FACTORS AFFECTING THE NUTRITIVE VALUE OF GRASS SILAGES, WITH SPECIAL REFERENCE TO PROTEIN NITROGEN

by

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ABSTRACT

Factors affecting the nutritive value of silage were highlighted in the Literature Review, along with silage biochemistry, additives, protein metabolism in the ruminant and the nylon bag technique.

The nutritive value of two silages (made from second-cut Perennial and Italian ryegrass), one pretreated with 6.0 lt^{-1} formic acid before ensiling (S) and the other with 7.5 lt^{-1} formalin (F), was assessed.

In Experiment 1, the two grass silages and a control complete ruminant diet (C.R.D.) were offered ad libitum to 24 Crossbred lambs. C.R.D. had a significantly greater ($p < 0.001$) daily DMI than the two silages between which there was no significant difference in intake. The high intake of C.R.D. was attributed to its high concentrate content and its pelleted form and explained the significantly higher ($P < 0.001$) liveweight gains than from the silage diets. The silages did not differ significantly in liveweight gain. Silage F and C.R.D. had higher efficiency of feed conversion ($P < 0.05$) than Silage S.

In Experiment 2, 12 Crossbred lambs were randomly allocated to the three diets and fed in metabolism cages. Silage S had similar apparent digestibility coefficients to an untreated control, but Silage F had lower apparent digestibility coefficients for OM, DM energy and crude protein. The latter was significantly lower than those of the two other diets. C.R.D. had significantly higher CP digestibility than Silage F and significantly greater N retention than F and S, but was lower in all other parameters, due to its high straw content. Silage F displayed better efficiency of nutrient utilization than Silage S.

In Experiment 3, the two silages and an untreated control silage were incubated in artificial fibre bags in the rumen for 1, 4, 12, 24

INTRODUCTION

Livestock production is an important source of animal protein for mankind. The production of ruminant livestock is favoured by their unique ability to digest a wide range of forages, undigestible by man, and by the fact that ruminants need not compete with humans for vital grain resources. Grasses and legumes form the main forage resources fed to ruminants.

The growth and production of forages is not uniform, and can be highly seasonal, which makes forage conservation a major agricultural activity throughout the world. In most areas of the tropics, the growing season of forages is mainly limited by low rainfall, and in the temperate zone, the principal limitation on the growing season is low temperature. It is for this reason that forage conservation becomes necessary, in order to feed animals during the drier period in the tropics and the colder temperate months.

There are three methods of forage conservation commonly employed in Europe: these are the artificial drying of grass, the making of hay and the making of silage. The artificial drying of grass involves the directing of a hot air stream through the herbage, to reduce the moisture content to 5-10%, for preservation and prevention of fermentation and spoilage. The making of hay involves the reduction of moisture content to about 10-15%, by natural and artificial means, to inhibit the action of plant and microbial enzymes, thus promoting preservation. Silage-making involves the controlled fermentation of a high moisture crop preservation (McDonald, Edwards and Greenhalgh, 1981).

In recent years, there has been marked increases in the price of support energy in agriculture, which contraindicates the artificial

drying of grass (White, 1979) and encourages the use of hay or silage. In the U.K., the quantity of silage produced is lower than that of hay. The reasons for this are that the farmer could easily assess the production potential of his crop, and could visually evaluate the success or failure of hay-making. In the case of silage, the farmer could not predict the type and extent of fermentation of ensiled material, and the fermentation products exert considerable influence on the production potential of silage. The latter engendered a lack of confidence in silage making and silage was being fed to ruminants in low quantities.

Over the period 1967-1973, silage making increased by an average of 15% per annum (Wilkinson, Wilson and Barry, 1976) and in the last five years, it has doubled, exceeding 13 million tonnes (Wilkins, 1980). Now, some 6 million tonnes of silage dry matter are produced annually in the U.K. at a value of at least £300 million and represents 45% of Britain's conserved fodder.

The increase in silage making was brought about by improved practical techniques for silage-making, the knowledge of the superior nutritive value of silage compared with hay, and a reduction on dependence on good weather.conditions, compared to hay-making.

The use of grass silages is not without problems, however. The nutritive value of silages for ruminants is affected by many factors, and the adverse factors must be overcome, before silage can be better exploited as an animal feed over the unfavourable season. There are crucial problems of intake (Dermarquilly, 1973) and utilisation of silage nitrogen (Wilkinson et al, 1976; Ohshima and McDonald, 1978). The latter problem has generated much interest recently, especially since the importance of the relative supply of

rumen degradable and undegradable protein has been recognized (ARC, 1980). Research has thus been directed at understanding how low silage nitrogen affects nitrogen utilisation, and how low silage additives can be used to alter favourably silage nitrogen fractions for improved animal performance. The research done in this dissertation is similarly motivated.

The Literature Review covers the factors which affect the nutritive value of grass silages. Its opening chapter reviews the fundamental principles of ensilage and silage biochemistry. The next chapter reviews the nutritive value of silage. The third chapter deals with the use and effects of silage additives. The fourth chapter deals with protein metabolism in the ruminant, and the final chapter reviews the nylon bag technique.

The experimental section covers experiments performed during the period June - September 1982 at the Rumen Metabolism Unit, Edinburgh School of Agriculture, Easter Howgate. The experiments were designed to assess the nutritive value of mixed grass silages treated with high levels of formic acid and formaldehyde additives. Intra-ruminal studies were performed in vivo, using the artificial fibre bag technique for the assessment of silage dry matter and protein degradability and to detect any interaction between diet and silage protein degradability.

LITERATURE REVIEW

2.0 THE ENSILAGE PROCESS

Historically, the making of silage appears to date back to the time of ancient Egypt and Carthage, 1000 B.C. Northern Europeans have made silage since the beginning of the nineteenth century, and since the later part of that century, silage was made in the U.K. (Wilkins, 1980; McDonald, 1981).

Silage may be defined as that material produced by the controlled fermentation of a crop of high moisture content. The process is called ensilage, and the container is called the silo (McDonald and Whittenbury, 1973; McDonald, Edwards and Greenhalgh, 1981). The principal aim of silage-making, is to preserve the forage material with minimum nutrient loss (McDonald and Whittenbury, 1973; Ohshima and McDonald, 1978; Wilkinson, Wilson and Barry, 1976). To achieve this aim, two main principles can be followed: firstly, the attainment and maintenance of anaerobiosis; and secondly, the inhibition of clostridial fermentation. Anaerobiosis is most efficiently achieved by storing of consolidated forage in a hermetically sealed container, and is important because continued aerobic respiration results in deliterious temperature rise in the ensiled mass, and also because the desirable microbes for good fermentation are facultative anaerobes (McDonald and Whittenbury, 1973). Clostridial inhibition may be achieved by reducing the moisture content of the herbage, as Clostridia sp. are sensitive to water availability, and by promoting a lactic acid fermentation, which increases H^+ ion and undissociated acid concentration, and inhibits clostridia (Wieringa, 1958). Clostridial inhibition is important because their activity causes catabolism of water soluble carbohydrates (WSC) and breakdown of

amino acids, and yields carbon dioxide, ammonia, and undesirable nitrogenous compounds such as amines (McDonald and Whittenbury, 1973). Silage nutritive value is thus affected.

The principal biochemical changes occuring during ensilage, arise mainly from the activities of plant enzymes, lactic acid bacteria and clostridia (McDonald and Whittenbury, 1973; Ohshima and McDonald, 1978; McDonald et al, 1981); as well as enterobacteriaceae and yeasts (McDonald, 1979). These micro-organisms are ubiquitous, and are present on herbage and cutting equipment (Henderson, McDonald and Woodford, 1972). The biochemical changes occurring during ensilage are discussed below, but are comprehensively reviewed in the book of McDonald (1981).

2.1 Action of plant enzymes

Immediately after harvesting, and in the early stages of ensilage, the major biochemical changes in the cut herbage arise due to the activities of plant enzymes (McDonald, 1979).

2.1.1 Carbohydrates

The main water soluble carbohydrates in herbage are glucose, fructose, sucrose and fructans. Pentose sugars may become available due to the action of hemicellulases on plant hemicellulose (Dewar et al, 1963). Polysaccharides may be a source of soluble sugars because of enzymatic breakdown. Under anaerobic conditions, plant respiratory enzymes oxidize sugars to carbon dioxide and water (McDonald and Whittenbury, 1973). The WSC content of herbage depends on the species of crop, the stage of growth of the crop, weather and fertilizer application.

2.1.2 Nitrogenous compounds

Extensive proteolysis occurs in the first few days of ensiling. Within the first 24 hours the non-protein nitrogen (NPN) content of herbage may increase from less than 20% to about 40% of the total nitrogen (TN); and this change is attributed to plant enzyme activity. The principal products of proteolysis are peptides and amino acids. Further breakdown of amino acids can occur by decarboxylation (e.g. aspartate to α-alanine), and some deamination can also occur due to plant enzyme activity. Free ammonia is rarely detected, although amides, glutanine, asparagine and proline may be detected (McDonald, 1979).

2.1.3 Control of plant enzyme activity

The extent of respiratory enzyme activity is dependent upon the presence of oxygen, and control is a question of silo design, chop length, rate of ensiling, sealing, and general management (McDonald, 1979). Proteolysis can be restricted by the addition of formaldehyde (Barry, 1976), or by rapid acidification (Virtanen, 1933; Carpintero et al, 1979). The rapid reduction of pH to 4.3 decreases proteolytic activity (MacPherson, 1952), as this pH is outside the optimum range of 5-6 for leaf proteases (Tracy, 1948).

2.2 Microbial flora

Aerobic micro-organisms are the dominant species on fresh herbage. Once aerobic respiration has ceased in the silo they are replacedby anaerobic bacteria and yeasts and species such as Lactobacillus, Escherichia, Pediococcus, Leuconostoc, Klebsiella, Bacillus and Clostridium multiply (McDonald and Whittenbury, 1973). This initial stage usually lasts for four days and is followed by a phase

of decreasing viable count. Initially, enterobacteriaceae dominate the silage microflora, but are soon displaced by lactic acid producing bacteria, such as Leuconostoc and Streptococci, which in turn, are finally dominated by two other species of lactic acid bacteria Lactobacilli and Pediococci (Gibson and Stirling, 1959). These latter bacterial species reduce the pH to about 4.0. Beck (1978) found that acidification in well preserved silage from fresh or wilted herbage, was initiated by homofermentative lactic acid bacteria, and after four days, these accounted for 85% of the Lactobacilli present, mainly as L. curvatus and L. plantarum. At the end of the 142 day ensiling period, heterofermentative bacteria made up 75 and 98% of the total Lactobacilli in silages of low and high dry matter, with L. buchneri and L. brevis dominating; possibly due to higher acetate tolerance.

Table 1 shows the species of bacteria commonly found in silage.

2.2.1 Action of lactic acid bacteria

These organisms are divided into two main categories. The homofermentative - whose sole metabolic product after sugar fermentation is lactate - and the heterofermentative, which produce other metabolic products in addition to lactate. According to Langston and Bouma (1960), the dominant species in the silage were L. plantarum, L. brevis and Pediococcus sp., but Stirling and Whittenbury (1963) found that Leuconostoc sp. have been found to represent over 80% of isolated lactic acid bacteria present on growing plants during the grazing season.

2.2.1.1 Water soluble carbohydrates (WSC)

The anaerobic pathways of carbohydrate sugar metabolism by lactic acid bacteria are documented in publications by McDonald (1979,

TABLE 1

Some species of lactic acid bacteria commonly found on fresh herbage and in silage

Homofermentative	Heterofermentative
Lactobacillus plantarum	Lactobacillus brevis
Pediococcus acidilactici	Lactobacillus buchneri
Streptococcus durans	Lactobacillus fermentum
Streptococcus faecalis	Lactobacillus viridescens
Streptococcus faecium	Leuconostoc mesenteroides
Streptococcus lactis	

Source: McDonald (1981)

1981; Bryan-Jones, 1969; Whittenbury, 1963). Homofermentative lactic acid bacteria can form two moles of lactate per mole of glucose or fructose fermented, by Embden-Myerhof pathway, yielding ATP. Heterofermentative organisms can produce one mole each of lactate, ethanol and carbon dioxide per mole of glucose fermented. Glucose and fructose, after degradation via pentose phosphate route, are fermented by different pathways, yielding different ratios of final products - mannitol and acetate produced from fructose instead of ethanol. The amount of lactic acid formed depends on the balance between the two types of bacteria and the concentration of WSC in the herbage (see Table 2).

2.2.1.2 Organic acids

Quantitatively, the most important in grasses are citric and malic, whereas in legumes, glyceric acid is a major organic acid, and is rapidly hydrolysed in silages (Whittenbury, 1968). Citric acid fermentation is undergone mainly by Streptococcus faecalis and L. brevis. Malic acid is fermented by S. faecium, S. faecalis, Pediococcus sp., L. plantarum, L. mesenteroids. The products of such fermentation are either neutral (acetoin, 2, 3, butanediol and ethanol), salts of organic acids (acetates, lactates and formates) or alkaline released cations (McDonald and Whittenbury, 1973).

2.2.1.3 Nitrogenous compounds

About 75-90% of the total nitrogen (TN) in fresh grass is present as protein, most of it in the form of metabolic protein, and much of the protein is cold water soluble. 10-25% of the total nitrogen in forage is in the form of non-protein nitrogen (NPN). This includes free amino acids, the amides, glutamine and asparagine,

TABLE 2

Effect on water soluble carbohydrate content (WSC) on fermentation

	Composition of crop silage						
	DM %	% WSC in crop DM	pH	Lactic acid % DM	Acetic acid % DM	Butyric acid % DM	NH_3-N % Total N
Ryegrass	16.3	16.4	3.8	15.4	3.4	0.0	8.5
Lucerne	16.3	4.5	6.4	2.1	6.5	2.9	24.4

Source: Wilkinson, Wilson and Barry, 1976

peptides of varying chain length, ureides, nucleotides, chlorophyll, nitrates, non-protein free amino acids (Ohshima and McDonald, 1977; McDonald, 1981).

After the initial proteolytic activity of plant enzymes has declined, a microbial fermentation occurs, which results in changes to amino acids and other nitrogenous compounds (Ohshima, McDonald, Acamovic, 1979). Lactic acid bacteria are non-proteolytic and have limited powers of protein synthesis, and need an extensive supply of amino acids for growth. For example, L. plantarum has an external requirement for glutamate, leucine, tryptophan and valine (Bhandari et al, 1955).

Amides have been reported to increase, decrease or disappear during formation of low pH silage. Amines have been found in low pH silages by several workers (McPherson and Violante, 1966; Hughes, 1970), and have been found to account for 9.3% of total nitrogen in low pH silage stored for six months. Putrescine and cadaverine were dominant amines.

Nitrates can be reduced to nitrite and thence to ammonia by some lactic acid bacteria (e.g. L. plantarum).

2.2.2 Action of Clostridia

Clostridia are obligate anaerobes. The activities of clostridial bacteria are well documented by Whittenbury et al (1967), Ohshima and McDonald (1979) and McDonald (1981). There are two main physiological groups of clostridia in silage: the saccharolytic clostridia and the proteolytic clostridia. The former ferment mainly carbohydrates and organic acids and very little protein e.g. Clostridium butyricum. The latter ferment mainly protein and

very little carbohydrate. Some organisms, like Cl. perfringens have both high saccharolytic and proteolytic activity.

Proliferation of clostridia is undesirable, as it acts against preservation by converting lactate to butyrate leading to a pH rise and results in the catabolism of amino acids. This in turn reduces the intake and nutritive value of silage.

2.2.2.1 Amino acids

Fermentation of amino acids by clostridia occurs via three major pathways:

(1) Deamination: deamination of single amino acids to fatty acids, carbon dioxide and ammonia.

(2) Decarboxylation: The decarboxylation of amino acids to form amines.

(3) Oxidation/Reduction: in this coupled Stickland type reaction, one amino acid is oxidized, whilst the other is reduced, with the liberation of ammonia. Of the important amino acids, those commonly oxidized are: alanine, leucine, serine, tyrosine, phenylalanine, histidine and tryptophan; and those commonly reduced are cysteine, glycine, arginine, proline, methionine and ornithine.

2.2.2.2 Carbohydrates and organic acids

Saccharolytic clostridia utilize sugar and lactic acid as energy sources, producing end products such as butyrate, carbon dioxide, and hydrogen. One mole of butyrate is formed from two moles of lactate, and this, in addition to the weaker acidity of butyric acid relative to lactic acid, causes a rise in pH (McDonald, 1981). Clostridia can be controlled by rapid acidification to pH 4 by the use of additives such as formic acid, or by the increase in dry matter content of herbage (>300 g kg^{-1}) before ensiling.

2.2.3 Action of enterobacteriaceae

These are gram negative, facultative anaerobes in silage; and are normally active in the early stages of ensilage, competing with lactobacilli for sugars. Acetic acid is a main fermentation product, but carbon dioxide, lactic acid and to a lesser extent, ethanol, 2, 3, butanediol and hydrogen are produced (Beck, 1978). These organisms do not normally attack proteins, but can deaminate and decarboxylate amino acids. Their presence reduces intake due to acetate production. Rapid acidification reduces growth of enterobacteriaceae.

2.2.4 Action of yeasts

Beck (1978) identified two main groups of yeast in silage. The first is 'sediment' yeasts, which grow on the ground and preferentially ferment sugars. The second is 'pellicle' yeasts, which have a high respiratory capacity for lactic acid. Fermentation products include lactic acid, VFA and alcohols. Yeasts are normally a minority group in silage, but are more active in formic acid treated grass silages (Henderson and McDonald, 1971), and in maize silage (Woodford, 1976). Yeasts are also associated with secondary aerobic deterioration under certain conditions.

