although in the case of animals on diet four, it was mediated partly through reduced digestibility of nitrogen. In the case of diet five, there was an abnormally high loss of nitrogen in the urine compared to the other diets containing the formaldehyde treated forage. Winter (1976) reported that the addition of sulphur to starter diets containing biuret reduced weight gains in calves by 20%, feed intake by 9% and feed efficiency by 12%. Brown and Arlyne (1970) reported that sulphate added to rat diets improved performances only up to 0.10% level of supplementation. Above that level, performance was decreased although not significantly.

Measuring nitrogen retention as a percentage of digested, all the diets containing the formaldehyde treated forage were significantly ($p < 0.05$) superior to the diet containing the untreated forage. This was because a greater amount of the nitrogen digested was lost in the urine with diet one compared to the others. Similarly, using nitrogen retained as a percentage of intake as an index of nitrogen retention, diets two, three, four and five were better than diet one ($p < 0.05$). Diet three was also superior to diet five ($p < 0.05$) using nitrogen retained as a percentage of intake as an index of nitrogen utilization. These measures did not follow closely nitrogen balance expressed as grams per day per unit of metabolic body size. This was because those measures do not take into account variations in nitrogen intake, and nitrogen digestibility with the animals on the different diets. They

also did not take into account the variations in the sizes of animals used. Amos et al. (1974) and Driedger and Hatfield (1972) also reported that nitrogen retained as a percentage of intake did not follow nitrogen balance with different diets. Nitrogen losses in the urine ($g/Wkg^{0.75}$/day or percentage of digested) did not follow closely the nitrogen balance figures. This was because the measured values did not take into account differences in intake and in digestibility. The differences in nitrogen excretion were not due to differences in output of urine. Daily excretion of urine ($ml/Wkg^{0.75}$) was not affected by treatment ($p > 0.05$, Table 4).

Growth rate

The growth rates of the animals during the first seventeen days (pre-metabolism study period) were not significantly ($p > 0.05$) affected by dietary treatment (Table 4). The growth rate figures were 154.76, 170.77, 170.77, 160.10 and 149.42g/day for diets one, two, three, four and five respectively. Rattray and Joyce (1970) reported a positive response of nitrogen retention but not wool growth or growth rate with formaldehyde treatment of their diets. Their experimental period was five weeks. It is possible that in the present experiment, as in the trial of Rattray and Joyce (1970), the period of the experiment was too short for a growth rate response to be demonstrated. Ames and Brink (1977) however measured growth rates of sheep at different environmental temperatures

for only twelve days and differences in responses could be assessed. Driedger and Hatfield (1972) also used a sixteen-day preliminary period and a six-day metabolism study period. They observed differences in responses to nitrogen retention during the metabolism study period and also growth rate during the sixteen-day preliminary period with tannin treatment of soybean meal. The growth rates of their animals for the sixteen-day period were 277g/day for the diet containing the tannin treated soybean meal and 177g/day for the diet containing the untreated soybean meal.

The growth rate figures reported in the present trial were lower than those reported by Tait (1972) for male lambs. His figures were 237g/day, 239g/day and 233g/day for animals fed 100% dried grass, 50% barley plus 50% dried grass and 100% barley rations respectively. The animals used by Tait (1972) weighed about (average) 19.2kg at the start of the experiment and were fed to attain the weight of about 45kg (average). In the present experiment the animals ranged in bodyweight of 29kg to 36kg at the start of the experiment.

The greater growth rates of the animals used by Tait (1972) compared to those of the animals used in the present trial could most likely be accounted for by the variations in their sizes at the start of the experiments. Adeleye (1972) using animals with average bodyweight of 30.65kg at the start of the experiment reported daily weight gain of 110g, 70g, 90g, 130g, and 70g when diets containing soybean meal, urea, biuret, poultry droppings and poultry litter were fed. All these trials (Tait, 1972; Adeleye, 1972; and present trial) were carried out on the same farm. The same breed of sheep (Dorset) was used. The other factor

which could cause variations in the growth rates of the animals in all the experiments apart from the stage of growth at which measurements were carried out , was type of feed and level of intake.

