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**CLASSIFICATION OF SILAGES AND THEIR INTAKE
WITH CONCENTRATES OF DIFFERENT TYPES**

by

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being

**a thesis submitted in part fulfillment of the requirements
for DOCTOR OF PHILOSOPHY**

**and comprising a report of studies undertaken at
the Scottish Agricultural College, Auchincruive
in the Faculty of Science, of the University of Glasgow**

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ABSTRACT

The aim of this study was to seek an improved understanding of the factors governing the intake by calves of silage as a sole feed and of the effect on silage intake of different types of concentrate fed with silages of different qualities. A large number of research silage analyses were obtained and the possibility of classifying them into groups of similar chemical composition investigated. Classification of a wide range of silages into distinct groups was found to be possible using the multivariate statistical method, cluster analysis. Silages could be classified into four distinct groups on the basis of toluene dry matter, pH, total nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid and lactic acid. Further silages could be easily allocated to these groups provided that they had been analysed for the above eight parameters.

Silages with a more limited analysis of oven dry matter, pH, total nitrogen and ammonia nitrogen were not found to have sufficiently detailed analysis for classification into the same groups.

Four linked trials were conducted where the intakes of silages of different types fed with different concentrates were investigated. A silage representative of each of the four classification types, namely a low pH, normal, high dry matter and poorly fermented silage, were fed with five different concentrate types: a standard concentrate, the standard concentrate plus bicarbonate, high starch, high fibre and high protein concentrates. Five twelve week old Friesian calves were allocated to each treatment. The type of silage fed significantly affected silage intake. Intake of the high dry matter silage was markedly higher than the rest. When offered any of these silages without a supplement the animals ate significantly more silage than the animals offered this silage plus a concentrate. There were no significant differences in silage intake between concentrate types but there was a trend, however, for the fibre and bicarbonate supplements to produce the lowest substitution rates.

The applicability of single variable prediction equations (based on NDF and crude protein) in predicting the intake of the four experimental silages as a sole feed was investigated. Subsequently more complex multi-variable equations (based on fermentation characteristics and energy values) were used to predict the intake of silage as part of a mixed diet. The single variable equation by LaForest *et al* (1986) based on NDF was found to be as accurate in predicting silage intake as the complex equations by Rook *et al* (1990) based on fermentation characteristics. This suggests that factors other than fermentation characteristics are important in determining silage intake.

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ABBREVIATIONS

| | |
|--------------------|---|
| DM | dry matter |
| tDM | toluene dry matter |
| CP | crude protein |
| TN | total nitrogen |
| WSC | water soluble carbohydrates |
| LA | lactic acid |
| AA | acetic acid |
| PA | propionic acid |
| BA | butyric acid |
| NDF | neutral detergent fibre |
| ADF | acid detergent fibre |
| IADF | indigestible acid detergent fibre |
| Bc | buffering capacity |
| HS | high starch |
| (H)F | (high) fibre |
| (H)P | (high) protein |
| S | standard |
| B | bicarbonate |
| ME | metabolisable energy |
| SR | substitution rate |
| kg | kilogram |
| NIR | near infra-red reflectance spectroscopy |
| MSPE | mean square prediction error |
| MPE | mean prediction error |
| SED | standard error difference |
| F pr. | probability |
| LW | liveweight |
| LW ^{0.75} | metabolic liveweight |
| LWG | liveweight gain |

CHAPTER 1

1.1 INTRODUCTION

The aim of this work is to examine the inter-relationship between silage and concentrate in the diet of calves with a view to predicting intake in a practical situation. This chapter reviews the current understanding of the factors affecting ruminant nutrition.

The term silage is given to a crop which has been preserved by partial fermentation for consumption by livestock. Whilst grass is the most commonly used raw material, silage is also made from lucerne and whole crop cereals. Subsequent discussions will be limited to grass silage only, which for simplicity will be referred to as silage. Different conditions at harvest and in the silo produce silages of varying characteristics, with factors such as air and the chemical composition of the grass affecting the fermentation pathways and thus the silage composition and ultimately forage intake.

The amount of silage a ruminant will eat voluntarily is determined by a complex interaction of physical processes and pathways. Silage intake is also governed by the amount and type of concentrate fed along with the silage. Feeding a concentrate generally depresses silage intake, this effect being known as substitution or replacement. Where a silage is supplemented with concentrate, the type of concentrate and its possible interaction with the silage has been found to play an important role in the voluntary intake of forage, with characteristic concentrate types differing in the extent of their depression of silage.