2.3 Silage classification

McDonald (1981) classified silages into six categories depending upon main fermentation characteristics. These categories are: lactate, acetate, clostridial, wilted, additive inhibited, aerobically deteriorated. Lactate and acetate silages exhibit low pH (c. 3.7-4.2), NH_3-N (c. 80 g/kg TN), protein N (c. 900 g/kg TN) and WSC (10 g/kg DM) levels, and differ only in relative concentrates for lactic and acetic acids. Butyrate silages have a higher pH (c. 5.2),

NH_3-N (250 g/kg TN) and butyrate (35 g/kg DM) levels. Wilted and chemically restricted silages have restricted fermentation acid formation, reduced protein breakdown and the WSC may be relatively unaltered. Aerobically deteriorated silages have a high NH_3-N content and mould growth.

2.4 Losses during ensiling

Watson and Nash (1960) summarised the losses during ensilage in a survey of 800 experiments done between 1938-1960. The data are shown in Table 3. Mean DM and N losses during ensiling were found to be 16.1% and 15.2% respectively. Zimmer et al (cited by McDonald, 1981), using sophisticated techniques, categorized the losses during ensilage, together with causative factors. Zimmer concluded that unavoidable energy losses (due to residual respiration, fermentation and effluent or field losses) during ensilage, need not exceed 7%, and that aerobic deterioration during storage and after opening, could be a major source of loss of up to 25%. Gross (1981) stated that there is a close correlation between the losses, water content and ensiling methods.

3.0 THE NUTRITIVE VALUE OF SILAGES

The nutritive value of silages has been reviewed by several workers including Wilkins (1974, 1978, 1981), Wilkinson, Wilson and Barry (1976), Tayler (1967), McDonald (1981) and Ferrier (1982).

The principal factors which determine the nutritive value of silages are intake, digestibility and the efficiency of utilization of digested nutrients (Blaxter, 1962; Raymond, 1966). These will depend upon stage of growth at harvest, changes in composition during ensiling, quality of preservation, silage dry matter content,

TABLE 3

Losses during ensilage summarized by Watson and Nash, 1938-60

Treatment	Dry Matter		Nitrogen	
	No.	% loss	No.	% loss
Untreated	162	19.5	148	20.1
Wilted	69	13.4	62	11.0
Stimulated	212	17.5	218	15.2
Acidified	233	13.1	347	13.6
Inhibited	66	16.9	37	18.9
WEIGHTED MEAN	742	16.1	812	15.2

Source: McDonald, 1981

(Flynn, 1981) and the method of feeding and supplementation (Dulphy, 1979).

3.1 Effect on changes in composition during ensiling

These changes are discussed in Section 2. In brief, they result in an increase in acids and non-protein nitrogen, and a decrease in water soluble carbohydrates, as shown in Table 4. The effect of these changes is frequently a higher gross energy content of silage compared to fresh crop (Waldo et al, 1965; Waldo et al, 1969; Alderman, Collins, Dougall, 1971; Beever et al, 1971; McDonald, Henderson and Ralston, 1973). Table 5 shows the gross energy values of some silage and grass constituents. The value for ethanol and butyrate is fairly high, whereas those for lactate and acetate are lower than for glucose and fructose. McDonald et al (1973) reported that the mean gross energy of six silages was 9.3% higher than for fresh grass with a range of increase from 3.4% to 14.7%.

3.2 Voluntary intake of silage

Comprehensive reviews of voluntary intake in ruminants have been written by Campling and Balch (1961), Campling (1964, 1969), Baumgardt (1969), Blaxter (1962); Balch and Campling (1964), Baile and Forbes (1974), Bines (1976), Rohr (1977) and Baile et al (1978). The reviews have shown that food intake in ruminants is influenced by a number of plant and animal factors. With high energy diets, chemostatic or thermostatic regulatory mechanisms appear to act as the satiety signal. On low energy diets, rumen load appears to limit intake. With a given rumen capacity, forage intake may vary considerably in relation to rate of breakdown or rate of passage. The faster the breakdown occurs, the sooner realimentation occurs. Digestibility,

TABLE 4

Generalised comparison of some components in fresh grass and grass silage.

	Grass	Silage
pH	6.0	3.7-5.2
Water-soluble carbohydrates (% DM)	10-25	0-5
Acids (% DM)	3	5-20
True protein N (% total N)	80	20-60
Ammonia-N (% total N)	0	5-30

Source: Wilkins, 1980

TABLE 5

Gross energy values (kJ/g) of some grass and silage constituents (McDonald, Henderson and Ralton, 1973)

Grass	Silage	Constituents common to grass and silage
Glucose - 15.64	Lactate - 15.16	Crude Fibre - 17.45
Fructose - 15.70	Acetate - 14.60	Protein - 24.60
	Butyrate - 24.93	Ether Extract - 38.53
	Ethanol - 29.80	

Source: Wilkins, 1981

chemical composition, saliva production, rumen pH, and the physical structure of the feed influence the rate of breakdown (Rohr, 1979).

Dermarquilly and Dulphy (1970) suggested that the intake of direct cut silage is generally lower than that of corresponding fresh herbage. Dermarquilly (1973) found that the intake of 87 silages by sheep was on average 33% lower than that of fresh grass and legumes from which the silages were made; the reduction in intake ranging from 1% to 64%. He also found that daily intake of grass silages was about 47 g DM/kg $W^{0.75}$, which agrees well with the mean proposed value by the ARC (1980).

The characteristics of silages which determine their intake has been reviewed by Wilkins (1974) and Tayler and Wilkins (1976). Ferrier (1982) summarized the changes in the forage during ensiling which reduce intake of silage as: the modification of forage structure by harvesting; the fermentation of WSC liberating organic acids; hydrolysis of plant protein to non-protein nitrogen (including free amino acids, ammonia, amines) and the relative increase in liquified material.

The intake of silage is not closely related to its digestibility (Wilkins et al, 1971; Dermarquilly, 1973; Wilkins, Hutchinson, Wilson and Harris, 1971); which contrasts to the close positive correlation between intake and digestibility of fresh and dried forages (Blaxter, 1962; Balch and Campling, 1962). Wilkins (1979) found, however, that in the absence of differences in silage fermentation, the intake of silage by sheep increases with increasing digestibility.

3.2.1 Effect of dry matter (DM) content on voluntary intake or silage

Murdoch (1960), Gordon et al (1961) and Jackson and Forbes (1970) found a positive correlation between DM intake and silage DM

content; when silages varying in dry matter content were fed to ruminants. The water content *per se* seems to have little influence on intake (Thomas *et al*, 1961; Clancy *et al*, 1977), but it determines fermentation quality, hence may be indirectly related to intake (Thomas, Moore, Okamoto, Sykes, 1961). High fermentation quality is usually associated with high silage intakes and low losses during ensilage (Jackson and Forbes, 1970). In studies with 70 silages fed to sheep, Wilkins *et al* (1971) found that DM intake was positively correlated with contents of silage DM, total N and lactic acid as a % of total acids; and negatively correlated with the contents of acetic acid and ammonia-N.

Wilting, prior to ensiling, produces a silage with high dry matter content, in which the content of ammonia-N and fermentation acids are low due to restricted fermentation, thus dry matter intake is increased (Harris and Raymond, 1963). Marsh (1979), in a recent review of the effect of wilting, stated that wilting increased the DM intake of long chop silages in sheep by 4.4% in 10 comparisons. Dermarquilly (1973) noted an increase of only 12.6% in 14 comparisons using silages of 4-5 cm particle length. Flynn (1981) concluded that wilting increases DM intake, but reduces efficiency of feed conversion. He associated this reduction with the fermentation pattern in the rumen affecting the efficiency of energy utilization.

3.2.2 Effects of fermentation products on voluntary intake

Harris *et al* (1966), McCullough (1966), McCarrick *et al* (1965), Raymond (1969), Saue and Brierem (1969), Wilkins *et al* (1971), Waldo (1977) and Dermarquilly (1977) have reported the influence of the quality of preservation of silages (as defined by acid and NH_3 content) on intake. Wilkins *et al* (1971) and Dermarquilly (1973) associated

differences in silage intake with the products of silage fermentation. Over a wide range of silages, they found that intake was negatively correlated with contents of ammonia and VFA, especially acetate. However, addition of ammonia did not decrease silage intake (Wilkins, 1974). There is a positive correlation between acetate levels and extensive protein degradation (Wilkins et al, 1971), as well as its negative correlation with intake, yet addition of acetic acid to made silage does not reduce intake (Hutchinson and Wilkins, 1971; Deswysen et al, 1978). It seems therefore that a fermentation producing high acetate levels may produce other products which limit intake.

There are conflicting reports about the influence of lactic acid per se on silage intake. McLeod et al (1970) found that lactic acid added to silage to raise the concentration from 54 g kg^{-1} DM to 113 g kg^{-1} DM decreased DM intake of sheep by 22%. On the other hand, Morgan et al (1980) fed two well preserved grass-clover silages of high (165 g kg^{-1} DM) and low (34 g kg^{-1} DM) lactic acid contents to sheep, and found no significant difference in intakes. Thomas, Gill and Austin (1978) found evidence that the effects of acid levels on silage intake and influenced by protein breakdown during ensiling. They found that although lactic acid reduced silage intake when fed alone, this effect did not occur when the silage was supplemented with fish meal (source of rumen undegradable protein).

With clostridial silages, there is low N retention and much N excretion in the urine; and intake may be limited by low levels of protein or specific amino acid entering the duodenum.

In maize silage of low total N content, the addition of an N supplement increased DM intake, the extent of the increase in intake

being related to the total nitrogen concentration in the silage (Dermarquilly and Weiss, 1971).

It appears that the reduction in feeding value of silages arise principally from changes in the nitrogen fraction (Wilkins, 1980), but more work is needed to elucidate the mechanism.

3.2.3 The effect of physical features of silage on intake

The effect of physical processing of forages has been reviewed by Greenhalgh and Wainman (1972) and Osbourn et al (1976).

Several workers have shown that silage intake can be increased by reducing the chop length of the herbage (Dulphy, 1979; Dermarquilly and Dulphy, 1977; Greenhalgh and McDonald, 1978; Murdoch, 1965; Deswysen et al, 1978; Wilkins et al, 1978; Thomas et al, 1976). Dulphy has reported in nine comparisons with sheep, that the dry matter intake of direct-cut grass silages was increased by 56%, when a precision chop machine was used, instead of a flail machine. Deswysen et al (1978) reported that the decreasing of the particle length of Italian ryegrass from 53 mm to 18 mm prior to ensiling, significantly increased silage DM intake by about 17%.

Chopping increases silage intake in two ways: firstly, through improving fermentation quality (Murdoch et al, 1955; Balch et al, 1955; Murdoch, 1965; Dermarquilly and Dulphy, 1973); secondly, through increasing the rate of passage of food through the rumen (Deswysen et al, 1978). But chopping per se is the main reason for increased intake (Dermarquilly and Dulphy, 1973, 1977). These workers also found that when sheep are fed long silage, there is a large increase in the latent period between one meal and the onset of rumination. Chop length appears to be more important in silages of low digestibility (Gill, Thomas, Gibb and England, 1981).

3.2.4 Effect of species ensiled

It appears that at a given level of digestibility, there may be distinct differences in intake between legumes and grasses. Legumes appear to have a superior intake to grass, and this is attributed to differences in cell wall contents and rate of breakdown.

3.2.5 Effect of saliva production and rumen pH

Kaufmann and Orth (1966) and Rohr (1969) have found that saliva production was higher with increased fibrousness and decrease in mass density of feedstuffs. Increased salivation may accelerate the rate of passage of feed either by increasing flow to the omasum (Phillipson, 1966) or by activating cellulosedegradation via a higher buffering capacity in the rumen (Orth and Kanfmann, 1966) or by increasing rumen pH (Cheng et al, 1955; Storry and Sutton, 1969; Terry et al, 1970).

3.2.6 Effect of silage digestibility on intake

Friis et al (1979) found that as the silage DM digestibility increased, silage DM intake increased, when fed a constant amount of concentrates.

3.2.7 Effect of animal species and age

Sheep intake is more affected than cattle intake on silage diets (ARC, 1980). The intake of silage relative to hay by seven month old lambs was found to be 71%, compared with 86% for mature sheep (Harris, Raymond and Wilson, 1966).

3.3 Silage digestibility

The digestibility of silage is primarily determined by the parent crop digestibility (Harris and Raymond, 1963), which in turn is affected mainly be the stage of maturity (leaf: stem ratio) of the

herbage.

Ensiling has little effect on the DM digestibility and evidence supporting this is given by McDonald and Edwards (1976), who found values of DM digestibility of 0.767 and 0.768 for 36 paired samples of fresh grass and the silage made from it.

Earlier cutting was found to increase silage digestibility substantially and McIlmoyle found that an increase in digestibility of 0.07 increased silage intake by 26%, and Steen and McIlmoyle (1982) recorded increased daily carcass gain in beef cattle.

Physical treatments (laceration and chopping) were not found to have consistent effects on digestibility (Harris and Raymond, 1963), but wilting caused a reduction.

The use of additives, for example formaldehyde, can reduce silage DM, OM and N digestibility (Wilkins, 1980).

3.4 Efficiency of utilization of silage

Ensiling usually results in only small changes in digestibility and the efficiency of utilization of digested energy, but it may decrease dry matter intake and the efficiency of utilization of nitrogenous compounds (Wilkins, 1978; Beever, 1979; Wilkinson, Wilson and Barry, 1976).

3.4.1 Utilization of energy in silage

The gross energy (GE) of forages is generally considered to be about 18.4 MJ kg^{-1} DM, but due to the influence of fatty acids and protein, the GE content of silages may increase to about 20 MJ kg^{-1} DM (McDonald and Edwards, 1976). Alderman *et al* (1971) found that from 45 grass silages, the mean gross energy for silage was 10% higher than the value for fresh forage.

Of the GE of silages, 7-9% appear to be lost as methane. Table 6

TABLE 6

Losses of energy as methane and urine from conserved forages

Forage type		No. of samples	Methane energy (% GE ± SE)	Urine energy (% GE ± SE)
Dried grasses:	chopped	15	7.6 ± 0.21	5.2 ± 0.23
	pelleted	11	7.1 ± 0.48	4.3 ± 0.24
Hays		16	8.0 ± 0.25	2.9 ± 0.12
Silages:	grass and clover	32	7.7 ± 0.15	4.8 ± 0.20
Silages:	maize	16	8.0 ± 0.25	2.6 ± 0.11

Source: Greenhalgh and Wainman, 1979

shows the mean values for urine and methane loss of silages. The values obtained for methane are remarkably constant and agree well with the common value used of 8%. Urine energy is much more variable, and is significantly related to the protein content of the feed. Metabolisable energy (ME) is commonly calculated from digestible energy for a factor of 0.81, which may be too low for silages, 0.82 or 0.83 being preferable (Greenhalgh and Wainman, 1979). Comparisons of hay and silage by Greenhalgh and Wainman (1979) have shown silage as having greater ME concentration but poorer energy utilization than hay. However, Van der Honing et al (1973) reported no differences in ME utilization between silage and hay by dry or lactating cows.

McCarrick (1966) suggested that cattle fed on silage stored a greater proportion of their energy retention as fat, than animals fed hay. The efficiency of utilization of ME by ruminants for maintenance (Km) or for fattening (Kf) is very variable, but does not appear to be affected by the ensiling process. Ekern and Sundstol (1974) found similar values of Km and Kf for hay and silages from the same parent material. Smith et al (1975) obtained a Kf value of 0.424 and a net energy value of 4.78 MJ kg^{-1} DM for a grass-clover silage, similar to that of dried grass. Van Es (1969) concluded after studying 280 balance trials, that Km and Kl (efficiency of utilization of ME for lactation) did not differ for hays and silages of similar ME values.

At the present time, information on the efficiency of utilization of silage ME is limited, and more research is needed in this area.

3.4.2 Utilization of silage nitrogen

The nitrogenous compounds ingested in food by the ruminant undergo proteolysis and deamination in the rumen by microbial enzymes

to yield ammonia-N, which is one of the major substrates for the growth of rumen microbes.

The efficiency of conversion of food-N into microbial-N depends on the relative rates of ammonia release and ammonia assimilation. If release rate is faster than assimilation rate, then a large proportion of the ammonia will be absorbed, converted into urea and excreted in the urine. Two important factors in this process are the rumen degradability of dietary N compounds, and the level of energy available for microbial growth. In lactate silages, hydrolysis of proteins to amino acids allows much of the silage N to be in a highly degradable form. Also, variable amounts of ammonia in the silage and low residual soluble carbohydrate content induce poor N utilization by the animal (McDonald, 1981).

Several authors have reported lower N retentions in ruminants fed silage compared to the same crop fed after freezing or drying (Waldo et al, 1965; Fatianoff et al, 1966; Forbes and Irwin, 1968 and Thomson, 1968). Wilted silages and silages made with certain additives (fermentation restrictors) have shown higher levels of N retention than unwilted silages without additives (Wilkins, 1974). Wilkinson, Wilson and Barry (1976) cited Lonsdale's claim that he found that the retention of N in perennial ryegrass silages given to young calves, ranged from 4-21% of the digestible N consumed, whereas in comparable calves given similar crops in dried form, the range was 34-39%. The explanation for the reduction in N retention was a combination of large amounts of soluble "degradable" N and concomitant low concentrations of WSC (McDonald et al, 1981).

This is confirmed by the work of Fatianoff et al (1966), El Shazly (1952) and Ciszuk and Eriksson (1973), who found that silage diets

produce high levels of ammonia in the rumen. At Edinburgh, McDonald and Edwards (1976) found that peak ammonia concentrations for six lactate silages fed to fistulated lambs, varied from 195 mg l^{-1} to 450 mg l^{-1}, and there was a highly significant correlation between peak NH_3 concentration and NPN and NH_3-N contents of the silages. Ciszuk and Eriksson (1973) stated that net losses of N occur when rumen ammonia-N concentration exceeds 150 mg l^{-1}.