SUMMARY AND CONCLUSIONS

Different levels of formaldehyde (0.0%, 0.8%, 1.0% and 1.2% on an air dry basis) were applied to a grass-clover forage to determine the optimum level for protection of the protein. In vitro nitrogen digestibility was reduced significantly ($p < 0.05$) as level of formaldehyde application was increased except between 1.0% and 1.2%, at the microbial stage of incubation (31.88%, 15.72%, 6.87%, and 5.69% for 0.0%, 0.8%, 1.0% and 1.2% levels of formaldehyde treatment). The ammonia nitrogen production, at the microbial stage of incubation, was also reduced significantly ($p < 0.05$) as level of formaldehyde application increased except between 0.8%, and 1.0% (228.79 ppm, 78.58 ppm, 65.27 ppm, and 30.04 ppm for 0.0%, 0.8%, 1.0% and 1.2% levels of formaldehyde treatment). Nitrogen digestibility for the combined microbial and acid-pepsin stages of incubation, was reduced significantly ($p < 0.05$) only at the 1.2% level of formaldehyde application (80.95%, 79.76%, 75.85%, and 71.01% for 0.0%, 0.8%, 1.0% and 1.2% levels of formaldehyde application). The optimum level chosen was 1.0% since nitrogen digestibility was significantly ($p < 0.05$) reduced at the microbial stage but not significantly ($p > 0.05$) reduced at the combined microbial and acid-pepsin stages, compared to the untreated.

Ram lambs ranging in body weights of 29kg to 36kg were then used in in vivo studies of nitrogen and carbohydrate utilization with treatment of the grass-clover forage at 1% level of formaldehyde. The effects of supplementation with isovaleric and isobutyric acids

and/or sulphur were also studied. The diets (14% CP on D.M. basis) contained 50% grass-clover forage, 38% cassava, 11% barley and 1% sheep mineral premix on dry matter basis. Sodium sulphate was added at 0.67% replacing an equal portion of the cassava in the diets supplemented with sulphur. Diet one contained the untreated forage while the others contained the formaldehyde treated forage. Diets three and five were supplemented with isovaleric acid (3.0g/kg diet) and isobutyric acid (2.3g/kg diet) and diets four and five were supplemented with sulphur. The volatile fatty acids were sprayed onto the diets just before feeding.

Dry matter intake ($g/Wkg^{0.75}/day$), apparent digestibility coefficients of dry matter and organic matter were not significantly ($p > 0.05$) affected by dietary treatments. Formaldehyde treatment of the forage significantly (p 0.05) increased the apparent digestibility coefficients of acid-detergent fibre and cellulose. (ADF Digestibilities: 32.57%, 36.97%, 36.91%, 36.45% and 36.59% for diets one, two, three, four and five respectively; Cellulose digestibilities: 42.95%, 49.10%, 49.04%, 49.33% and 48.76% for diets one, two, three, four and five respectively. Greater amounts of the acid-detergent fibre and cellulose were digested in the hindgut with formaldehyde treatment of the forage.

Rumen pH, dry matter content in the rumen, rumen levels of volatile fatty acids, propionic, butyric and acetic acids were not affected significantly ($p > 0.05$) by the dietary treatments. There were however, significantly ($p < 0.05$) increased levels of isovaleric and isobutyric acids in the rumens of animals fed diet three compared to those fed diets two and four. The isovaleric acid and isobutyric acid levels

tended to decrease with the diets containing the formaldehyde treated forage without volatile fatty acid supplementation compared to the diet containing the untreated material, most likely because of greater degradation of proteins in the rumen with the latter than with the former (isovaleric: 2.53, 0.60, 2.58, 0.39 and 2.18% for diets one, two, three, four and five respectively; isobutyric: 1.54, 0.71, 2.11, 0.66 and 1.73% for diets one, two, three, four, and five respectively). Formaldehyde treatment of the grass-clover forage resulted in significant ($p < 0.05$) increases in n-valeric acid levels in the rumen and this could be due to reduced microbial growth due to limitation of nitrogen (1.82, 3.30, 3.19, 3.34 and 3.15 for diets one, two, three, four and five respectively). Rumen ammonia-nitrogen levels were significantly ($p < 0.05$) higher for animals fed diets containing the untreated forage than for animals on the other diets (21.14, 14.36, 14.30, 12.90 and 13.54 ppm for diets one, two, three, four and five respectively). The higher levels of rumen ammonia-nitrogen in the case of animals fed the diet containing the untreated forage compared to the others was most likely due to a greater rate of protein breakdown unaccompanied by efficient utilization in the case of the former compared to the latter.