The prediction of silage intake is of paramount importance to farmers when planning both the quantities of forage necessary and the amount of silage to be fed for optimum performance. Due to the wide range of silages of varying chemical composition, prediction of intake is difficult. Hence, to simplify the task of predicting intake, different researchers have constructed classification schemes which group silages of similar composition together, each group tending to have originated from one type of fermentation pathway. Many methods of predicting intake have thus been established, some of which are discussed below.

Each of the factors which directly or indirectly affect the intake of silage by ruminants will be discussed more fully in the subsequent sections, the first factor to be considered being fermentation pathways and the elements that influence these.

1.2 FERMENTATION PATHWAYS

Silage is made by the anaerobic fermentation of a crop, usually grass. The fermentation is influenced by the multiplication and growth of different species of bacteria, e.g. lactic acid bacteria, clostridia and enterobacteriaceae which are naturally present on the crop, and by the wilting of the crop or the use of chemicals prior to ensiling which either inhibit or stimulate the fermentation. In order to achieve a successful fermentation it is necessary to obtain and maintain anaerobic conditions. As it is the end products of the fermentation that affect intake, the mechanisms, causes and conditions of the major fermentation pathways will be examined first, followed by the consideration of factors affecting these pathways.

1.2.1 Lactic Acid Bacteria

Immediately after grass has been harvested the plant respiratory enzymes begin to oxidise the water soluble carbohydrates to carbon dioxide and water. This process continues once a silo has been filled, consolidated and sealed, with the production of some heat. Many species of bacteria grow and multiply until the oxygen has been used up and anaerobic conditions exist. Lactic acid bacteria then dominate the fermentation, other species being unable to survive without the presence of oxygen. There are two main types of lactic acid bacteria, homofermentative and heterofermentative, the former producing twice as much lactic acid from the fermentation of a given quantity of water- soluble carbohydrates.

Lactic acid bacteria are facultative anaerobes i.e. they can grow both aerobically and anaerobically. Where air is present the oxygen combines with hydrogen to form hydrogen peroxide whilst pyruvate is converted to lactate. This reaction provides twice as much energy to be used in further chemical reactions as that without oxygen (Stryer 1975).

Homofermentative bacteria can reduce carbohydrates to lactate via the glycolytic pathway. For each molecule of glucose reduced two molecules of lactate are formed.

Other water soluble carbohydrates e.g. fructose and pentose can also be fermented with the production of lactate.

Glucose --> 2 Lactic acid

Fructose --> 2 Lactic acid

Pentose --> Lactic acid + Acetic acid

(McDonald 1981)

Heterofermentative lactic acid bacteria produce a wide number of products depending on the type of sugar fermented. As a result they are not as efficient at producing lactic acid as the homofermentative lactic acid bacteria. For example, in the fermentation of glucose not only is lactic acid produced but also ethanol and carbon dioxide. Fermentation of fructose involves a long reaction with the production of mannitol, whilst the fermentation of pentose gives the same products as the homofermentative fermentation of pentose, namely lactic acid and acetic acid but by a different mechanism.

Glucose --> Lactic acid + Ethanol + carbon dioxide

3Fructose --> Lactic acid + 2 Mannitol + Acetic acid + Carbon dioxide

2 Fructose + Glucose --> Lactic acid + 2 Mannitol + Acetic acid + Carbon dioxide

Pentose --> Lactic acid + Acetic acid

(McDonald 1981)

1.2.3 **Enterobacteriaceae**

This group of bacteria is active mainly in the early stages of ensilage competing with the lactic acid bacteria. They multiply until about the seventh day after ensilage then decrease in numbers, progressively being replaced by lactic acid bacteria.

Under certain conditions, however, they may persist, for example when fermentation is delayed or when formic acid is applied, and they may also increase during aerobic deterioration. Their presence is undesirable as they compete with the lactic acid bacteria for nutrients. They also produce endotoxins and may be responsible for much of the ammonia produced during ensiling (McDonald *et al* 1991).

These bacteria, which range in species from aerobic to facultatively aerobic, ferment

glucose and other carbohydrates and alcohols to acids, particularly acetic acid with lactate, ethanol and 2,3-butanediol produced in small quantities (Stanier *et al* 1977).