Donaldson and Edwards (1979) observed that the efficiency of utilization of silage nitrogen may be improved by more frequent feeding of silage and subsequent levelling off of rumen ammonia concentration. Beever et al (1977) showed that about 85% of the amino acid N in silage is degraded in the rumen; compared with 52% for fresh grass and 29% in dried grass (Osbourn, 1976). Proud (1972) showed that the quantity of N and total amino acid N reaching the small intestine was markedly greater with dried herbage than with wilted silage, fed to sheep. Armstrong (1973) calculated that the supply of amino acid N to the host when a silage diet was fed, was only 76% of that when fresh grass was used. The lower value for silage may be due to changes in the rate of microbial protein synthesis and/or ammonia release. Beever et al (1971) concluded that amino acid N flow to the duodenum of sheep fed fresh grass was 63% of N in original crop, and amino acid absorbtion was 41%. The corresponding values for silage were 41% and 31% respectively, and for unwilted silage were 54% and 41% respectively. Examination of events occurring in the rumen (Table 7) revealed large differences in amounts of degraded carbohydrate and nitrogen, which were available for microbial synthesis and capture of degraded N was high. With unwilted silage, both degraded carbohydrate and N were reduced, and although the

TABLE 7

The influence of ensiling on the ruminal degradation of forage carbohydrate and nitrogen and the efficiency of microbial nitrogen synthesis. (All values per kg DM intake unless stated.)

	Fresh Grass	Wilted Silage	Unwilted Silage
Degraded carbohydrate (g)	331	238	248
Degraded nitrogen (g)	24.3	24.1	16.5
Ratio of degraded carbohydrate: degraded N	13.6	9.8	15.0
Apparent capture of N by the microbes (g/100 g degraded dietary N)	89	69	89
Microbial N synthesis (gN/kg rumen degradable OM)	53	39	41

Source: Beever, 1979

efficiency of capture of degraded N remained high, the overall efficiency of microbial N synthesis was reduced.

Both protein and energy supplementation increased N retention in ruminants fed lactate silages. Gill and Ulyatt (1977) showed that the flow of amino acids into the duodenum of sheep was increased from 50 g day^{-1} to 77 g day^{-1}, when a silage diet was supplemented with an energy (starch-sucrose) supplement. When a protein supplement was used (formaldehyde treated casein), the amino acid flow to the duodenum was increased to 111 g day^{-1}. A substantial part of the increased duodenal flow of amino acid on this protein supplemented silage was due to the formaldehyde protection of the casein from rumen degradation.

4.0 SILAGE ADDITIVES

Improvements in the nutritive value of silage may be attained by wilting or by the addition of chemical additives - to reduce the nutrient loss by altering the pattern of fermentation in silo and degradation in the rumen. Wilting has been discussed in Section 3.0.2.1.

The use of additives in Northern Europe is widespread, and has been established for about 50 years but in Britain, only in the post-war era did chemical additives make a serious impact.

4.1 Rationale

Fermentation acids and fermentation products from protein degradation have been shown to reduce dry matter intake of silage (see Section 3.0.2). Also, protein degradation in the rumen is wasteful.

Chemical additives can thus be used in these efforts to preserve forage without fermentation acid production and resultant DMI depression.

4.2 Classification of additives (see Table 8)

Classification of additives has been recently discussed by

TABLE 8

Silage additive classification

SILAGE ADDITIVES

Fermentation Stimulants

BACTERIAL CULTURES

- Lactic acid Bacteria

CARBOHYDRATE SOURCES

- Glucose
- Sucrose
- Molasses
- Whey
- Beet pulp
- Citrus pulp
- Potatoes
- Cellulases

Fermentation Inhibitors

ACIDS

- Mineral
- Formic
- Acetic
- Lactic
- Benzoic
- Acrylic
- Glycollic
- Sulphamic
- Citric
- Sorbic

OTHERS

- Formaldehyde
- Paraformaldehyde
- Sodium nitrite
- Sulphur dioxide
- Sodium metabisulphite
- Ammonium bisulphate
- Sodium chloride
- Antibiotics
- Carbon dioxide
- Carbon disulphide
- Hexamethylenetetramine
- Brono pol
- Sodium hydroxide

Aerobic Deterioration Inhibitors

- Propionic acid
- Caproic acid
- Sorbic acid
- Pimaricin
- Ammonia

Nutrients

- Urea
- Ammonia
- Biuret
- Minerals

Source: McDonald, 1981

McDonald (1981). This author has classified additives into four main categories:

(1) Fermentation stimulants - which control fermentation by encouraging lactic acid production.

(2) Fermentation inhibitors - which control fermentation by inhibition of microbial growth.

(3) Aerobic deterioration inhibitors - which control deterioration of silage exposed to the air.

(4) Nutrients - which are used to imporve the nutritive value of silage.

An additive must be non-toxic to animals and must have no adverse effect on rumen fermentation (McDonald, 1981), and must leave no harmful residues in animal products.

4.2.1 Fermentation stimulants

These may be either bacterial cultures of carbohydrate sources.

Cultures of lactic acid bacteria, especially L. plantarum and S. faecalis can be used to overwhelm resident bacteria and ensure lactic acid fermentation. The addition of soluble carbohydrate, such as sugars, molasses or cereals have been successfully used for encouraging lactate fermentation (McDonald, 1981).

4.2.2 Fermentation inhibitors

4.2.2.1 Mineral acids

A.I. Virtanen (1933) in Finland, suggested the use of mineral acids as silage additivies. By adding small amounts of inorganic acids, the pH of herbage fell rapidly to between 3 and 4, and he found that at this pH, bacterial and enzyme action were greatly reduced; and conversion of lactate to butyrate prevented. He advocated the use of HCl at the rate of 5 kg/tonne ensiled and observed total abolition of butyrate production and severe restriction of normal fermentation. Problems of acidosis, intake depression and hypomagnesaemia were not

observed unless the pH fell to 2.5 or H_2SO_4 was used alone.

The AIV process has declined in popularity in Europe mainly due to difficulty in handling the corrosive acids (Ekern et al, 1975; McDonald et al, 1973; McDonald and Whittenbury, 1973; Owen, 1971; Wilkinson et al, 1976). However, in Scandinavia, it has persisted, due to the development of better handling techniques for acids (Ekern et al, 1975; McDonald and Whittenbury, 1973) and partly due to accident-free usage of the dangerous chemicals (Drysdale, 1979).

Mineral acids appear to function mainly by pH reduction effects, since, in an extensive antimicrobial screening experiment by Woolford (1978), there was no specific antimicrobial effects of HCl and H_2SO_4.

Phosphoric acid has also been used as a silage additive with some success (McDonald, 1981).

4.2.2.2 Formic acid

Since the 1960's, there has been a marked changeover to the use of organic acids as silage additives. Formic acid was advocated originally by Dirks (1926), but distribution problems and long chop caused disappointing results. It was only after the development of suitable flail harvester and spray application technology that formic acid became popular in U.K. as "Add-F" (produced by BP Nutrition). This product has a formic acid concentration of 850 g kg^{-1} and is applied undiluted to herbage from a container attached to a forage harvester. The rate of application recommended is 2.3 l $tonne^{-1}$ (2.8 kg t^{-1}) - equivalent to 2 l t^{-1} pure formic acid. Higher levels are recommended (5-6 l t^{-1}) for legumes with their high buffering capacity.

4.2.2.2.1 Effect on pH

Formic acid may be applied to herbage to prevent clostridial fermentation by pH reduction but its potent antimicrobial properties

enable it to restrict a lactate fermentation, if applied at high concentration. With ryegrass crops of DM content 170 g kg^{-1}, an application rate of 2.8 g kg^{-1} would result in pH fall to about 4.6 (McDonald, 1981).

4.2.2.2.2 Effect on microflora

Formic acid has a bacteriostatic action, due partly to a H^+ ion concentration effect, and also to the selective bacteriocidal action of undissociated acid (Norgaard, Pederson et al, 1968). In antimicrobial studies by Woolford (1975), it was found that formic acid was more effective in inhibiting bacterial growth at pH4 and in the pH range 5-6, propionic acid inhibited more effective by growth of Clostridia, Bacillus sp. and the gram negative bacteria.

Yeasts were found to be especially tolerant of formic acid and high counts have been noted on silages treated with formic acid (Henderson, McDonald, Woodford, 1972). Pederson et al (1973) found enterobacteriaceae inhibition early in ensilage, but lactobacilli were active. Beck (1978) found that the pronounced inhibitory effect of formic acid on clostridia and enterobacteriaceae was proportional to the concentration used.

4.2.2.2.3 Effect on silage composition

The addition of formic acid has been observed to improve silage quality as assessed by chemical analysis, digestibility, voluntary intake, and liveweight gains in cattle and sheep (Castle and Watson, 1970; Waldo, Keys and Gordon, 1973; Hinks and Henderson, 1977).

The effect of formic acid on the chemical composition of silage varies with the level applied, the DM content of the ensiled material, the species of crop used and its WSC content. The effect of formic

acid on a crop low in soluble carbohydrates is shown in Table 9, and on a crop high in soluble carbohydrate is shown in Table 10.

Formic acid has the greatest benefits when used where high levels of ammonia and VFA are likely to be produced (e.g. in an immature grass) (Barry, 1975) - immature forages are likely to be low in WSC, than can benefit from formic acid addition.

Henderson and McDonald (1976) studied the effects of formic acid on silage at three stages of ear energence: 17, 50, 100% and found that silage pH values were p 4.87, 4.78, 4.69 ± 0.02 respectively. This indicates that as grass matures, the buffering capacity falls. At higher dry matter levels (30%) reduction in fermentation occurred and at 35% DM, about 54% of original WSC was preserved. Carpintero et al (1979) also obtained suppression of fermentation and from results of test-tube experiments, produced regression equations relating level of application of formic acid to protein-N and ammonia-N concentration.

Formic acid decreases proteolysis and deamination when (cf Table 9) the rate of application is increased. This is due to low pH conditions which suppress the rate of proteolysis and clostridial fermentation (Thomas et al, 1961; Harris, 1966; Waldo, Keys, Smith and Gordon, 1971; Wilson and Wilkins, 1973; Carpintero, Henderson and McDonald, 1979).

Increased intake on formic acid silages may be due to low fermentation acid levels and ammonia-N, but also to increased presence of nutrients, as shown by higher OM content and OM digestibility in treated silages (Henderson and McDonald, 1970). Waldo et al (1971) observed marked improvement in liveweight gain (LWG), from adding formic acid to forage before ensiling, and suggested that this

TABLE 9

The effect of formic acid and formalin additives on the composition of lucerne harvested by two methods

FLAIL HARVESTED	pH	DM g kg^{-1}	OM* g kg^{-1}DM	Total-N g kg^{-1}OM	Protein-N g kg^{-1}TN	Ammonia-N g kg^{-1}TN	WSC g kg^{-1}OM	Lacitic acid g kg^{-1}OM	Acetic acid g kg^{-1}DM	Propionic acid g kg^{-1}OM	Butyric acid g kg^{-1}OM
(1) Untreated	5.53	176	839	37.7	312	209	7	18	115	13	4
(2) Formic acid (85%)											
Low (1.5 l tonne^{-1})	5.35	201	768	37.2	326	194	6	41	88	7	5
Medium (3.0 l t^{-1})	4.62	220	784	33.3	403	143	6	64	56	4	1
High (6.0 l t^{-1})	4.20	229	789	33.4	489	93	20	51	33	2	1
Formalin (8 l t^{-1}) (35%)	4.92	189	831	36.6	635	107	35	37	46	3	7
PRECISION CHOPPED											
(1) Untreated	4.74	191	882	34.4	371	129	6	42	92	7	1
(2) Formic acid											
Low (1.5 l t^{-1})	4.19	190	894	34.3	425	83	8	56	38	6	1
Medium (3.0 l t^{-1})	3.96	200	898	33.1	505	42	35	39	29	1	0
High (6.0 l t^{-1})	4.25	198	901	34.2	543	45	60	21	17	1	1
Formalin (8.0 l t^{-1})	5.16	199	890	35.1	727	72	102	16	19	1	1

*OM = Organic matter

Source Barry, Cook and Wilkins, 1978

TABLE 10

The effect of different levels of formic acid on the composition of rygegrass-clover silages after a 50-day ensiling period

Parameter	Level of formic acid					
	0	0.4	1.0	2.0	4.1	7.7
pH	3.87	3.77	3.67	3.81	3.88	3.80
WSC (g kg^{-1}DM)	12	33	72	124	211	250
Total-N (g kg^{-1} DM)	18.2	17.8	18.5	19.3	19.2	18.6
Protein-N (g kg^{-1} TN)	265	285	325	358	401	462
Acetic acid (g kg^{-1} DM)	28.8	24.1	18.9	13.3	4.5	3.1
Propionic acid "	0.18	0.27	0.22	0.36	0.28	0.19
Butyric acid "	0.19	0.04	0.04	0.16	0.23	0.03
Lactic acid "	122	153	115	117	66	5
Ammonia-N (g kg^{-1} TN)	95	79	59	46	12	12

Source: Carpintero et al, 1979

improvement was due to an improvement in dry matter recovery from the silo, increased energy intake, increased energy digestibility and efficiency of utilization by the animal.

Formic acid applied to ryegrass and clover silages by Siddons, Beever and Kaiser (1982) was observed to have either no effect or to reduce protein degradability by a small amount. Møller and Thomsen (1977) found rumen N degradability of formic acid silage to be 64%, compared with 79% of untreated silage.

Wilkinson, Le Du, Cook and Baker (1981) fed Italian ryegrass conserved with formic acid or dehydrated to cattle, and found that although intakes were similar, rates of liveweight gain were much lower for formic acid silage. They concluded that this was due to poor silage protein utilization.

4.2.2.3 Formaldehyde

Formaldehyde is a well known sterilant sold commercially as formalin, which contains 40% of the gas in aqueous solution. Virtanen (1933) reported the use of formaldehyde as a silage additive, but did not foresee any practical application for it in silage-making. Silage research for a long time concentrated on fermentation enhancement. However, McLeod et al (1970) found that the nutritive value of fermented forage limited animal performance, and research was encouraged for finding ways of preservation without fermentation acid production.

Around that time, Ferguson et al (1967) demonstrated that casein could be protected from rumen degradation but digestible in intestine, if treated with formaldehyde. Thus, interest was generated in the protection of forage proteins and soluble protein supplements from rumen degradation.

Laboratory studies by Wilkins demonstrated the antimicrobial activity of formaldehyde, but the inhibitory concentrations identified were several times smaller than concentrations found necessary in the field. Wilkins and his colleagues (1974) performed test-tube scale analysis of the effects of formalin. Ryegrass was harvested and treated with five levels of formalin: 0, 2, 3, 4.5, 9.1, 18.3 l tonne^{-1} At low levels for formalin application, clostridial fermentation occurred and high NH_3-N levels (sign of extensive proteolysis) were found. At intermediate levels, the silage was similar to control but without butyrate, and at high levels of formalin, there was severe restriction of fermentation, and very low levels of fermentation acids and NH_3-N were found. Wilkinson et al (1976) proposed a critical effective level of 3-5 g HCHO/100 gm. Ferrier (1982) summarized the effects of formaldehyde as follows:

(a) Formaldehyde reduces the proportion of organic acids.

(b) Formaldehyde reduces protein degradation.

(c) Animal intake may be improved.

(d) Superior performance may be obtained because digestibilities of organic matter and energy may be improved.

(e) Formaldehyde treated silage does not undergo extensive degradation in the rumen.

(f) Formaldehyde silage is likely to heat rapidly and becomes mouldy due to presence of large amounts of residual fermentable carbohydrates.

Formalin applied at 6.8 l tonne^{-1} fresh weight to grass restricted fermentation almost completely and acted as a sterilant - high pH silage with low NH_3-N and butyric acid was obtained (Wilkins et al 1974). This is confirmed by Wilkins et al (1974) who found restriction of fermentation with slightly higher levels of formalin. These

workers also found that formalin at levels between 6.5–11 l tonne^{-1} significantly increased OM intake, but at high levels (c 12.3 l tonne^{-1}), intake was depressed, and rumen function was disturbed as indicated by reduction in cellulose digestion, and VFA production in the rumen. They suggested that free formaldehyde may suppress microbial action, and this effect is found to be less marked with lucerne and grass with high N content. However, they felt that mixtures of formalin and formic acid may be more likely to produce silage of high nutritive value.

The degradation of silage N in the rumen was reduced from 85% to 22%, in an experiment reported by Beever et al (1977), when formaldehyde was added at ensiling at 6 g HCHO per 100 g crude protein to ryegrass. The total amino acid flow to the small intestine increased by 33% compared with control non-additive silage, and a marked change occurred in the proportions of microbial and feed protein in the total duodenal protein. The reduced N degradation in the rumen led to reduction in degraded N precursors for microbial protein synthesis, which declined from 167 g/kg rumen degradable organic matter to 66 g/kg RDOM. The change in composition of duodenal protein caused a decline in overall amino acid availability from 75% (control) to 67% ($^{+}$HCHO), thus amino acid uptake on treated diet increased by only 13%.

Ammonia concentration in the rumen has been reported to decrease when formaldehyde treatment of herbage occurs at ensiling by Barry and Fennessy (1973), Siddons et al (1979) and Wilkins et al (1975). An explanation for these observations might be the large reduction in protein degradability in the rumen of sheep fed formaldehyde treated silage.

The intake and digestibility of formaldehyde treated silages

have been found to be sensitive to the level of application, declining at high levels of formaldehyde application in studies with sheep (Wilkins, Wilson and Cook, 1975; Brown and Valentine, 1972) and cattle (Kaiser, Tayler, Gibbs and England, 1981).

Kaiser, Osbourn and England (1982) found that urea supplementation of formaldehyde treated red clover silages fed to calves, increased rumen ammonia concentrations, but not to a great extent at the high level of formaldehyde treatment; and increased the rate of dry matter and cellulose disapperance from silage in nylon bags. Behavioural observations showed that eating time was increased on formaldehyde treated silages.