Abomasal pH, abomasal digesta concentrations of acid-detergent fibre, cellulose, total nitrogen, ribonucleic acid nitrogen, and microbial protein nitrogen were not affected by treatment. The ratios of % RNA-N: % total abomasal digesta nitrogen (0.060, 0.036, 0.044, 0.034 and 0.033 for diets one, two, three, four and five respectively) and % microbial nitrogen: % total abomasal digesta nitrogen (0.786, 0.480, 0.583, 0.451 and 0.439 for diets one, two, three, four and five

respectively) were reduced significantly ($p < 0.05$) except for diet three by the formaldehyde treatment of the forage. The abomasal digesta concentration of non-protein-nitrogen was significantly ($p < 0.05$) higher for the diet containing the untreated forage compared to the others. The higher ratio of microbial-nitrogen: total abomasal digesta nitrogen (except for diet three) and the higher levels of non-protein-nitrogen in the abomasal digesta for the diet containing the untreated forage compared to the others indicated that there was a greater degree of degradation of dietary protein by rumen microbes. For diet three, there might have been a greater degree of degradation of dietary protein as for diet one but in the case of diet three the conversion of the degraded protein to microbial protein was more efficient.

Sulphur balance ($g/Wkg^{0.75}/day$) was significantly ($p < 0.05$) improved by the formaldehyde treatment of the forage except for the diet supplemented with both sulphur and VFAS (diet five). The values were 0.032, 0.061, 0.061, 0.056 and 0.050g for diets one, two, three, four and five respectively. Sulphur intake per unit of metabolic body size per day was significantly ($p < 0.05$) higher for the diets supplemented with sulphur than for the other diets. The values were 0.157, 0.168, 0.188, 0.247 and 0.251g for diets one, two, three, four and five respectively. Sulphur retained to nitrogen retained ratios were however not affected significantly ($p > 0.05$) by dietary treatments. The amount of sulphur excreted in urine per day ($g/Wkg^{0.75}/day$) was significantly ($p < 0.05$) higher for the sulphur supplemented diets than for the rest (0.050, 0.024, 0.034, 0.102, and 0.103g for diets one, two, three, four and five respectively).

Sulphur excreted in urine as a percentage of intake was significantly ($p < 0.05$) higher for diets one, four and five than for diets two and three. The values were 31.38, 14.17, 17.41, 41.43 and 42.23% for diets one, two, three, four and five respectively. The loss of sulphur in urine and faeces as a percentage of intake was significantly ($p < 0.05$) higher for diets one, four and five than for diet two and for diets one and five than for diet three. The values were 79.67, 63.69, 67.57, 76.89 and 80.28% for diets one, two, three, four and five respectively.

Nitrogen intake (g/day or $g/Wkg^{0.75}$/day) was not significantly ($p > 0.05$) affected by dietary treatments. The apparent digestibility coefficient of nitrogen was significantly ($p < 0.05$) higher for diet one than for diets two, four, and five and for diet three than for diet four. The values were 54.13, 47.06, 51.25, 44.90, and 47.24% for diets one, two, three, four and five respectively. Nitrogen balance ($gN/Wkg^{0.75}$/day) was significantly ($p < 0.05$) better for diets two and three than for diet one. The nitrogen balance values were ($g/Wkg^{0.75}$/day) 0.459, 0.802 , 0.843, 0.752 and 0.672g for diets one, two, three, four and five respectively. Formaldehyde treatment of the forage significantly ($p < 0.05$) reduced the amount of nitrogen excreted in the urine per day per unit of metabolic body size (0.805, 0.461, 0.467, 0.417 and 0.518g for diets one, two, three, four and five respectively). Nitrogen excreted in urine as a percentage of digested and nitrogen excreted in urine as a percentage of intake were also reduced significantly ($p < 0.05$) by formaldehyde treatment of the forage. The values for nitrogen excreted in urine as a percentage of digested were 72.10, 44.65, 39.11, 44.25 and 50.39% for diets one, two, three, four and five respectively. The values for

nitrogen excreted in the urine as a percentage of intake were 39.07, 21.07, 19.97, 19.86 and 23.94% for diets one, two, three, four, and five respectively. Nitrogen retained as a percentage of digested (27.90, 55.39, 58.93, 55.75, and 49.61% for diets one, two, three, four and five respectively) and nitrogen retained as a percentage of intake (15.06, 26.00, 30.24, 25.04 and 23.30% for diets one, two, three, four and five in that order) were significantly ($p < 0.05$) greater for the diets containing the formaldehyde treated forage compared to the diet containing the untreated forage. Nitrogen utilization was improved for all the diets containing the formaldehyde treated forage as a result of lower urinary nitrogen losses. The addition of sulphur to the diets containing the formaldehyde treated forage tended to offset the beneficial effects of the formaldehyde treatment. The addition of the volatile fatty acids to the diets containing the formaldehyde treated forage did not further enhance nitrogen utilization.