The particular type of fermentation which proceeds and the ratio of products formed vary considerably, both from one bacterial strain to another and also within strains grown under different environmental conditions.

Enterobacteriaceae are able to deaminate and decarboxylate amino acids and reduce nitrate but possess only weak proteolytic properties (Woolford 1984).

1.2.3 Clostridia

Clostridia are bacteria which grow without the presence of oxygen and can ferment sugars or organic acids. Unlike lactic acid bacteria they are also able to degrade proteins. There are two distinct types, each varying in their substrates:

1. saccharolytic clostridia ferment mainly carbohydrates and organic acids with only limited ability to degrade proteins
2. proteolytic clostridia utilise mainly proteins with a limited ability to ferment carbohydrates and organic acids

(McDonald 1981)

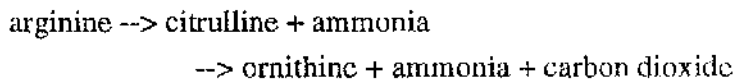
Clostridia do not compete with lactic acid bacteria in the early stages of fermentation, but will proliferate in certain conditions. Saccharolytic clostridia utilise sugars and lactic acid to produce butyric acid, and since this acid is weaker than either lactic acid or acetic acid, there will be a rise in pH. Conditions may then become favourable for the proliferation of proteolytic clostridia, which are less tolerant of acid than their saccharolytic counterparts, resulting in the formation of amines, amides and ammonia from proteins and amino acids, thus causing a further increase in pH. Therefore where saccharolytic fermentations dominate, the pH will be high with low levels of lactate and sugars whilst being high in butyrate and often acetate. Hence the suppression of the growth of clostridia is a prerequisite to a well fermented silage. There are two ways of ensuring that they do not dominate the fermentation. Firstly, clostridia do not grow in acidic conditions, the optimum pH for their multiplication being 7.0 to 7.4 (Pelczar and Reid 1972). Hence, if sufficient lactic acid is produced, keeping the environment acidic, clostridial growth will be suppressed. The dry matter of the crop is the second factor determining clostridial growth. These bacteria proliferate in wet conditions.

hence, if crops are wilted to greater than 300 g/kg, clostridial growth is inhibited solely by lack of moisture. However, where the crop has been wilted to between 200-300 g/kg, clostridial growth is only partially inhibited by moisture availability (Woolford 1981).

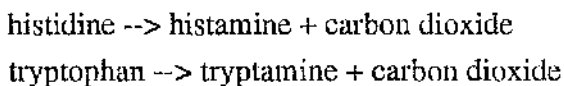
There are three types of reactions in which the proteolytic clostridia participate:

1. deamination in which ammonia is released leaving an organic residue
2. decarboxylation where an amine is formed
3. oxidation/reduction in a Stickland reaction where one amino acid is oxidised while the other is reduced

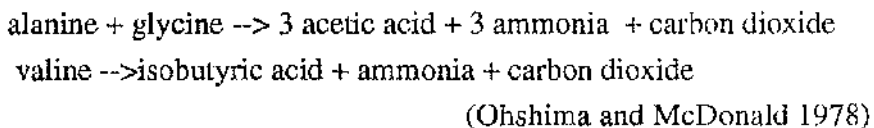
The amount of ammonia present in a silage is a good indicator of the extent of proteolytic clostridial activity as it is only produced in very small quantities by other microorganisms (Kemble 1956) e.g. enterobacteria. An example of a deamination reaction is:



Decarboxylation reactions include:



Stickland type reactions include:



In conclusion, the type of fermentation that will proceed in a silo depends on the rate of growth and proliferation of lactic acid bacteria with a concomitant fall in pH. Such growth will inhibit enterobacteriaceae and clostridia and the resultant silage will be of good quality if a low pH is maintained.

1.2.4 Organisms Involved in Aerobic Deterioration

Aerobic deterioration in silage is prevalent in carbohydrate rich silages, heavily wilted silages and those in which the fermentation has been restricted by the use of additives

(Woolford 1978) but may also occur in low dry matter, lactate silages (Henderson *et al* 1979).

Although of little importance during fermentation, the yeasts are the main causes of aerobic deterioration (McDonald *et al* 1991), particularly yeasts which utilise both acids and sugars as their substrate.

Moulds also play a significant role in the aerobic deterioration of silage, their growth generally following that of yeasts (Ohyama *et al* 1979). Not only do they break down sugars and lactic acid, they also hydrolyse and metabolise cellulose and other cell wall components. Furthermore, some moulds produce mycotoxins.