The chemistry of formaldehyde action on protein has been described by Barry (1976). The reaction is a two stage process, the initial step involving the rapid formation of a methylol group (Barry, 1976; Ohshima and McDonald, 1978), and the second step involves the condensation reactions at a slower rate which result in the formation of stable methylene cross linkages between protein chains (Barry, 1976) (see Figure 1).

There are major losses of formaldehyde in silages and Barry (1976) found that the recovery of formaldehyde from treated silage declines progressively with time and that after 100 days, may be as low as 20%.

4.23 Aerobic deterioration inhibitors

Aerobic deterioration refers to the deterioration of a silage on exposure to air. Yeasts, bacteria and moulds are all thought to be responsible for this occurrence. The losses of DM from deterioration can be as high as 30% or more (Honig and Woolford, 1979). As deterioration proceeds, decreases were observed in silage ethanol

FIGURE 1

Chemistry of the reactions between formaldehyde and protein

STEP 1: Rapid methylol group formation during protein: HCHO interaction

$$R - XH + HCHO = R - X - CH_2OH$$

(-XH may be terminal amino groups $-NH_2$, α amino groups of lysine, primary amide groups of asparagine and glutamine, the guanidyl group of arginine, the hydroxy groups of threonine and serine, the sulphydryl group of cysteine, the phenol group of tyrosine, the phenyl group of phenylalanine, the indole group of tryptophan and the imadazole group of histidine.)

STEP 2: Slow condensation reactions resulting in formation of stable methylene cross linkages between protein chains.

$$R - X - CH_2OH + R\overset{\alpha}{N}H_2 \quad R - X - CH_2 - \overset{\alpha}{N}H - R + H_2O;$$

$$R - XH + R\text{-}\overset{\alpha}{N}H - CH_2OH \quad R - X - CH_2 - \overset{\alpha}{N}H - R + H_2O.$$

Source: Barry, 1976

and acid contents and the pH value rose. Variable losses of ammonia-N were obtained. Recent studies indicate that bacteria may play a significant role in deterioration (Woolford et al, 1977, 1978, 1979).

The presence of oxygen, the microbial population, the substrate and temperature can all influence aerobic deterioration (Honig and Woolford, 1979). Propionic acid used at 1-3% of DM has had a reasonable degree of success.

4.24 Nutrients

When added to silages, nutrient additives improve their nutritive value. Nitrogenous compounds can be added an ensiling to a crop to improve protein, which may be lacking. Urea addition was found to have a marked effect on silage nitrogenous components, resulting in increases in crude protein, true protein, free amino acids and ammonia. Thus true protein was spared, and microbial protein synthesis increased.

5.0 PROTEIN METABOLISM IN THE RUMINANT

Nitrogen metabolism in the ruminant has been recently reviewed (Smith, 1975; Buttery, 1976; Mercer and Annison, 1976; Armstrong, 1976; Hogan, 1975; Sattler and Roffler, 1975; Clark, 1975).

The first step in the metabolism of nitrogen in the ruminant after feed intake is the digestion of feed protein and non-protein nitrogen in the rumen, which is followed by normal protein digestion in the abomasum. This is followed by protein digestion and absorbtion of amino acids from the small intestine. By the time the undigested nitrogen reaches the large intestine as illeal nitrogen, it is either incorporated into micro-organisms or remains undigested and is voided through the anus. Absorbed amino acids enter the blood

circulation to the liver (but some are utilized during absorbtion) and are used as precursors for protein synthetic pathways or as energy sources at cellular level. Some are deaminated and converted to urea, which is either excreted through the kidneys or recycled via saliva.

5.1 Rumen digestion of nitrogen in protein

In the ruminant, protein digestion is complicated by the fact that before dietary protein reaches the site of host animal proteolytic enzyme secretion, it has to pass through the rumen, where the microbes exert a considerable influence on its digestion.

Dietary protein entering the rumen is often extensively degraded by bacteria and protozoa. This degradation involves two steps: firstly, the protein chain is broken by hydrolysis of peptide bonds (proteolysis) which results in peptides and amino acids. Secondly, amino acids are deaminated. Proteolysis may be the rate limiting step, as immediately after feeding, increased levels of free amino acids appear in the rumen (Demeijer, 1976 cited by Tamminga, 1978). Proteolysis and deamination were both found to be affected by pH, with the optimum pH for these processes reported to be between 6-7 (Lewis and Emergy 1962). However, under most nutritional circumstances, pH in the rumen will allow breakdown of dietary protein.

Bacteria breakdown protein into smaller parts by hydrolysis outside the bacterial cell. The resulting amino acids and peptides are transported inside the bacterial cells and hydrolysed to amino acids, then incorporated into bacterial protein or degraded to VFA, ammonia, carbon dioxide, methane, with some fermentation heat produced. End products are excreted back into the surroundings

(Tamminga, 1978). Protozoa differ in that they engulf bacteria or feed particles and proteolysis occurs within the protozoal cell. Amino acids are either incorporated into protozoal protein or excreted. Degradation of protein in the rumen provides microbes with required precursors for their own protein synthesis: either ammonia and presumably α keto-acids or even intact amino acids. Degradation of amino acids yields energy which is used by microbes for their synthetic processes. Prins (1977) found that at least one strain of bacteria needs amino acids as energy sources. Important biochemical reaction mechanisms in the further degradation of amino acids by microbes are deaminations, transaminations and decarboxylations. The most important pathway of amino acid degradation is thought to be deamination of the amino acid, followed by decarboxylation of the resulting α-keto acid (Demeijer, 1976;* Prins, 1977). The end products of rumen fermentation contain a high proportion of acetate.

5.2 Factors affecting protein degradation in the rumen

Protein solubility and rate of flow of digesta are two major factors governing the rate of breakdown of dietary protein in the rumen (Chalmers and Synge, 1954; Annison, 1956; Kempton et al, 1977) and they can have considerable influence on the quantity of dietary protein passing intact from rumen to duodenum, and the subsequent availability of amino acids. The molecular structure of the protein may be another important factor (Nugent and Mangan, 1978). Factors influencing passage rate out of the rumen are: specific gravity, particlesize and level of intake (Church, 1979). Solubility of protein is determined by the relative amount of albumins and globulins relative to the less soluble prolamins and glutelins (Tamminga, 1978). Isaacs and Owens (1972) reported an effect of pH on protein solubility.

*cited by Tamminga (1978)

Solubility of protein may also be affected by treatments - physical or chemical - before feeding to the animal.

With respect to silage, some of the proteins and carbohydrates are degraded in the rumen. The N-containing end products of fermented protein will then be found in the soluble fraction. Substantial increases in N- solubility may result if a clostridium type of fermentation occurs. Jarrige et al (1978) calculated that 65% of the insoluble dietary protein escapes degradation.

Formaldehyde treatment of silages protects silages from degradation in the fermentation in silo and in the rumen. A furtherreduction in degradation can be obtained by drying the formaldehyde treated silage at high temperature (Beever et al, 1977). At high levels, over protection occurs, causing a reduced degradation of protein in the rumen, but also a decreased susceptibility to proteolytic enzymes in the abomasum and small intestine (Tamminga, 1978). An inadequate N supply to the microbes due to increased protein flow to the small intestine (by dietary protein) may have a negative effect on degradation and on microbial protein synthesis from ammonia. There is evidence for a reduced microbial protein synthesis following feeding of formaldehyde treated grass silage (Beever et al, 1977). Zelter et al (1970) showed that the minimum protecting dose of formaledhyde reduced cellulolysis by 13-20%.

5.3 Digestion and absorbtion of protein from the small intestine

When the digesta leaves the rumen, it enters the abomasum where the mixture of dietary and microbial protein comes under the influence of the digestive enzymes of the host animal. MacRae (1979) quoted that 55-75% of the protein entering the small intestine disappears there. Exceptions are denatured dietary proteins or protected protein.

The optimal pH for intestinal proteases in ruminants is between 7.5-8.8. Pancreatic proteases may not digest protein until the final two-thirds of the small intestine.

The amino acid composition of digesta entering the small intestine is remarkably constant, even though the accuracy of estimation of undegraded N is suspect, and is complicated by unknown endogenous secretions of N (Clark, Ellinger, Phillipson, 1966; Coelho da Silva et al, 1972; Harrison et al, 1973). The constancy can be explained by the appreciable amounts or microbial protein and its uniformity of amino acid make-up; and the endogenous secretions have a constant amino acid composition. There is a high correlation ($r = 0.985$) between intake of organic matter and duodenal amino acid flow in cows (Tamminga and Van Hellemond, 1977), and ME intake is a major determinant of optimal non-ammonia-N (protein) supply. About 20% of the microbial non-ammonia nitrogen is present as nucleic acid.

Amino acids entering the small intestine become hydrolysed in the abomasum and small intestine due to the action of proteolytic enzymes (Armstrong and Hutton, 1975) and the resulting peptides and amino acids are transported across the gut wall to the portal blood. Peptides are hydrolysed further, possibly as part of the transport process (Matthews, 1975). In the gut wall, significant amounts of amino acids are metabolized (Tamminga, 1981). Apparent absorbtion is high (70-80%) and only small differences have been observed between essential and non-essential amino acids in this respect.

Nucleic acids, RNA and DNA, are degraded by the action of pancreatic nucleases in the small intestine to nucleosides and are absorbed.

5.4 Synthesis of microbial protein

The synthesis of microbial protein is governed essentially by the energy supply (ATP) and by the availability of nutrients. The amount of microbial crude protein, which contains 70-80% amino acid-N has been related to the amount of organic matter apparently digested in the rumen. Values reported in the literature range from 2-4 g microbial N/100 g OM apparently digested (Armstrong, 1976). The efficiency of microbial synthesis is influenced by the dilution rate (Harrison et al, 1975) and by the proportion of roughage in the diet (Cole et al, 1976).

Nolan (1975) showed that for forage diets, 50-70% of bacterial nitrogen and 31-55% of protozoal nitrogen is derived from ammonia, and that the uptake of preformed amino acids makes a substantial contribution to protein synthesis. The synthesis is of methionine and cysteine by rumen microbes requires a sulphur source.

5.5 Protein metabolism in the large intestine

Few reports have been made of amino acid absorbtion from the large intestine. It is assumed that absorbtion is passive in nature and trivial in significance. The digestion in the large intestine accounts for 5-10% of overall N digestion of ruminants (MacRae, 1979). The significance of this to the host animal is not clearly understood. It is not generally believed that amino acids can be absorbed by the large intestine and any losses from the caecum to the faeces is thought to be due mainly to ammonia-N absorbtion, the bacterial protein produced in caecal fermentation being excreted in the faeces.

5.6 Protein availability and animal performance

In fresh herbage experiments of MacRae and Ulyatt (1974), digestive parameters*were measured in sheep given a range of herbage intakes.

*energy and protein digestion

They were found to be linearly correlated with intake. When these data were related to liveweight gain with sheep on the same herbages, there was a very good relationship between absorbed protein and production ($r = 0.79$), but none between absorbed energy and production ($r = 0.02$). In these experiments the availability of protein apparently absorbed from the small intestine appeared to be a limiting factor to animal performance. Armstrong and Annison (1973) warned that methionine and threonine could be inadequate to meet requirements, on forage based diets.

6.0 THE NYLON BAG TECHNIQUE

This technique has been reviewed by Orskov et al (1980). As early as the 1930's, Quinn et al (1938) used the technique to investigate the digestion of feeds in the rumen of fistulated sheep, using cylindrical bags from fine silk. The fabric of the bag has varied, as has the name of the technique (Mmbaga, 1981). Originally, the method was meant to provide a single technique for estimation of forage digestibility to replace expensive, time consuming digestibility trials, which require much forage (Van Keuren et al, 1962) or to measure DM degradation in the rumen (McAnally, 1942) and measures protein degradation of feeds in the rumen at present.

In its simplest form, the technique consists of the incubation of suspended nylon (fibre) bags in the rumen liquor of trained fistulated sheep (Henderson,personal communication; Schoeman et al 1972; Mehrez, 1976; Mehrez and Orskov, 1977; Mathers et al, 1977).

Degradability of nitrogen is measured as the difference between initial and final nitrogen in the bag, stated as a proportion of the initial nitrogen (McDonald et al, 1981).

6.1 Pore size of the bag

Orskov et al (1980) in a review of the technique, stated that early workers put a lot of emphasis in the pore size of the bag, as it regulates the passage of solid particles from the bags. Materials with pores of 20 μ and 30 μ were found to give smaller DM losses than those with 53 μ pores (Uden, Parra and Van Soest, 1974). None of the materials currently in use meet the pore size of 10 μm recommended by Van Hellen (Crawford et al, 1978). Orskov et al (1980) used material with 12 μ pore and emphasized that the important thing was to use the same material in any one trial.

6.2 Size of bags

The optimum size of bag has been investigated (Rodriguez, 1968; Mahrez et al, 1976). It appeared that the best solution was to have a bag which allowed rumen fluid to enter, but which was small enough for easy removal from the rumen through the cannula (Orskov et al, 1980). They also recommended that the bag should have a double stitch using polyester thread, and the bottom corners rounded to prevent trapping of the sample. The double stitch ensures no gain or loss of sample particles in the rumen.

6.3 Sample preparations

Orskov et al (1980) noted that the samples should represent as far as possible the materials as they would appear in the rumen; had they been eaten by the animal. Playne et al (1978) suggested that the ideal sample would be masticated ingesta from animals fitted with oesophageal fistula, but in practice this cannot be done. Orskov et al (1980) found that the use of a laboratory hammer mill fitted with 2.5-3.0 mm screen for milling dry feeds was adequate. Compared

with the 1 mm used by Crawford et al (1978) and the 2 mm used by Playne et al (1978), this value seems large, although Proven (1980) emphasizes the observation of Playne et al (1978) that when using a nylon bag pore size of 25 μm, and 1 mm screen, much particulate loss occurred.

With fresh forage, chopping followed by shredding with a domestic liquidizer was adequate (Santana and Hovell, 1979).

6.4 Sample size

Variable results have been found when the sample size was altered. Rodriguez (1968) reported no effect of increasing sample size, Playne et al (1978) reported small effect on digestibility but Van Keuren et al (1962) and Mehrez et al,(1977) reported reduced digestibility. Orskov et al (1980) recommends the use of a sample size which yielded enough material after incubation for weighing on precision balances available.

6.5 Position and number of bags in the rumen

Variation in digestibility has been reported as a result of changing of position of the bag. With regard to number of bags, Orskov et al (1980) recommended the use of enough bags to ensure convenience of withdrawal, which is a major constraint on the number of bags which can be used.

6.6 Effects of animals, days and diets

Variations between animals have been reported by Rodriguez (1968) and Mehrez et al (1977) reported that by using three sheep (replicates), the variation was reduced. Variation between days occurred and Mehrez et al (1977) recommended replications in time to reduce the variation between days. Variation between diets was pronounced, and

high concentrate diets have reduced cellulolytic activity in the rumen whereas legumes increased diet digestibility (Orskov et al, 1980; Hopson et al, 1963).

6.7 Assessment of the technique

McDonald et al (1981) stated that there were several inherent sources of error, which had to be controlled, if reproducible results were to be obtained. These were sample size, bag size and porosity of the bag material. The need for standardization of the technique has been emphasized by Proven (1980).

A basic assumption of the technique is that the disappearance of nitrogen from the bag (reflecting virtually solubility in rumen fluid) is synonymous with degradation. Available evidence tends to confirm this basic assumption, but difficulty is found in correlating rate of disappearance of N with degradability data in vivo (McDonald et al, 1981; Mohammed and Smith, 1977).

Another factor affecting the extent of degradation is the time of incubation - as some diets are rich in soluble nitrogen, which disappears rapidly with two hours and then there is a slower rate of disappearance for less soluble material.

Also the protein in the dacron bag is not subjected to the dynamic system characteristic of digestive metabolism in the ruminant (Tamminga, 1978), but this problem can be overcome to a certain extent by estimating protein degradation at the time when 90% of the truly digestible organic matter has disappeared from the bag, thus simulating the in vivo situation in a noramlly fed animal (Orskov, 1977).

The technique is not suitable for predicting the extent of feed protein degradation with a high level of precision, but can give general valid conclusions about protein degradation of feeds. More-

over, its simplicity and speed enable it to be used in routine forage evaluation (Mmbaga, 1981).

EXPERIMENTAL

7.1 Objective of the Study

The main objective of this study was to compare the nutritive value and solubility in the rumen of silages prepared from similar grass, but treated with different chemical additives, namely formaldehyde and formic acid. In Section 3, it was observed that proteolysis and fermentation acid production reduced the nutritive value of silage. The use of high levels of formaldehyde or formic acid is justified because these can restrict proteolysis and lactate fermentation.

Experiment 1 was designed to assess the dry matter intakes and performances of the chemically-treated silages by lambs and to compare them with a control diet - Complete Ruminant Diet (C.R.D.) from a commercial feed mill.

Experiment 2 was designed to assess and compare the intake, digestibility, nitrogen-retention and ME of the two silage diets and the control, when fed to lambs.

Experiment 3 was designed to assess the solubility of the dry matter and protein of two treated silages and a control untreated silage, in fistulated adult sheep fed the two treated diets and the C.R.D. control.

The silages were made from second cut grass taken on July 25th, 1981 (Lolium multiflorum Lam 55%, 95% ear emergence; Lolium perenne L. 34%, 25% ear emergence) with a drum mower and lifted with a class 40 precision chop forage harvester.

Formaldehyde (40% w/v) was applied uniformly to one batch of grass before ensiling at a rate of 7.5 litres per tonne (7.5 l t^{-1}). To another batch of harvested grass, formic acid (under the commercial brand name 'Add-F', 'Add-F' is 85% formic acid w/w.) was added at 6.0 l t^{-1}

7.2 Experiment 1

Intake trial with lambs

Twenty-four Suffolk x Border Leicester x Cheviot wethers of approximately 31 kg liveweight were ranked according to their weight and divided into three groups (eight lambs per treatment), each group having a similar mean liveweight. After an introductory period on silage, the lambs in each group were randomly allocated to individual pens, to reduce position effects in the intake house.