Duodenal flow was measured over a twenty-four-hour period using a sheep fitted with a re-entrant cannula. The quantities of total digesta, dry matter, organic matter, nitrogen, acid-detergent fibre, and cellulose flowing through the duodenum daily were markedly higher for the diets containing the formaldehyde treated forage than for the diet containing the untreated forage. The daily amounts of microbial protein and non-protein-nitrogen arriving at the duodenum were markedly higher for the diet containing the untreated forage than for the rest.

Growth rate of the animals during the seventeen day pre-metabolism period and their metabolic body sizes at the beginning of the metabolism studies were not significantly ($p > 0.05$) different for dietary treatments. The lack of a response in growth rate may have been due to the relatively

short period of measurement.

Formaldehyde treatment of the forage portion of the diet had beneficial effects in terms of the nitrogen economy of the animal. The treatment of the forage portion of the diet with formaldehyde resulted in changes in the sites of digestion of both protein and fibre. The digestion of these fractions was depressed in the rumen but increased in the lower sections of the digestive tract.

This study indicated that supplementation of the diets containing the formaldehyde treated grass-legume forage with either sulphur and/or branched chain volatile fatty acids was not necessary as no beneficial effects were observed.

There was an indication from this study that formaldehyde treatment of the forage portion of the diet might result in improved efficiency of utilization of dietary protein by ruminants. However, production experiments assessing its effects on such parameters as growth rate, carcass quality, or milk production need to be undertaken before recommendations regarding its practical application can be made.

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APPENDIX TABLES I - XXX

Table I. ANOVA percent N digestibility, 1st stage of *in vitro* digestion of rye-grass-clover forage with the different levels of formaldehyde treatments.

Source	SS	df	Variance	Fcal
Total	1350.47	11	-------	-----
Treatment	1314.8181	3	438.2727	98.27**
Error	35.6519	8	4.46	-----

** $p < 0.01$ S.E. $\pm$ 1.219

Table II. ANOVA percent N digestibility 2nd stage of *in vitro* digestion of rye-grass-clover forage with the different levels of formaldehyde treatment.

Source	SS	df	Variance	Fcal
Total	228.83062	11	-------	-----
Treatment	181.22628	3	60.40876	10.1518**
Error	47.60434	8	5.9505425	-----

** $p < 0.01$ S.E. $\pm$ 1.408

Table III. ANOVA *in vitro* ammonia-nitrogen production (ppm) per gram dry matter of rye-grass-clover forage with the different levels of formaldehyde treatment.

Source	SS	df	Variance	Fcal
Total	145794.2618	23	-------	-----
Treatment	138869.9399	3	46289.98	133.70**
Error	6924.3219	20	346.22	

** $p < 0.01$ S.E. $\pm$ 7.596

Table IV. ANOVA metabolic body sizes of animals at the beginning of the metabolism studies (kg).

Source	SS	df	Variance	Fcal
Total	26.157944	24	-------	-----
Treatment	5.108064	4	1.277016	1.93
Block	10.493344	4	2.623336	3.98*
Error	10.556536	16	0.6597835	-----

* $p < 0.05$ S.E. $\pm$ 0.3632584478

Table V. ANOVA apparent digestibility coefficient of nitrogen (%).

Source	SS	df	Variance	Fcal
Total	465.15122	24	-------	-----
Treatment	275.4159	4	68.853975	10.03**
Block	79.90234	4	19.975585	2.91
Error	109.83298	16	6.86456125	-----

** $p < 0.01$ S.E. $\pm$ 1.171713382

Table VI. ANOVA apparent digestibility coefficient of acid-detergent fibre (%).

Source	SS	df	Variance	Fcal
Total	169.55554	24	-------	-----
Treatment	70.09646	4	17.524115	4.41*
Block	35.91918	4	8.979795	2.26
Error	63.5399	16	3.97124375	-----

* $p < 0.05$ S.E. $\pm$ 0.8912063454

Table VII. ANOVA apparent digestibility coefficient of cellulose (%).

Source	SS	df	Variance	Fcal
Total	312.12734	24	-------	-----
Treatment	149.96314	4	37.490785	4.21*
Block	19.5969	4	4.899225	0.55
Error	142.5673	16	8.91045625	-----

* $p < 0.05$ S.E. $\pm$ 1.334949905

Table VIII. ANOVA nitrogen excreted in urine per unit metabolic body size per day (g).

Source	SS	df	Variance	Fcal
Total	0.71007224	24	-------	-----
Treatment	0.48606944	4	0.12151736	18.94**
Block	0.12136904	4	0.03034226	4.73*
Error	0.10263376	16	0.00641461	-----

** $p < 0.01$ * $p < 0.05$ S.E. for treatment and block means $\pm$ 0.0358179005

Table IX. ANOVA % nitrogen excreted in urine over intake.

Source	SS	df	Variance	Fcal
Total	2712.62046	24	-------	-----
Treatment	1329.91614	4	332.479035	4.88**
Block	293.50034	4	73.375085	1.08
Error	1089.20398	16	68.07524875	-----

** $p < 0.01$ S.E. $\pm$ 3.689857687

Table X. ANOVA % nitrogen excreted in urine over digested.

Source	SS	df	Variance	Fcal
Total	4826.9737	24	-------	-----
Treatment	3343.26462	4	835.816155	14.28**
Block	547.19962	4	136.799905	2.34
Error	936.50946	16	58.53184125	-----

** $p < 0.01$ S.E. $\pm$ 3.421457036

Table XI. ANOVA % nitrogen retained over intake.

Source	SS	df	Variance	Fcal
Total	885.5855	24	-------	-----
Treatment	622.11626	4	155.529065	14.17**
Block	87.91154	4	21.977885	2.00
Error	175.5577	16	10.97235625	-----

** $p < 0.01$ S.E. $\pm$ 1.481374784

Table XII. ANOVA % nitrogen retained over digested.

Source	SS	df	Variance	Fcal
Total	4354.29162	24	-------	-----
Treatment	2945.51022	4	736.377555	12.70**
Block	480.9983	4	120.249575	2.07
Error	927.7831	16	57.98644375	-----

** $p < 0.01$ S.E. $\pm$ 3.405479225

Table XIII. ANOVA nitrogen balance g/day.

Source	SS	df	Variance	Fcal
Total	141.9019326	24	-------	-----
Treatment	82.6064218	4	20.65160545	5.57**
Block	6.731593	4	1.68289825	0.45
Error	59.2955108	16	3.70596425	-----

** $p < 0.01$ S.E. $\pm$ 0.8609255775

Table XIV. ANOVA nitrogen balance (g) per unit of metabolic body size per day.

Source	SS	df	Variance	Fcal
Total	0.72847696	24	-------	-----
Treatment	0.43781416	4	0.10945354	8.20**
Block	0.07698256	4	0.01924564	1.44
Error	0.21368024	16	0.013355015	-----

** $p < 0.01$ S.E. $\pm$ 0.0516817472

Table XV. ANOVA sulphur intake per day (g).

Source	SS	df	Variance	Fcal
Total	9.539896	24	-------	-----
Treatment	6.336296	4	1.584074	9.36**
Block	0.494776	4	0.123694	0.73
Error	2.708824	16	0.1693015	-----

** $p < 0.01$ S.E. $\pm$ 0.1840116844

Table XVI. ANOVA sulphur intake per unit of metabolic body size per day (g).

Source	SS	df	Variance	Fcal
Total	0.05090784	24	-------	-----
Treatment	0.03885544	4	0.00971386	16.09**
Block	0.00239544	4	0.00059886	0.99
Error	0.00965696	16	0.00060356	-----

** $p < 0.01$ S.E. $\pm$ 0.0109869012

Table XVII. ANOVA sulphur excreted in urine per day per unit of metabolic body size (g).

Source	SS	df	Variance	Fcal
Total	0.03454384	24	-------	-----
Treatment	0.02941184	4	0.00735296	27.59**
Block	0.00086824	4	0.00021706	0.81
Error	0.00426376	16	0.000266485	-----

** $p < 0.01$ S.E. $\pm$ 0.0073004794

Table XVIII. ANOVA % sulphur excreted in urine over intake.

Source	SS	df	Variance	Fcal
Total	4392.9128	24	-------	-----
Treatment	3443.66224	4	860.91556	22.45**
Block	335.63716	4	83.90929	2.19
Error	613.6134	16	38.3508375	-----

** $p < 0.01$ S.E. $\pm$ 2.769506725

Table XIX. ANOVA total amount of sulphur lost in urine and faeces as a percentage of intake.

Source	SS	df	Variance	Fcal
Total	1884.5519	24	-------	-----
Treatment	1134.6117	4	283.652925	10.07**
Block	299.1533	4	74.788325	2.65
Error	450.7869	16	28.17418125	-----

** $p < 0.01$ S.E. $\pm$ 2.373781003

Table XX. ANOVA sulphur balance per day (g).