The individual pens (1.3 x 0.75 M) were on raised mesh floors, with mesh sides and a metal grill door with space for a galvanized iron feeder, and a bucket holder for a plastic bucket. Water was constantly available throughout the experiment, and one scoop (about 14 g) of mineral supplement was fed with the diets daily.

The diets were: (1) Commercial complete ruminant diet (C.R.D.). (2) Formaldehyde-treated ryegrass silage. (3) Formic acid-treated ryegrass silage.

Following a seven day introductory period, intakes were measured daily and the animals fed 10% in excess of what they had eaten the previous day. Refusals were removed and weighed each morning, and the animals were fed at 09.00 hours and 16.00 hours. The lambs were weighed at 08.30 hours and days 1, 8, 15, 22. Samples of each diet were taken daily and bulked over a week. From the bulked weekly sample, representative samples were taken for dry matter determination. These weekly dry matter values were corrected as necessary, using the toluene dry matter (Dewar and McDonald, 1961) from the bulked sample for the whole period. The weekly dry matter values are given in Table 11.

TABLE 11

Weekly DM values for diets in intake trial

Diet	Dry Matter Values ($g\ kg^{-1}$)		
	Week 1	Week 2	Week 3
Formalin-treated silage	20.50	20.87	20.63
Formic acid-treated silage	20.72	20.26	21.00
C.R.D.	88.72	88.26	88.89

7.3 Experiment 2

Metabolism trial with lambs

Twelve Suffolk x Border Leicester x Cheviot wethers of approximately one year age and 46 kg liveweight, were randomly allocated to one of the three diets (i.e. four animals per treatment), on which they were fed for the duration of the trial. The diets are described in Experiment 1.

Daily feeding allowances of the silages and C.R.D. were weighed, and placed in individual, labelled polythene bags, sealed and placed in a deep freeze at -18°C. At the same time as bagging, representative sub-samples of each diet were taken and analysed for organic matter, nitrogen, gross energy and pH. The analyses are given in Table 12. The silage diets were allowed to thaw at room temperature prior to feeding.

The feeding period was of 10 days duration with a lag of 2 days for collection of faeces. About ten days before the beginning of the trial, the lambs were housed in metabolism crates and fed on the diet to which they had been allocated for the duration of the trial. In addition, these lambs were given one scoop of the mineral mix (about 14 g) daily, and water was constantly available. The lambs were fed twice daily, at 08.30 hours and 16.00 hours.

The metabolism crates used were of metal, measuring 1,370 mm x 560 mm. Faeces collection was done by means of a harness, which secured a canvas faeces bag in position. The faeces bag was lined with polythene, thereby eliminating any errors and inconveniences caused by transferring wet, fresh faeces into a bulked container. The faeces were collected daily and stored in a deep freeze. The bulked faeces collected over the trial were weighed and sampled.

TABLE 12

Composition of diets used in digestibility and intake trials

Parameters	DIET		
	C.R.D.	Formaledhyde-treated silage (F)	Formic acid treated silage (S)
pH	n.d.	4.26	3.82 (3.86)
Total DM (g kg^{-1})	869	204	213 (207)
Components of DM (g kg^{-1} DM)			
OM	900	916	918 (908)
WSC	n.d.	72	54 (0)
Total nitrogen	22.0	26.4	26.1 (26)
Crude protein	137.5	165	163 (162.5)
Protein nitrogen	n.d.	17.0	12.2 (11.8)
Protein N (g/kg TN)	n.d.	644	466 (452)
NH_3-N	n.d.	1.04	1.47 (1.9)
NH_3-N (g/kg TN)	n.d.	39	56 (75)
Ethanol	n.d.	16.7	5.8 (6.7)
Modified acid detergent fibre	n.d.	303	299 (304)
Free formaldehyde	n.d.	3.64	.016 (n.d.)
GE (MJ kg^{-1} DM)	17.1	17.9	18.2 (19.2)
Lactic acid	n.d.	62	92 (110)

( ) Bracketed values are those quoted by Ferrier (1982) when using untreated silage made from similar harvested grass at same time as the two silages used. The original grass had the following composition: DM = 176 g kg^{-1}; WSC = 171 g kg^{-1} DM; N = 25.9 g kg^{-1} DM: CP = 162 g kg^{-1} DM.

n.d. = not determined

TABLE 13a

pH and content of dry matter, nitrogen, protein nitrogen and ammonia nitrogen of the three silages used in the bags in the artificial fibre bag experiment (Expt. 3)

Silage	DM content g kg^{-1}	N content g kg^{-1} DM	pH	Protein N g kg^{-1} TN	g kg^{-1} TN
Control	199	26.6	4.01	442	75
Formalin	207	25.2	4.58	641	26
Formic	212	26.1	3.93	434	51

TABLE 13b

Composition of C.R.D. diet according to manufacturer's description

Formula	30% roughage A.A.6	
Barley Straw	30.00	
Ex. Groundnut Meal	7.00	
Barley	22.25	
Wheatfeed	22.00	
Molasses	10.00	
Urea	1.00	
50% Fat Premix	2.50	
Salt	1.50	
Dical Phosphate	1.50	
Sodium Bicarbonate	2.00	
Lime	0.25	
Additives	**gms/ton**	
Advitamix AD_3E	200	
Cobalt Sulphate $5H_2O$	17	
Ferrous Sulphate $7H_2O$	36	
Manganese Sulphate H_2O	18	(Barley base
Potassium Iodide	2	ESCA No. 2)
Zinc Oxide	20	
AD_3E (50,000 i.u.A p.g.; 10,000 i.u.D p.g.; 5.25 i.u.E p.g.)		
Analytical Data		
Oil	3.0	
Protein	13.5	
Fibre	15.7	
Theoretical (Bulletin 48 values)		
Starch Equivalent	46.80	
Starch Equivalent in dry matter	53.50	
ME (R)	8.85	

Source: Agric. Biochem. Dept., Bush Estate

Urine collection was done in plastic bottles situated beneath the metabolism crates. Sufficient 25% sulphuric acid was added daily to the bulked urine kept in large covered plastic dustbins, in order to reduce the pH to 2-3 (as shown by indicator paper). The urine was acidified to prevent loss of ammonia.

Refusals were collected each morning and dried overnight in an oven at 100°C, and dry matter values subsequently calculated for loss of volatile fatty acids (Dewar and McDonald, 1961). Samples of each dried silage were milked and then asked to determine by difference the organic matter content. This was repeated for the faeces.

Energy balance figures were calculated from the results of gross energy determinations made on samples of diet, faeces and urine, using and Adiabatic bomb calorimeter. The gross energy of the faeces was also calculated using a fresh sample which was first macerated. The metabolisable energy of the diets were calculated by subtraction of the gross energies in the faeces, urine and methane from the gross energy in the diet. Methane was estimated using the equation of Blaxter and Clapperton (1965): $CH_4 = 3.67 + 0.06 \times D$ for energy 100 kg of food fed where D = % digestibility of energy. Nitrogen determinations were carried out on the fresh faeces, urine and diets using the Kjeldahl method (Kjeldahl, 1883). From these, values for nitrogen (N) and crude protein (CP) were determined (CP = N x 6.25).

7.4 Experiment 3

In sacco degradability of silage dry matter and protein using fistulated sheep

Diets. The diets were the same as those described in Experiments 1 and 2, and were fed ad lib. A third silage was examined in this experiment. This was the untreated control ryegrass silage made from similar material as the other silages.

Bags. The bags used were made with material woven from artificial fibre of high tensile strength and chemical resistance, but low water absorbtion (Brady, 1971, cited by Proven, 1980). This material was HSO 13 filter cloth of approximately 47 μ pore diameter - supplied by Henry Simon at Stockport. Bag size was approximately 200 x 150 mm. The bags were double seamed with round corners.

Animals. Nine adult male sheep (numbers 1190, 135, 550, 91, 728, 1280, 487, 218, 353), fistulated at the rumen and fitted with small diameter cannulae, were used in the experiment. The animals had been fistulated for about 4-5 years, and weighed 60-70 kg at the time of the experiment. The nine animals were randomly allocated to three groups of three animals. After an introductory period of about two weeks, each group were fed the same diet throughout the experiment. Group 1 was fed C.R.D., Group 2 was fed formaldehyde-treated silage and Group 3 was fed formic acid-treated silage.

Procedure. On each of the first days of each test period, 45 bags (15 containing control untreated silage, 15 containing formaldehyde-treated silage and 15 containing formic acid-treated silage) were prepared. 15 g of the appropriate silage was accurately weighed into each of the pre-weighed white artificial fibre Dacron bags, which were numbered with black indelible ink.

The bags were tied tightly at the neck using 10 kg breaking strain nylon fishing line. Using the ends of the fishing line, which had been deliberately left long, five Dacron bags (containing the weighed appropriate silages) were securely tied in numerical order onto successive loops on a piece of insulating cable about 45-50 cm long, which was connected at one end to a screw cap. The procedure was repeated with other cables using different silages

with each cable. Thus, at the end of tying, there were nine cables each with fine Dacron bags fitted on it in numerical sequence.

After ½ hour of soaking in cold water, each cable with bag was introduced into the rumen liquor and pushed down until all the artificial fibre bags were totally submerged, and the screwcap cover was put on in order to prevent leakage of digesta and/or rumen liquor. The introduction of each cable was done at 09.00 hours.

One hour after insertion, the screwcaps were removed and the cables extracted from the rumen of all nine animals. One bag, the nearest to the screwcap, was detached from each cable and the cables and screwcaps were replaced. The removed bags were then subjected to a standard washing procedure. These bags were washed for twenty minutes under cold running water and the contents were squeezed and manipulated until the draining water ran clear. The bags were then placed on a drying tray in a 60°C forced draught oven overnight and removed and stored in a dessicator until ready for reweighing. The procedure was repeated at 4, 12, 24, and 48 hours after insertion time.

The washed dried bags, complete with their contents were reweighed, accurately, after removal of the piece of nylon fishing line and DM losses were calculated. Part of the dried samples were weighed out accurately into small weighed polythene bags and put into the Kjeldahl flasks for determining N (Horwitz, 1955). The remainder of the dried samples were stored in a refridgerator.

Since the weight and TN concentration of the material inserted and material removed were known (expressed as % of TN originally present), then the TN loss could be determined.

Statistics. The basic statistical design of the experiment is shown in Figure 2.

FIGURE 2

Basic statistical design of the artificial fibre bag experiment (Experiment 3)

	Pellets (C.R.D.)	Silage F (Formalin)	Silage S (Formic)
DIETS 9 sheep	3 sheep	3 sheep	3 sheep
BAGS			
PERIOD 1	Control Silage	Silage S (Formalin)	Silage F (Formic)
PERIOD 2	Silage F Formic	Control	Formalin
PERIOD 3	Silage S Formalin	Formic	Control

Mean % TN and DM disappearance for the nine animals were determined for each treatment/incubation time combination. The significance of inter-treatment dry matter and TN disappearance differences was assessed using the analysis of variance methods (Rothamsted Experimental Station, 1977).

Chemical analysis. Analyses of all diets and samples were provided by the Agricultural Biochemistry Department and the Central Analytical Laboratory, Edinburgh School of Agriculture.

FIGURE 3

The relationship between % DM loss, and intra-ruminal incubation period for control silage when diets of C.R.D. □ ; Formic acid silage △ and Formaldehyde-treated silage ● are fed.

CONTROL SILAGE IN THE BAG

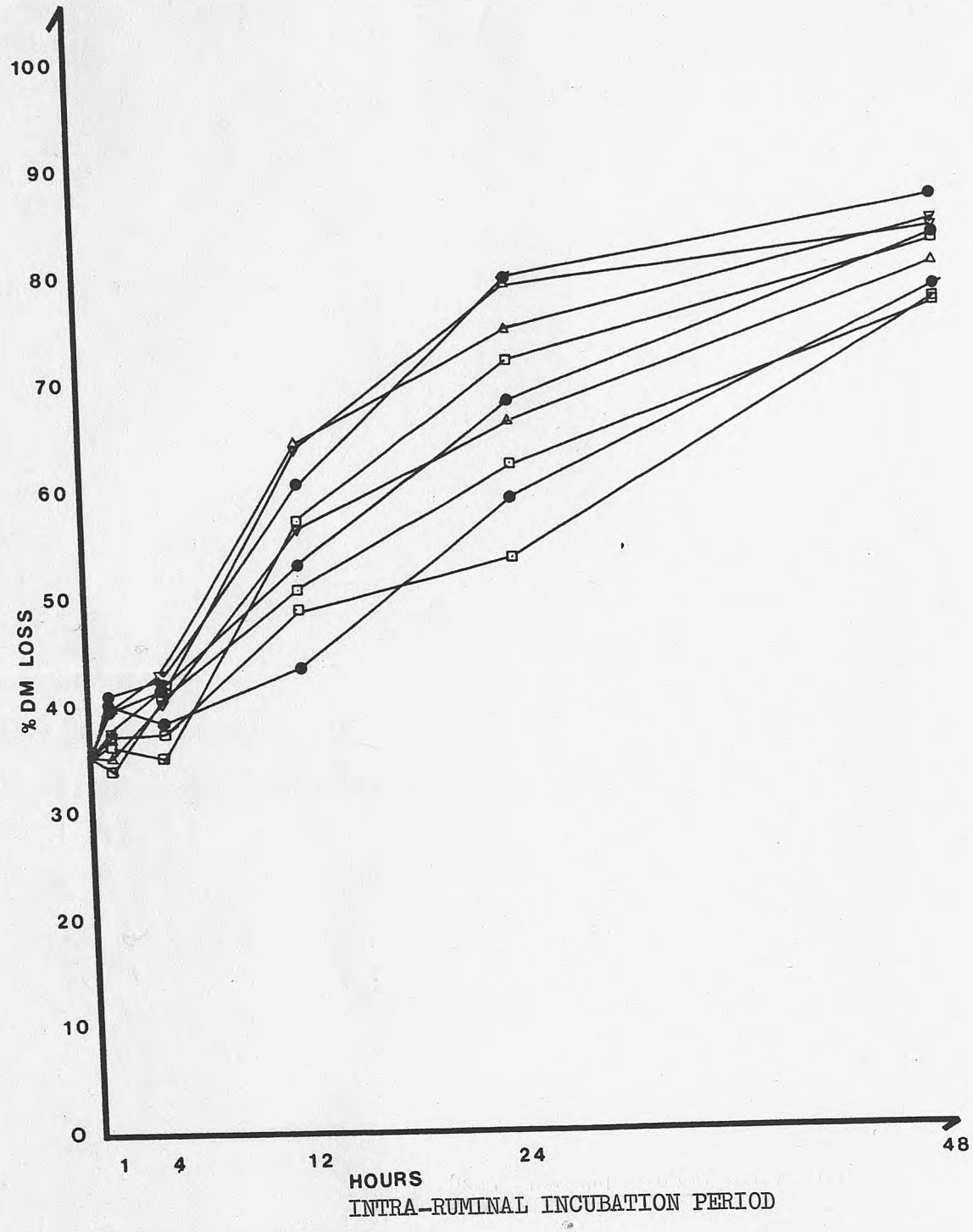


FIGURE 4

The relationship between % N loss and intra-ruminal incubation period for control silage when diets of C.R.D. □ ; Formic acid silage △ and Formaldehyde-treated silage ● are fed.

CONTROL SILAGE IN THE BAG

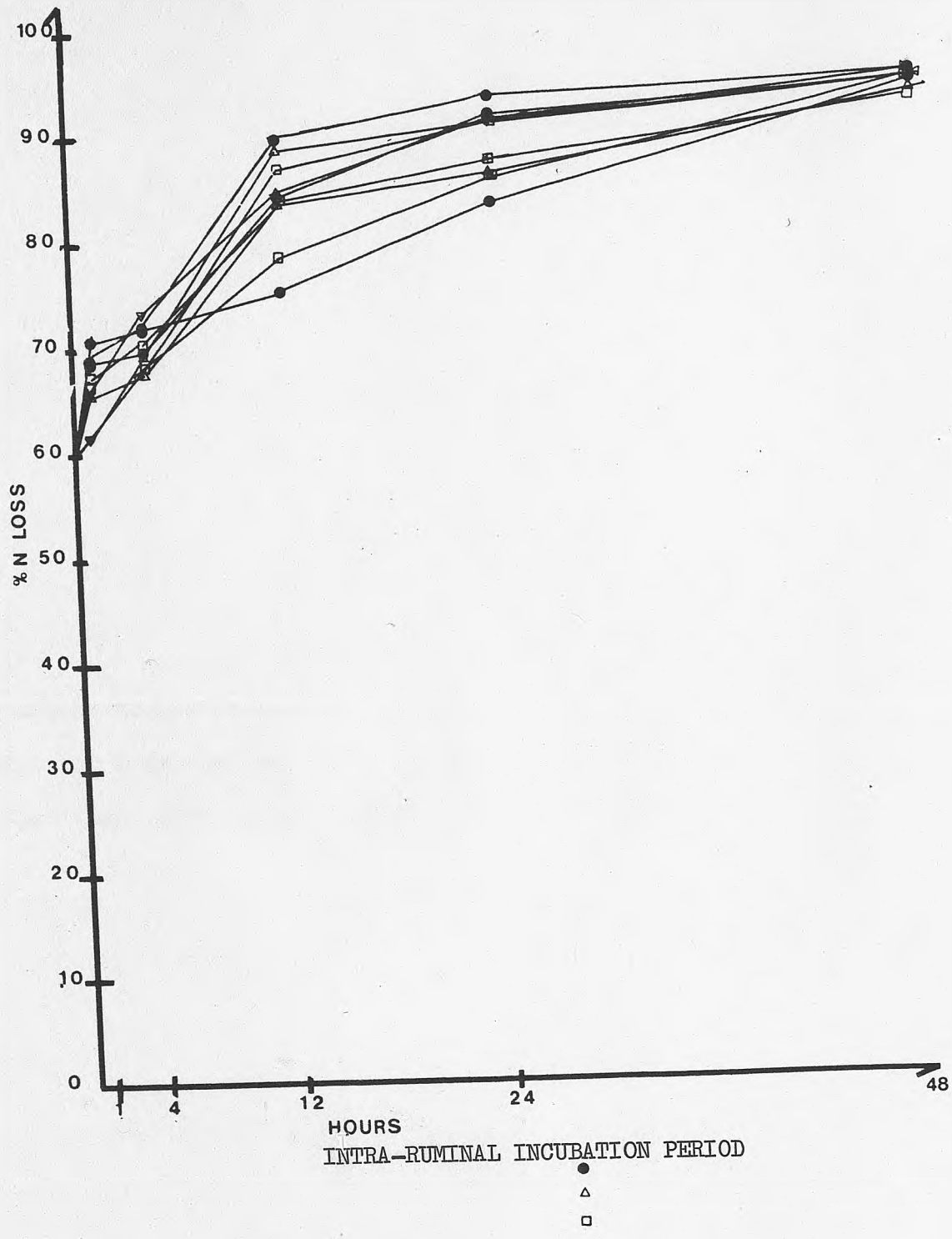


FIGURE 5

The relationship between % DM loss and intra-ruminal incubation period for formic silage when diets of C.R.D. □ ; Formic acid silage △ and Formaldehyde-treated silage ● are fed.

FORMIC SILAGE IN THE BAG

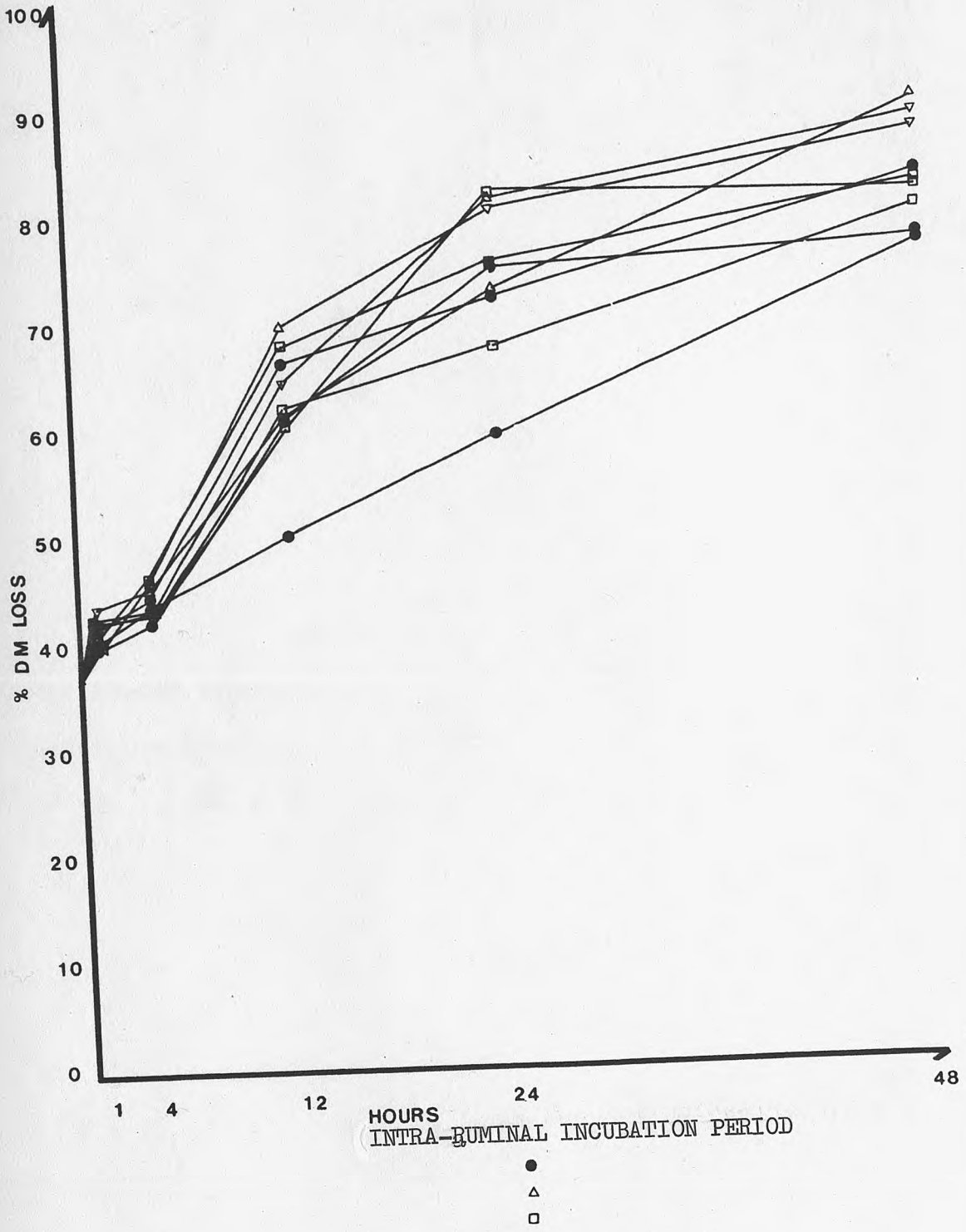
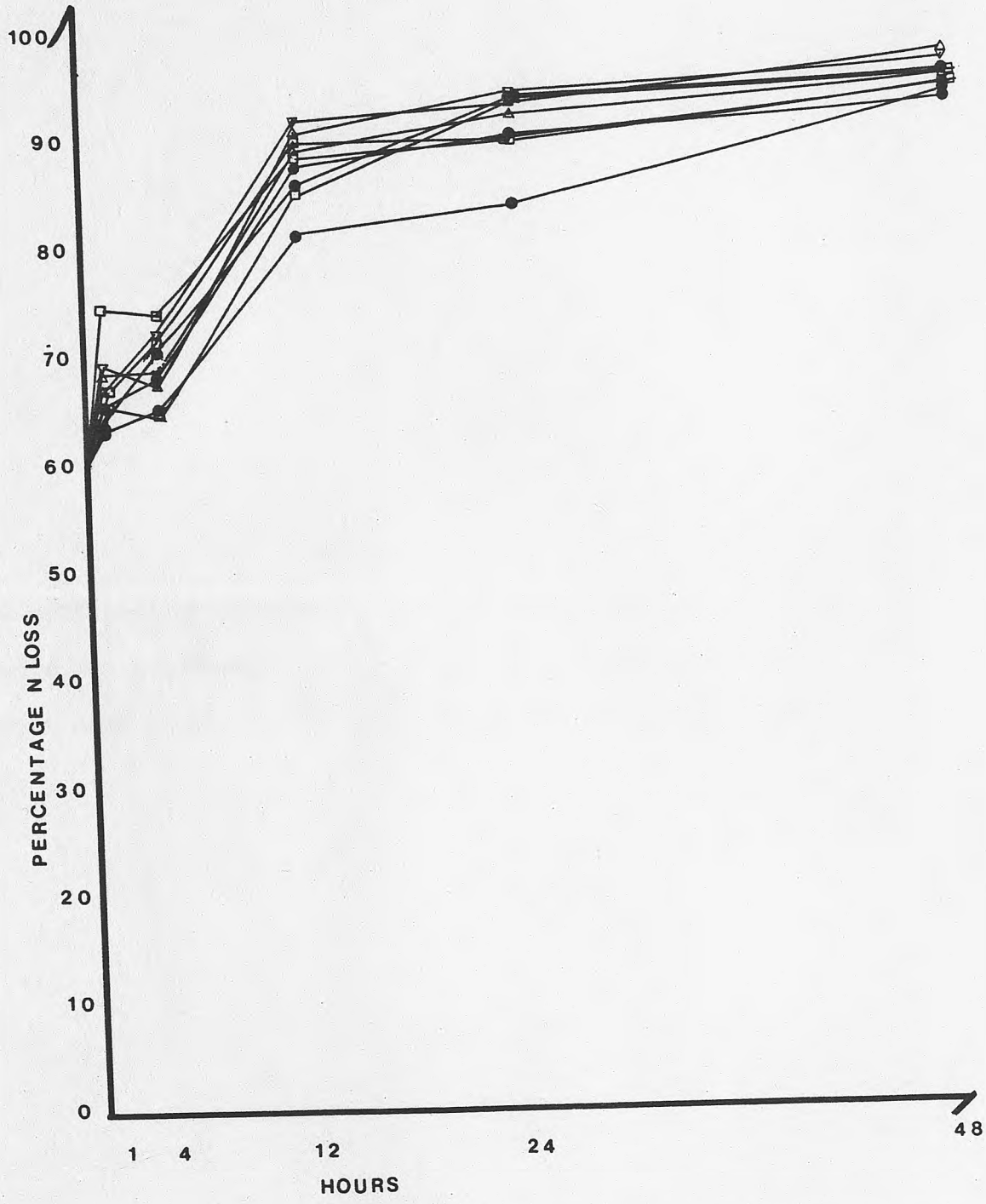


FIGURE 6

The relationship between % N loss and intra-ruminal incubation period for formic silage when diets of C.R.D. □ ; Formic acid silage △ and Formaldehyde-treated silage ● are fed.

FORMIC SILAGE IN THE BAG

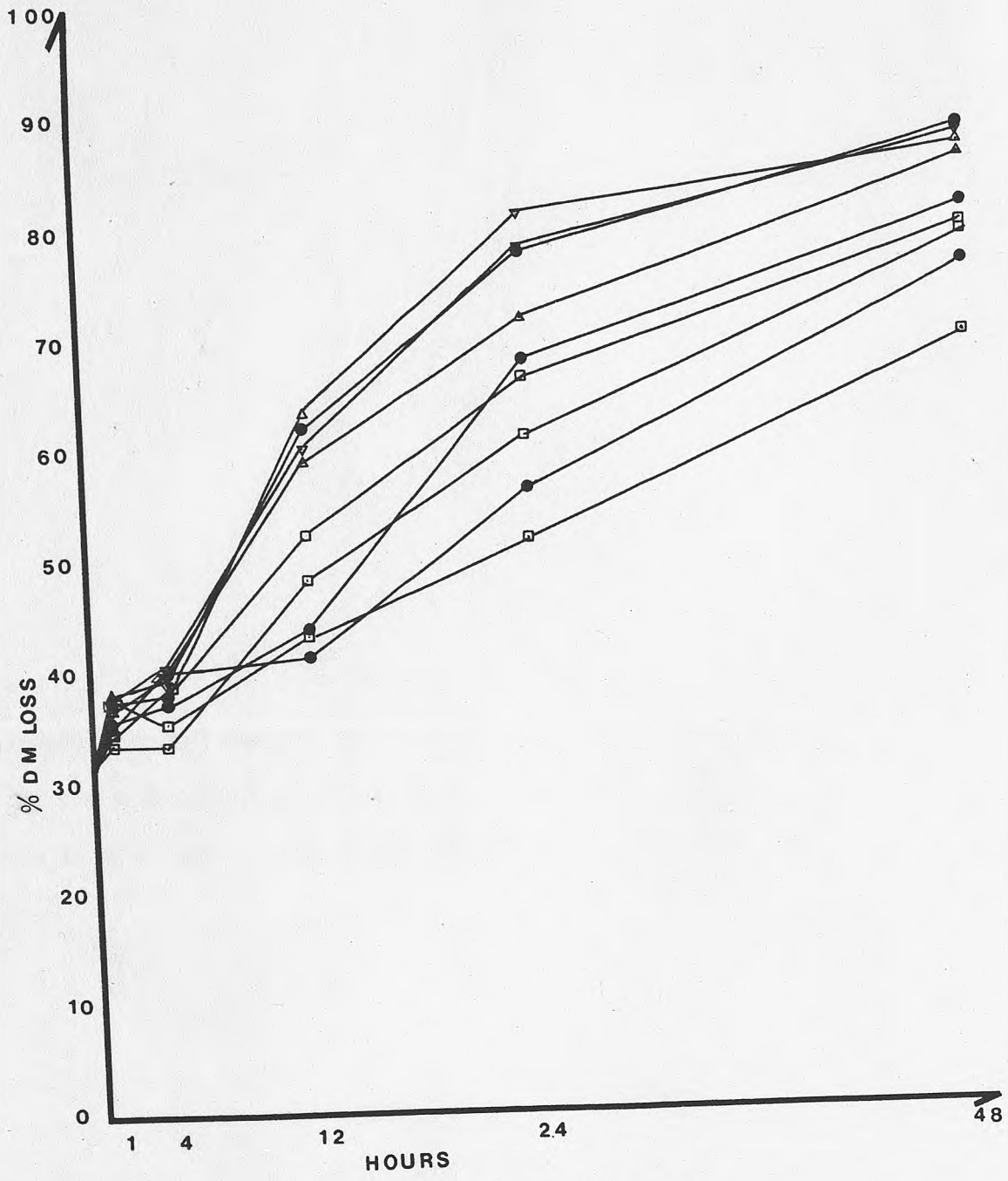


INTRA-RUMINAL INCUBATION PERIOD

FIGURE 7

The relationship between % DM loss and intra-ruminal incubation period for formaldehyde silage when diets of C.R.D. □ ; Formic acid silage △ and Formaldehyde-treated silage ● are fed.

FORMALIN SILAGE IN BAG

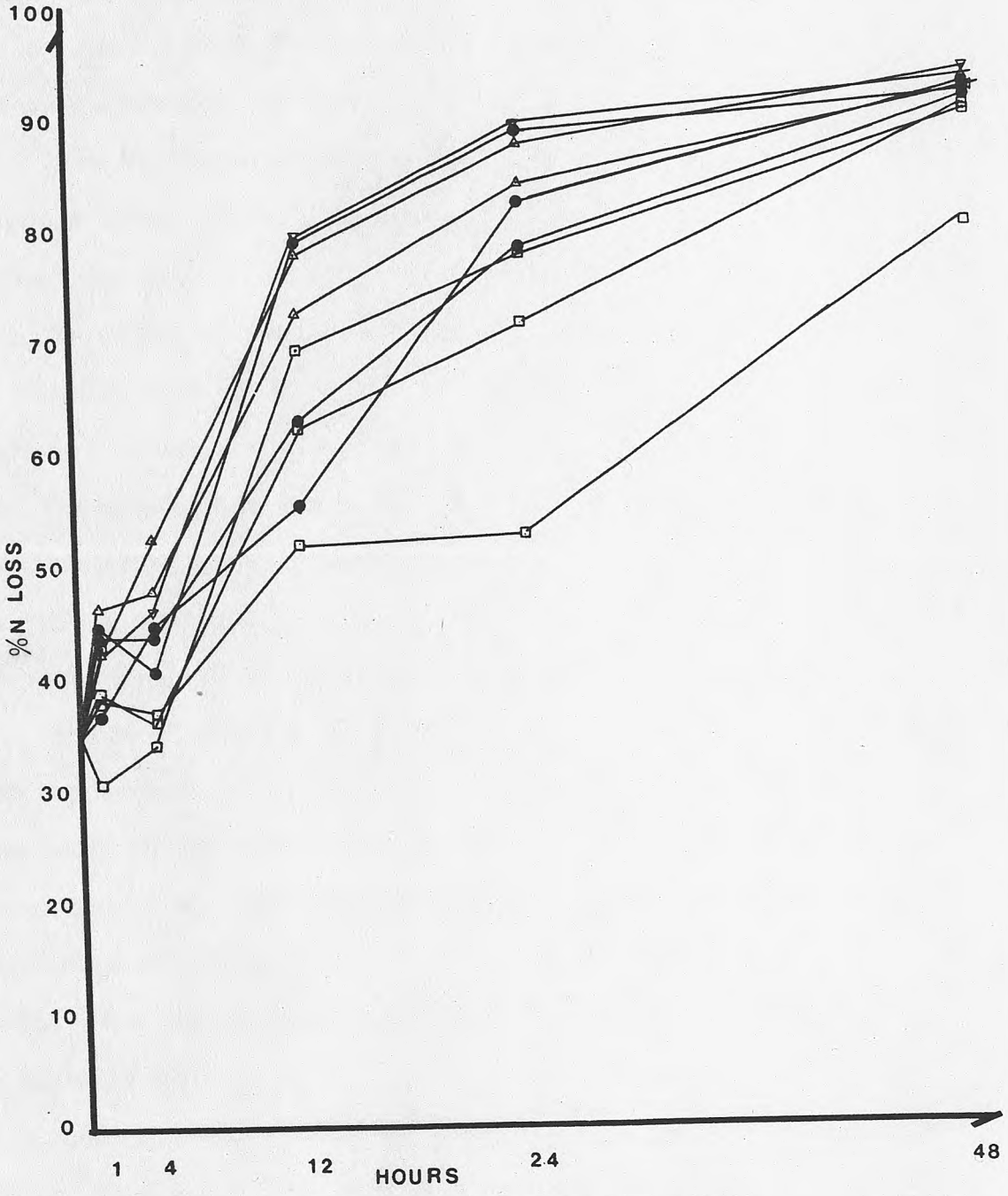


INTRA-RUMINAL INCUBATION PERIOD

FIGURE 8

The relationship between % N loss and intra-ruminal incubation period for formaldehyde silage when diets of C.R.D. □ ; Formic acid silage △ and Formaldehyde-treated silage ● are fed.