Source	SS	df	Variance	Fcal
Total	0.99126424	24	-------	-----
Treatment	0.46094104	4	0.11523526	5.67**
Block	0.20486304	4	0.05121576	-----
Error	0.32546016	16	0.02034126	-----

** $p < 0.01$ S.E. $\pm$ 0.0637828503

Table XXI. ANOVA sulphur balance per day per unit of metabolic body size (g).

Source	SS	df	Variance	Fcal
Total	0.005612	24	-------	-----
Treatment	0.0028912	4	0.0007228	6.80**
Block	0.00102	4	0.000255	2.40
Error	0.0017008	16	0.0001063	-----

** $p < 0.01$ S.E. $\pm$ 0.0046108567

Table XXII. ANOVA molar proportion of acetic acid in rumen fluid (%).

Source	SS	df	Variance	Fcal
Total	521.26974	24	-------	-----
Treatment	106.9635	4	26.740875	2.17
Block	217.39594	4	54.348985	4.42*
Error	196.9103	16	12.30689375	-----

* $p < 0.05$ S.E. $\pm$ 1.568878182

Table XXIII. ANOVA molar proportion of propionic acid in rumen fluid (%).

Source	SS	df	Variance	Fcal
Total	891.38178	24	-------	-----
Treatment	74.0105	4	18.502625	0.82
Block	454.64618	4	113.661545	5.01**
Error	362.7251	16	22.67031875	-----

** $p < 0.01$ S.E. $\pm$ 2.12933411

Table XXIV. ANOVA molar proportion of isobutyric acid in rumen fluid (%).

Source	SS	df	Variance	Fcal
Total	13.77958095	24	-------	-----
Treatment	6.18916095	4	1.547290238	4.29*
Block	1.814484283	4	0.4536210708	1.26
Error	5.775935717	16	0.3609959823	-----

* $p < 0.05$ S.E. $\pm$ 1.163467023

Table XXV. ANOVA molar proportions of isovaleric acid proportion in rumen fluid (%).

Source	SS	df	Variance	Fcal
Total	40.99798095	24	-------	-----
Treatment	17.13082595	4	4.282706488	4.39*
Block	8.272439283	4	2.068108921	2.12
Error	15.59471572	16	0.9746697323	-----

* $p < 0.05$ S.E. $\pm$ 0.4415132461

Table XXVI. ANOVA molar proportion of valeric acid in rumen fluid (%).

Source	SS	df	Variance	Fcal
Total	15.930784	24	-------	-----
Treatment	8.315384	4	2.078846	5.39**
Block	1.445024	4	0.361256	0.94
Error	6.170376	16	0.3856485	-----

** $p < 0.01$ S.E. $\pm$ 0.2777223434

Table XXVII. ANOVA rumen ammonia-nitrogen levels (ppm).

Source	SS	df	Variance	Fcal
Total	537.0824	24	-------	-----
Treatment	224.1664	4	56.0416	4.7176*
Block	122.8504	4	30.7126	2.585
Error	190.0656	16	11.8791	-----

* $p < 0.05$ S.E. $\pm$ 1.541369521

Table XXVIII. ANOVA abomasal digesta non-protein-nitrogen concentration (%).

Source	SS	df	Variance	Fcal
Total	0.33877336	24	-------	-----
Treatment	0.17597416	4	0.04399354	4.83**
Block	0.01710296	4	0.00427574	0.47
Error	0.14569624	16	0.009106015	-----

** $p < 0.01$ S.E. $\pm$ 0.042675555

Table XXIX. ANOVA % RNA-N: % total N in abomasal digesta.

Source	SS	df	Variance	Fcal
Total	0.005168	24	-------	-----
Treatment	0.0025188	4	0.0006297	4.30*
Block	0.0003084	4	0.0000771	0.53
Error	0.0023408	16	0.0001463	-----

* $p < 0.05$ S.E. $\pm$ 0.0054092513

Table XXX. ANOVA % microbial-protein-nitrogen: % total digesta nitrogen for abomasal digesta (x:1).

Source	SS	df	Variance	Fcal
Total	0.850612	24	-------	-----
Treatment	0.4185084	4	0.1046271	4.53*
Block	0.0633512	4	0.0158378	0.69
Error	0.3687524	16	0.023047025	-----

* $p < 0.05$ S.E. $\pm$ 0.067892599

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