FORMALIN SILAGE IN THE BAG



INTRA-RUMINAL INCUBATION PERIOD

RESULTS

EXPERIMENT 1: Intake trial

The composition of the diets is shown in Table 12. The silages were well preserved with relatively low ammonia-nitrogen (NH_3-N) contents. The pH value of the formaldehyde-treated silage (F) was higher than the pH value of the formic acid-treated silage (S). The WSC contents were higher in the additive-treated silages, compared to the untreated silage of Ferrier (1982). The values reflect the inhibitory effect of the additives on lactic acid bacteria, and, the values of lactic acid indicate a greater inhibitory effect of formalin than formic acid. The protein-N level was considerably higher in silage F than silage S.

The C.R.D. had about four times the dry matter content of the two treated silages, but had a slightly lower protein content, and a lower G.E. content than these silages, which in turn had lower G.E. contents than the untreated control silage of Ferrier (1982).

The data in Table 14 summarises the results of the intake trial. The Dry Matter Intakes (DMI) of all diets were relatively high and the daily DMI of the C.R.D. (46.1 g kg^{-1}) was significantly greater than that of the formaldehyde-treated silage, F,(22.29) or the formic acid-treated silage, S (23.20) at the 0.1% significance level. However, there was no significant difference between the silages F and S in daily DMI.

Lamb liveweight gain was significantly greater ($P < 0.001$) on the C.R.D. (363 g day^{-1}) than on silage F (153) or silage S (101), when adjusted for initial liveweight. There was no significant difference between silage F and silage S in promoting liveweight gain. However, silage S (115 g gain kg^{-1} DM) had a significantly lower feed efficiency

TABLE 14

Summary of results of intake trial (Expt. 1)

	DIETS				Interpretation
	C.R.D.	Formalin (F) silage	Formic acid (S) silage	s.e. of diff. (21 df)	
Dry Matter Intake (g/day)	1743	800	836	65.9	C.R.D. > Formalin Formic
" (g/day/kg)	46.10	22.29	23.20	1.367	(P <0.001)
" (g/day/kg$^{0.75}$)	114.24	54.54	56.82	3.479	
Mean initial liveweight (kg)	34.34	34.46	35.07		
Liveweight gain (g/day)	366	155	97	48.3	C.R.D. > others (P <0.001)
Feed efficiency (g gain/kg DMI)	205	195	110	39.3	Formic < others (P <0.05)
ME intake (MJ/day)	15.27	7.59	9.20 (estimated)		
Adjusted for covariate - Initial Weight (not significant)					
Liveweight gain (g/day)	363	153	101	49.3	As above
Feed efficiency (g gain/kg DMI)	202	193	115	39.5	

	DIET X WEEK				
		Week 1	Week 2	Week 3	s.e. of diff within diets (42 df)
Dry Matter Intake (g/day)					Wk 1 <Wk2 (P <0.001) Wk 2 <Wk3 (P <0.0001)
DIET	C.R.D.	1503	1799	1927	46.1
	Formalin	794	787	818	No differences significant
	Formic	823	818	865	
	s.e. of diff within weeks (35 df)	75.9			
		C.R.D. > Formalin Formic in each week	(P <0.001)		

The untreated silage of Ferrier (1982) fed to lambs over 5 weeks had DMI of 17.82 g/day/kg LN or 45.21 g/day/kg $LW^{0.75}$; Liveweight gain of 69 g/day, and feed efficiency of 93 g LWG/kg DMI.

($P < 0.05$) than C.R.D. (202) or silage F (193). The latter two diets had similar feed efficiencies.

The dry matter intakes of all diets increased from week 1 to week 3, but this increase was due almost entirely to the C.R.D. Diet x week interactions were significant only for C.R.D. Dry matter intakes of C.R.D. in week 1 was significantly less ($P < 0.001$) than in week 2, and similarly, DMI for C.R.D. in week 2 was significantly less ($P < 0.001$) for week 3. There were no significant differences between weeks in DMI for silage F and silage S. Within weeks, the C.R.D. dry matter intake was significantly greater ($P < 0.001$) than that of silage F and silage S. Within weeks, there was no significant difference in DMI between silage F and silage S.

EXPERIMENT 2: Metabolism trial

The composition of diets used in this experiment and the results of the metabolism trial are shown in Table 15.

Intake data in the digestibility trial confirm the findings of the intake trial i.e. the DMI of C.R.D. was significantly greater than that of the two silages ($P < 0.001$) and there was no significant difference in intake between silage F and silage S.

With regard to apparent digestibility coefficients, silage F had greater DM and OM digestibility than C.R.D. ($P < 0.01$), and greater energy digestibility than C.R.D. ($P < 0.05$). However silage F had significantly lower ($P < 0.001$) crude protein digestibility than the C.R.D., mainly because of formaldehyde treatment, and its protecting effect on protein (discussed in Section 4). The C.R.D. had significantly greater content ($P < 0.01$) of digestible crude protein in the dry matter than silage F, but had significantly lower digestible organic matter ($P < 0.001$) in the dry matter. Silage F had significantly

TABLE 15

Composition of diets and summary of results of digestibility trial (Expt. 2)

Parameter	DIET mean (n=4) C.R.D. ± Se	mean (n=4) Formaldehyde silage ± Se	mean (n=4) Formic silage ± Se	Se_m	Comparison with Ferrier's 1982 untreated silage
DM g kg^{-1}	869	204	213	-	207
OM g kg^{-1} DM	900	916	918	-	208
N g kg^{-1} DM	22.0	26.4	26.1	-	26.0
GE MJ kg^{-1} DM	17.1	17.9	18.2	-	19.2
INTAKE g kg^{-1} $LW^{0.73}$	37.0±1.88	24.2±1.12	26.0±0.66	±1.321	19.3
" g kg^{-1} $LW^{0.73}$	96.8±3.68	62.9±2.17	66.9±1.44	±2.60	n.d.
	APPARENT DIGESTIBILITY				
(1) DM digestibility	0.627±0.006	0.667±0.009	0.739±0.007	±0.0073	0.726
(2) OM "	0.638±0.006	0.682±0.009	0.760±0.006	±0.0069	0.758
(3) CP "	0.665±0.009	0.592±0.008	0.718±0.008	±0.0083	0.752
(4) E "	0.608±0.008	0.636±0.010	0.720±0.007	±0.0084	0.728
	DIGESTIBLE NUTRIENTS				
(5) Dig. OM in DM g kg^{-1}	573±5.12	625±7.93	698±5.45	±6.2922	688
(6) Dig. CP "	91.2±1.17	97.9±1.30	117.1±1.29	±1.2547	122.2
(7) Dig. E in DM (MJ kg^{-1})	10.4±0.13	11.4±0.18	13.1±0.13	±0.1509	13.98
(8) N retention (g day^{-1})	11.97±0.311	6.50±0.782	7.29±0.438	±0.5477	5.05
(9) ME (MJ kg^{-1} DM)	8.76±0.128	9.49±0.151	11.00±0.119	±0.1867	11.40
ME/DE	0.842	0.834	0.839	n.d.	0.815

greater digestible energy in the dry matter than C.R.D. ($P < 0.01$) Silage S was significantly greater than both C.R.D. and silage F on all parameters ($P < 0.001$) except nitrogen retention. C.R.D. had significantly greater nitrogen retention (11.97 g day^{-1}) than silage S (7.29) and silage F (6.50) at the 0.1% significance level, the two silages having no significant difference in nitrogen retention.

EXPERIMENT 3: In sacco silage degradability in the rumen of sheep

The results of this experiment are given for dry matter degradability (expressed as %DM loss) in Tables 16-20, and for nitrogen degradability (expressed as %N loss) in Tables 21-26, and the important features of the silages used in Table 13a.

Table 16 shows % dry matter loss after 4 hours incubation of bags in the rumen. The effect of diet was not significant and there was no diet x bag interaction+. However, there was a significant bag effect* ($P < 0.001$), with the highest mean value of 44.68% with silage S and the lowest of 38.34 with silage F.

Table 17 shows % dry matter loss after 12 hours incubation of bags in the rumen. The effect of diet was not significant and there was no diet x bag interaction. However, there was a significant bag effect ($P < 0.001$) with the highest mean value of 63.16% with silage S and the lowest of 53.12% with silage F.

Table 18 shows the per hour % dry matter loss between 4 and 12 hours incubation of bags in the rumen. There was no significant effect of diet, bags, nor diet x bag interaction. The mean per hour loss was greatest on formic silage (2.31) and the lowest was 1.76 on control silage.

Table 19 shows % DM loss after 24 hours incubation of bags in the

+diet x bag interaction refers to effects on DM or N loss caused by a combination of both.

*bag effect refers to the effect of the silages themselves contained in the bags on loss of N or DM

TABLE 16

% Dry matter loss after 4 hours from rumen incubated artificial fibre bags containing grass silage in sheep fed different diets.

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	38.63	44.77	36.33	39.91)	
Formic silage	42.27	45.20	39.37	42.28)	0.883
Formalin silage	41.33	44.07	39.33	41.58)	
MEAN	40.74	44.68	38.34	41.26	
SE diff	1.022				

(See Appendix 2 for Analysis of Variance)

TABLE 17

% Dry matter loss after 12 hours from rumen incubated artificial fibre bags containing silage in sheep fed different diets

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	52.73	64.03	48.60	55.12)	
Formic silage	62.20	65.70	61.30	63.07)	4.555
Formalin silage	52.23	59.73	49.47	53.81)	
MEAN	55.72	63.16	53.12	57.33	
SE diff	1.784				

(See Appendix 3 for Analysis of Variance)

TABLE 18

Per hour Dry matter loss % between 4-12 hours of rumen incubated artificial fibre bags containing silage in sheep fed different diets

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	1.76	2.41	1.53	1.90)	
Formic silage	2.07	2.56	2.74	2.46)	0.484
Formalin silage	1.45	1.96	1.27	1.56)	
MEAN	1.76	2.31	1.85	1.97	
SE diff	0.278				

(See Appendix 4 for Analysis of Variance)

rumen. The effect of diet and diet x bag interaction was not significant. However, there was a significant bag effect ($P < 0.05$), with the highest mean value of 74.44 on silage S and the lowest of 68.51 on silage F.

Table 20 shows the per hour % DM loss between 12-24 hours incubation in the rumen. The effect of diet, bags and diet x bag interaction is not significant. The highest mean value is that of silage F (1.282) and the lowest on formic acid (1.007).

Table 21 shows the % N loss after 4 hours incubation of bags in the rumen. There was no significant diet effect, however there was significant bag effect ($P < 0.001$) and diet x bag interaction ($P < 0.01$). The lowest mean value of N loss was 43.20 for the silage F, and the values for silage F and control silage were very similar.

Table 22 shows % N loss after 12 hours incubation of bags in the rumen. Only bag effect was significant ($P < 0.001$). The highest mean value of N loss was 88.48 for silage S and the lowest 68.41 for silage F.

Table 23 shows the per hour % N loss between 4-12 hours incubation of bags in the rumen. Only bag effect was significant ($P < 0.01$). The highest mean value of 3.15 was on silage F and the lowest 1.74 on control silage.

Table 24 shows the % N loss after 24 hours incubation of bags in the rumen. Diet effect was not signiciant, but there was a significant bag effect ($P < 0.001$) and a significant diet x bag interaction ($P < 0.05$). The highest mean value of N loss was 91.60% for silage S and the lowest 79.82 for silage F.

Table 25 shows the per hour % N loss between 12-24 hours incubation of bags in the rumen. Only bag effect was significant ($P < 0.01$).

TABLE 19

Percentage DM loss at 24 hours in rumen incubated artificial fibre bags containing grass silage in sheep fed different diets.

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	62.83	75.17	60.23	66.08)	
Formic silage	73.70	78.87	77.43	76.67)	5.709
Formalin silage	69.23	69.30	67.87	68.80)	
MEAN	68.59	74.44	68.51	70.51	
SE diff	2.322				

(See Appendix 5 for Analysis of Variance)

TABLE 20

Per hour % DM loss between 12-24 hours in rumen incubated artificial fibre bags containing silage in sheep fed different diets

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	0.842	0.928	0.969	0.913)	
Formic silage	1.103	1.097	1.344	1.181)	0.494
Formalin silage	1.417	0.994	1.534	1.315)	
MEAN	1.120	1.007	1.282	1.136	
SE diff	0.186				

(See Appendix 6 for Analysis of Variance)

TABLE 21

% Nitrogen (N) loss after 4 hours in rumen incubated artificial fibre bags containing silage in sheep fed different diets

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	68.67	70.73	36.57	58.66)	
Formic silage	70.23	69.73	49.43	63.13)	1.50
Formalin silage	70.87	68.53	43.60	61.00)	
MEAN	69.92	69.67	43.20	60.93	
SE diff	1.175				

(See Appendix 9 for Analysis of Variance)

TABLE 22

% N Loss after 12 hours in rumen incubated artificial fibre bags containing silage in sheep fed different diets

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	83.20	89.03	61.83	78.02)	
Formic silage	87.53	91.03	77.07	85.21)	4.101
Formalin silage	83.03	85.37	66.33	78.24)	
MEAN	84.59	88.48	68.41	80.49	
SE diff	1.974				

(See Appendix 8 for Analysis of Variance)

TABLE 23

Per hour % N loss between 4-12 hours in rumen incubated artificial fibre bags containing silage in sheep fed different diets

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	1.55	2.06	3.16	2.25)	
Formic silage	2.16	2.39	3.45	2.67)	0.531
Formalin silage	1.52	2.11	2.84	2.16)	
MEAN	1.74	2.19	3.15	2.36	
SE diff	0.338				

(See Appendix 9 for Analysis of Variance)

TABLE 24

% N loss at 24 hours in rumen incubated artificial fibre bags containing silage in sheep fed different diets.

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	88.27	91.27	68.00	82.51)	
Formic silage	89.70	93.77	87.60	90.36)	3.475
Formalin silage	89.67	89.77	83.87	87.77)	
MEAN	89.21	91.60	79.82	86.88	
SE diff	2.182				

(See Appendix 10 for Analysis of Variance)

TABLE 25

Per hour % N Loss between 12-24 hours in rumen incubated artificial fibre bags containing silage in sheep fed different diets.

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	0.422	0.264	0.514	.400)	
Formic silage	0.181	0.228	0.878	.429)	0.1657
Formalin silage	0.553	0.367	1.461	.793)	
MEAN	0.385	0.286	0.951	.541	
SE diff	0.1477				

(See Appendix 11 for Analysis of Variance)

TABLE 26

% N loss at 90% DM disappearance in rumen incubated artificial fibre bags containing silage in sheep fed different diets

BAGS	CONTROL	FORMIC SILAGE	FORMALIN SILAGE	MEAN	SE diff
DIET					
C.R.D.	89.00	89.33	70.67	83.00)	
Formic silage	88.33	90.33	76.00	84.89)	1.19
Formalin silage	89.00	88.67	77.33	85.00)	
MEAN	88.78	89.44	74.67	84.30	
SE diff	0.979				

(See Appendix 12 for Analysis of Variance)

The highest mean value was 0.951 on silage F and the lowest 0.286 on silage S.

Table 26 shows the % N loss at the time when 90% DM has disappeared from bags incubated in the rumen. There was a significant bag effect ($P < 0.001$) and diet x bag interaction ($P < 0.05$). The mean values were 88.78%, 89.44% and 74.67% for control silage, silage S and silage F respectively.

Figures 3-8 show graphically the degradability of DM and N over the intra minimal incubation periods for each silage in the bags when the different diets are fed.

DISCUSSION

The composition of silages and C.R.D. used in the intake trial is shown in Table 12 and the manufacturer's description of the C.R.D. in Table 13b. By inspection, we can reasonably compare the values in these tables with that of the untreated control silage of Ferrier (1982).

According to the findings of Barry et al, (1978) and Carpintero et al, (1979), the high level of formic acid (6.0 lt^{-1}) used in silage S would have been expected to restrict the production of fermentation acids and to conserve most of the soluble carbohydrates in the silage, especially if the WSC content of the grass was high. In fact, Table 12 shows only a moderate level of conservation of WSC and a slight lowering of the concentration of fermentation acid, in the form of lactic acid. This may be due to the relatively low DM content of the grass (176 g kg^{-1}), and it is possible that some formic acid was lost in the effluent, as is usually the case with low DM grasses, or lost by volatilisation at application. The high level of formic acid used would have been expected to conserve protein-N to a greater degree than the value in Table 12 (466 g kg^{-1}TN), when compared with the untreated control silage (452 g kg^{-1}TN). The level of NH_3-N for formic acid silage (56 g kg^{-1}TN) is also higher than the value expected with such a high level of application, when compared with the untreated control silage (75 g kg^{-1}TN). The GE content of the untreated control, compared to silage S is higher, but this may be due to the high GE of ethanol and other products of fermentation.

With regard to the formaldehyde silage (F), the level of application was 7.5 lt^{-1} (90 g kg^{-1}CP) or twice the recommended level.

At this level, in accordance with the findings of Barry et al (1978) and Wilkins et al (1974), it was expected that the pH value would be fairly high, most of the WSC would be conserved, lactic acid content would be very low, NH_3-N level would be relatively low and protein-N level would be high due to protein protection. In fact, the pH value was 4.26. The WSC was conserved to a greater extent than with formic acid treatment, but was still only moderate. The lactic acid content (62 g kg^{-1}DM) was higher than expected (c.20), but the NH_3-N level (39 g kg^{-1}TN) was low. The protein-N level (644 g kg^{-1}TN) was very high compared to the untreated control, as expected. Thus the extent of fermentation restriction obtained from the use of high levels of formic acid and formaldehyde may be less than the desired extent of restriction.

The C.R.D. had about a third of its composition as barley straw (roughage), and about a third as barley and molasses (fermentable carbohydrates). The overall fibre content was about 16% (Table 13b).

In the intake trial, the main finding was that the DMI of all three diets were fairly high, but that the daily DMI of the C.R.D. was significantly greater than the two silage diets ($p < 0.001$), and that there was no significant difference in DMI between silage S and silage F. This finding was later substantiated in the digestibility trial. The high DMI of the C.R.D. was expected because, although the C.R.D. is 30% roughage, the remaining 70% is chiefly concentrates, which would be easily digested in the rumen; hence the rate of passage of digesta may be quicker and realimentation could occur sooner (Rohr, 1979), with less latent time between rumination. The high moisture content of the silages probably meant that less DM could be eaten for a given gut capacity than the C.R.D. This conflicts with

the findings of Thomas et al, (1961) and Clancy et al, (1977) that water intake per se has little influence on silage intake, but may give credence to the finding of Dermarquilly and Dulphy (1977) that silages containing long particles are ingested very slowly when wet, due to chewing difficulty. Another reason for the high DMI of C.R.D. may be that it was fed as pellets, and pelleting has been shown to improve intake on forage diets (Wilkinson, 1978), though it may not necessarily improve efficiency of feed utilization.

With regard to liveweight gain the C.R.D. promoted significantly greater gains (363 g day^{-1}) than the two silages (153 and 101). The difference between the two silages in liveweight gain promotion was not significant. The high concentrate content of the C.R.D. and its relatively high content of soluble carbohydrate and good quality crude protein, (some of which may be undegraded in the rumen), in addition to the higher intake, was responsible for the better gains on C.R.D. compared with the silages. Also, increased gutfill with weekly increasing C.R.D. intakes, may be partly responsible for the higher final weight of animals fed C.R.D., compared to those with steady silage intake. When compared to the untreated silage of Ferrier (1982), the two treated silages both had higher DM intakes and higher liveweight gains than the untreated silage.

The efficiencies of utilisation are similar in the C.R.D. and silage F, both of which are significantly higher than that of silage S, and this is an important finding in this experiment, especially since silage S has a higher ME intake than silage F. This leads us to suspect that if the digestibility of nutrients of silage S is similar to or greater than that of silage F, then the fundamental difference between the two treated silages must be in the efficiency of utilisation of digested nutrients. One factor which may have influenced intake of silage F is the level of free formaldehyde,

because at high rates of application (as in this experiment), free formaldehyde in the rumen can severely depress intake, possibly by affecting the function of microbes (Wilkinson et al, 1976; Kaiser, England and Gibbs, 1978). The protection of protein could also depress microbial growth by reducing the supply of easily fermentable N to the microbes to sub-optimum levels (Beever et al, 1977). Furthermore, the high level of formaldehyde may have caused some 'overprotection' of protein, and intake as well as liveweight gain may be affected, as such protein may not even be digested in the small intestine.

With regard to the digestibility trial, the principal findings were the preservation of the apparent digestibility of most nutrients by the addition of formic acid at this high level, and a depression in the apparent digestibility of OM, DM, energy and particularly crude protein, by the high level of formaldehyde added, when compared to the untreated silage of Ferrier (1982).

Waldo (1978), cited by McDonald (1981), carried out a comprehensive survey of the literature dealing with the effects of formic acid treatment. He found that for direct-out silages, formic acid treatment increased the apparent digestibility of the organic matter by about 7%, of digestible energy by about 2%, improved liveweight gains on average by 71% and milk production by 2%, when compared to untreated controls.

Compared to Ferrier's untreated control silage, the OM digestibility determined in experiment 2 improved by a negligible amount, the digestible energy fell by 1%, CP digestibility fell by 4.5%, the liveweight gain (from experiment 1) improved by about 46% and efficiency of feed conversion by about 24%. This data comes from different groups

of animals and a comparison may not be justified. Some amino acids may have been protected from deamination and, in accordance with the findings of Carpintero et al (1979) and Wilson and Wilkins (1973), and may have contributed to the efficiency of silage S. It is possible that the formic acid may have improved silage N-utilisation, although it did not improve digestibility of silage markedly. This supports the evidence of Dermarquilly and Dulphy, (1977).

Formaldehyde has been shown to decrease intake and digestibility of silages markedly when fed to sheep (McDonald, 1981). Valentine and Brown (1973) observed significant reduction in in vivo N digestibility, and Wilkins et al (1974) found that high levels of formalin significantly decreased DM and cellulose digestibility, as well as intake. The main effect of formaldehyde was to depress the digestibility of crude protein, so that it was significantly less than that of the other two diets. Comparison with Ferrier's untreated control shows depressions of 21% for crude protein digestibility, 8% for DM digestibility, 10% for OM digestibility and 13% for energy digestibility. Depression in crude protein digestibility was due to protein protection by formaldehyde, whereas that of DM, OM and energy digestibility may be a reflection of CP digestibility, but may also be caused by depressed cellulose digestibility by formaldehyde. In the absence of cellulose data, this cannot be supported with evidence.

An interesting feature of the digestibility trial was that the results tend to strengthen the earlier suspicion that the difference between silage S and silage F is in the utilisation of digested nutrients. The nitrogen retentions from silage F and silage S are similar, in spite of "similar" intakes, lower energy and protein digestibility of silage F relative to silage S and a higher ME

content of silage S.

C.R.D. had significantly higher CP digestibility than silage F and significantly greater N retention than silage F and S, but is lower on all other parameters, probably due to the high content of barley straw of low digestibility. This leads us to the conclusion that higher intake of C.R.D. was chiefly responsible for the higher N retention.

The composition of the samples of silages placed in the artificial fibre bags in Experiment 3 are given in Table 13a. As in earlier experiments, the protein-N content was very much greater on silage F than silage S or control silage.

The results of the in sacco rumen degradability of the silages in sheep fed different diets are shown in Tables 16-25 and Figures 3-8. The limitations of this in sacco technique have been discussed already in Section 6. However, it should be pointed out, that what is actually measured is 'solubilisation' of the constituents of the incubated silages in bags in the rumen, rather than total degradation (Henderson, 1982; personal communication). The results are expressed as %DM loss or%N loss at, between or after chosen periods of time. The periods selected were 1 hour, 4 hours, 12 hours, 24 hours and 48 hours after the incubation of bags, because previous workers have found that most of the DM and nutrients disappear in the first 24 hours of incubation. Proven (1982, personal communication) suggested that the loss of N was very rapid in the first few hours and that measurement should be made at 1 and 4 hours. It was also found that there might be a slight time lag before effective loss of DM or N occurs, similar in concept to the activation of enzymes for reactions in biochemical systems, probably for proper substrate-enzyme interaction and contact. This

may cause 1 hour readings to be very similar on all silages, and similar to the 0 hour values (blank controls) obtained after thorough washing of bag contents in water. This is brought out very clearly in the graphs in Figures 3-8. Thus, in an examination of the results, losses of DM and N after 4,12 and 24 hours were thought to give a better interpretation of events in the rumen. Rate measurements were done by assessing the per hour percentage DM and N losses between 4-12 and 12-24 hours; for it was felt that absolute loss percentages do not tell whether the rate of loss is increasing or decreasing. Loss of N when 90% of DM had disappeared, was determined because by that time most of the N would be lost as in the in vivo situation, and comparison could be done with previous data quoted by the ARC (1980).

If the diet fed is to influence DM or N loss from the silages in bags, it can do so in several ways. It can change the rumen pH; affect or alter the rumen microflora, change the pattern of fermentation or affect the rate of passage of digesta. Since the silages are suspended in bags, it is not expected that rate of passage of digesta would be important. Thus, it appears that diet would only be able to influence DM or N loss by pH changes or alteration of microflora in the rumen. C.R.D. diet appeared most capable of changing the pH and/or microflora in the rumen, because it has 70% concentrates, which could be rapidly digested and rumen pH could fall. The fall in pH could alter the microbial population. Silage F could influence DM or N loss if fed because free formaldehyde depressed microbial activity. However, in all time periods examined, diet effects were not significant, though there were significant diet x bag interactions. For DM loss, no diet x bag interactions were significant. However for N loss, there were significant diet x bag interactions at 4 hours and

24 hours. The nature of the diet x bag interaction at 4 hours may be observed when C.R.D. is fed. It depresses the % N loss of control silage and formalin silage, but formic silage is not affected. Also, when silage F is fed, N loss from formalin silage in the bag is also depressed, possibly due to the influence of free formaldehyde. Control silage N loss is also depressed when silage F is fed. This pattern is repeated at 24 hours, but not at 12 hours.

There were significant bag effects at all periods for both DM and N loss. Bag effect, used in this sense, means that the contents of the bags themselves were responsible for the loss of DM or N recorded, due to their own properties. As far as DM loss is concerned, silage S had greater mean loss values than silage F or control silage, a pattern which was repeated at 4, 12 and 24 hours. With regard to N loss, silage F had definitely much lower loss than silage S or control silage at 4, 12 and 24 hours. Thus formalin treatment depressed silage degradability in terms of N and DM loss, and formic acid treatment appeared to enhance silage degradability.

There were changes in the rate of loss of DM and N by the three silages as the incubation proceeded. The rate of loss of DM between 4-12 hours showed silage S as having the higher rate and silage F, the lowest. But N loss rate over the same period was the reverse. Between 12-24 hours, the DM loss had reversed its earlier pattern and resembles the N loss pattern, with silage F having greater rates than silage S or control. There are two main explanations for this. The first is that the protection of protein waned sometime after 4 hours due to breaking of formaldehyde-protein bonds, and led to a sizable increase in the rate of DM or N loss. Secondly, the other silages had lost much of their DM and N very rapidly in the first few

hours, and later on, had little material left to lose, compared with silage F, which lost less material in the first few hours. It was thus able to attain and exceed the loss rate of the other silages. In spite of this higher rate of loss of silage F after a few hours, the actual loss of N and DM from silage F remained slightly lower.

The implications of a lower rate of N loss are that it is less wasteful because ammonia may be produced in quantities that the rumen microbes could readily assimilate, whereas with higher rates of N loss, ammonia is produced rapidly, and if the concentration of NH_3 exceeds 5-10mM, then much is wasted by conversion to urea and excretion via the kidneys. However, some salivary recycling of urea does occur. Also, slower release of N enables better matching of energy to N supply, and there is better utilisation of N and energy.

Formaldehyde treatment of silage has definitely reduced DM and N degradability in the rumen in this experiment, when compared to formic acid treatment. Formic acid treatment appeared to enhance DM and N degradability compared to the control, thus contradicting the findings of Møller and Thomson (1977). The reduction in N degradability by formaldehyde was much less than that quoted by Beever et al, (1977). The degradability values for N in these silages (Silage F 74.67, control 88.78) compares favourably with those quoted by ARC (1980) of timothy silages (range: .70-.82). The important questions from here are: how much is the degraded DM and N utilized lower down the tract and small intestine for various physiological functions, and how can the maximum level of undegraded forage protein be achieved with additives economically? The level of formaldehyde used was high, and aerobic deterioration and moulding still occurred. It is uneconomic to use such levels of formaldehyde or formic acid.

A mixture of formic acid and formaldehyde may be a better alternative. An integrated multi-disciplinary approach may be critical to answering such questions in the future.

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APPENDIX 1

Table of means, minima and maxima for all parameters measured in Experiment 3 (Shown as percentages)

PARAMETER	MEAN	MINIMUM	MAXIMUM	n
DM loss 24 hours	41.2554	33.9000	46.7000	27
DM loss 12 hours	57.3332	41.6000	70.3000	27
DM loss 4–12 hours	1.9725	0.1625	3.2250	27
DM loss 24 hours	70.5147	52.3000	33.3000	27
DM loss 12–24 hours	1.1364	0.3580	2.0170	27
N loss 4 hours	60.9295	34.8000	74.6000	27
N loss 12 hours	80.4925	52.6000	92.3000	27
N loss 4–12 hours	2.3605	0.5370	4.7900	27
N loss 24 hours	86.8777	53.1000	94.3000	27
N loss 12–24 hours	0.5408	0.0080	2.2580	27
N loss 90% DM	84.2963	67.0000	91.0000	27

APPENDIX 2

Analysis of Variance

Varia te: % DM loss after 4 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum					
Diet	2	26.607	13.303	4.258	NS
Residual	6	18.747	3.124	0.664	
Total	8	45.353	5 .669	1.205	
Sheep units stratum					
Bags	2	184.027	92.013	19.561	Sig (P<0.01)
Diet bags	4	14.920	3.730	0.793	NS
Residual	12	56.447	4.704		
Total	18	255.393	14.189		
Grand Total	26	300.746	100.000		
Grand Mean		41.26			
Total number of observations		27			

APPENDIX 3

Analysis of Variance

Variate: % DM loss at 12 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum					
Diet	2	451.50	225.75	2.418	NS
Residual	6	560.27	93.38	6.522	
Total	8	1011.77	126.47	8.833	
Sheep units stratum					
Bags	2	488.05	244.02	17.042	Sig (P<0.001)
Diet bags	4	96.64	24.16	1.687	NS
Residual	12	171.82	14.32		
Total	18	756.51	42.03		
Grand Total	26	1768.28			
Grand Mean		57.33			
Total number of observations		27			

APPENDIX 4

Analysis of Variance

Variate: Per hour % DM loss between 4-12 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum	2	3.7356	1.8678	1.775	NS
Diet	6	6.3144	1.0524	3.017	
Residual	8	10.0500	1.2562	3.601	
Total					
Sheep units stratum	2	1.5661	0.7831	2.245	NS
Bags	4	1.1569	0.2892	0.829	NS
Diet bags	12	4.1862	0.3489		
Residual	18	6.9092	0.3838		
Total					
Grand Total	26	16.9592			
Grand Mean		1.97			
Total number of observations		27			

APPENDIX 5

Analysis of Variance

Variate: % DM loss after 24 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum	2	544.26	272.13	1.856	NS
Diet	6	879.94	146.66	6.047	
Residual	8	1424.19	178.02	7.340	
Total					
Sheep units stratum	2	208.49	104.25	4.298	Sig (P<0.05)
Bags	4	219.99	55.00	2.268	NS
Diet bags	12	291.03	24.25		
Residual	18	719.52	39.97		
Total					
Grand Total	26	2143.71			
Grand Mean		70.51			
Total number of observations		27			

APPENDIX 6

Analysis of Variance

Variate: % DM loss after 12 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum	2	0.7542	0.3771	1.347	NS
Diet	6	1.6792	0.2799	1.798	
Residual	8	2.4333	0.3042	1.954	
Total					
Sheep units stratum	2	0.3460	0.1730	1.111	NS
Bags	4	0.2818	0.0705	0.453	NS
Diet bags	12	1.8679	0.1557		
Residual	18	2.4957	0.1387		
Total					
Grand Total	26	4.9290			
Grand Mean		1.136			
Total number of observations		27			

APPENDIX 7

Analysis of Variance

Variate: % N Loss at 4 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum	2	90.294	45.147	4.459	NS
Diet	6	60.749	10.125	1.630	
Residual	8	151.043	18.880	3.039	
Total					
Sheep units stratum	2	4243.879	2121.939	341.586	Sig (P<0.001)
Bags	4	173.728	43.432	6.992	Sig (P<0.01)
Diet bags	12	74.544	6.212		
Residual	18	4492.148	249.564		
Total					
Grand Total	26	4643.191			
Grand Mean		60.93			
Total number of observations		27			

APPENDIX 8

Analysis of Variance
Variate: % N loss at 12 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum					
Diet	2	300.79	150.40	1.988	NS
Residual	6	454.00	75.67	4.317	
Total	8	754.79	94.35	5.383	
Sheep units stratum					
Bags	2	2038.54	1019.27	56.152	Sig (P<0.001)
Diet bags	4	155.33	38.83	2.215	NS
Residual	12	210.33	17.53		
Total	18	2404.21	133.57		
Grand Total	26	3159.00			
Grand Mean		80.49			
Total number of observations		27			

APPENDIX 9

Analysis of Variance
Variate: % N loss between 4-12 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum					
Diet	2	1.3354	0.6677	0.526	NS
Residual	6	7.6187	1.2698	2.471	
Total	8	8.9541	1.1193	2.178	
Sheep units stratum					
Bags	2	9.3298	4.6649	9.077	Sig (P<0.01)
Diet bags	4	0.2122	0.0530	0.103	NS
Residual	12	6.1673	0.5139		
Total	18	15.7093	0.8727		
Grand Total	26	24.6634			
Grand Mean		2.36			
Total number of observations		27			

APPENDIX 10

Analysis of Variance

Variate : % N loss at 24 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum					
Diet	2	287.58	143.79	2.646	NS
Residual	6	326.06	54.34	2,536	
Total	8	613.63	76.70	3,580	
Sheep units stratum					
Bags	2	697.72	348.86	16.280	Sig (P<0.001)
Diet bags	4	390.79	97.70	4.559	Sig (P<0.05)
Residual	12	257.14	21.43		
Total	18	1345.65	74.76		
Grand Total	26	1959.29			
Grand Mean		86.88			
Total number of observations		27			

APPENDIX 11

Analysis of Variance

Variate: Per hour N loss abetween 12-24 hours incubation in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum					
Diet	2	0.86532	0.43266	3.502	NS
Residual	6	0.74123	0.12354	1.259	
Total	8	1.60654	0.20082	2.046	
Sheep units stratum					
Bags	2	2.31550	1.5775	11.797	Sig (P<0.001)
Diet bags	4	0.74871	0.18718	1.907	NS
Residual	12	1.17768	0.09814		
Total	18	4.24189	0.23566		
Grand Total	26	5.84844			
Grand Mean		0.541			
Total number of observations		27			

Analysis of Variance APPENDIX 12

Variate: N Loss at 90% DM disappearance in the rumen

SOURCE OF VARIATION	DF	SS	MS	VR	Level of significance
Sheep stratum					
Diet	2	22.741	11.370	1.785	NS
Residual	6	38.222	6. 370	1.476	
Total	8	60.963	7.628	1.766	
Sheep units stratum					
Bags	2	1253.852	626.926	145.296	Sig (P<0.001)
Diet bags	4	57037	14.259	3.305	Sig (P<0.05)
Residual	12	51.778	4.315		
Total	18	1362.666	75.704		
Grand Total	26	1423.629			
Grand Mean		84.30			
Total number of observations		27			

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