Recently, species of bacteria have been found to play a greater role in aerobic deterioration than was previously thought. Proteolytic species and both lactic acid bacteria and acetic acid bacteria have been particularly involved (McDonald *et al* 1991).

1.2.5 Factors Affecting Fermentation Pathways

There are several factors which affect the fermentation pathway, some of which have been mentioned briefly above. The two most important are the nature of the crop at the time of harvesting and the changes which occur during microbial fermentation in the silo (McDonald 1981, McDonald *et al* 1981). These two factors can be expanded as follows:

- chemical composition of the raw material
- amount of moisture and extent of prewilting
- mechanical treatment
- rate of acidification
- presence of air
- temperature
- amount of preservatives added

with factors affecting aerobic stability of silage being:

- presence of air
 - available substrate
 - temperature
- (Woolford 1984)

The importance of each of these factors will now be examined.

1.2.5.1 Chemical Composition

The amount of water soluble carbohydrates in the grass crop prior to ensiling is a major factor determining fermentation type; concentrations of water soluble carbohydrates in grass vary greatly from 16.0 to 285.0 g/kg DM (MAFF 1990). The level of water soluble carbohydrates present in grass prior to ensiling differs with the species of grass used, ryegrasses having the highest water soluble carbohydrate content (McDonald 1981). Where there are low levels of water soluble carbohydrates in the grass crop, less than 3% of fresh weight (McDonald *et al* 1991), the conversion of sugar to lactic acid, a prerequisite for good quality silage, proceeds only slowly.

The amount of water soluble carbohydrates present in the grass at the time of ensiling affects the fermentation pattern and the resultant silage chemical composition; low grass water soluble carbohydrate contents tending to produce silages of poorer quality than where the water soluble carbohydrate content was higher. Where sugar levels are low initially enterobacteria compete with the lactic acid bacteria for substrate thus, the fall in pH is slow, and generally is not fast enough to prevent the saccharolytic clostridia multiplying and fermenting the lactic acid and residual sugars to butyric acid causing the pH to rise (McDonald *et al* 1991). The less acid-tolerant proteolytic clostridia then usually become active, leading to a further increase in pH caused by the production of ammonia.

The proportion of dry matter in the crop immediately prior to ensiling can affect fermentation pathways. In particular, in crops of high moisture content it can be difficult to obtain a lactic fermentation. One problem of ensiling a low dry matter silage is that the pH below which clostridial growth is inhibited varies with the moisture content of the grass and, unless water soluble carbohydrate levels are very high, the ensiling of wet crops will encourage a clostridial fermentation resulting in high dry matter losses and a silage of low nutritional value (McDonald 1981).

The main effect of wilting is to restrict fermentation as dry matter content increases. This is reflected in the higher pH and levels of water soluble carbohydrate and the lower levels of fermentation acids of wilted silages as compared to unwilted silages (Jackson and Forbes 1970).

1.2.5.2 Buffering Capacity

The buffering capacity of plants, or their ability to resist pH changes is an important factor in successful ensilage. This effect is due to the balance between inorganic acids and their salts; as acid is added or produced, it reacts to give its salt and water, thus not reducing the pH to any great extent. This effect can alter fermentation pathways: as lactic acid is produced, which should lower the pH and encourage a good fermentation, it is converted into its salt, thus resisting any change in pH (McDonald 1981) and allowing undesirable bacteria and microorganisms to grow. The use of additives can partially overcome this problem, provided sufficient quantities are added to maintain the balance in favour of the acids.

1.2.5.3 The Effect of Air

A well preserved silage originates from a silo where the air has been excluded and rapid proliferation of lactic acid bacteria ensures a rapid fall in pH and the suppression of enterobacteria and clostridial growth. Whether the fermentation of the grass is homofermentative or heterofermentative depends on the species of lactic acid bacteria native to the cut crop. Lactobacillus plantarum and Lactobacillus brevis are amongst the most prevalent microorganisms present in most silages and are homofermentative and heterofermentative respectively (Langston and Bouma 1960).

In silos which are not well sealed, continual seepage of oxygen into the silage enables plant respiratory enzymes to oxidise sugar substrates with the production of heat. The growth of lactic acid bacteria is at a minimum as the optimal temperature for growth of most species is 15 degrees centigrade (McDonald 1976). Due to the warm, high pH conditions and the presence of oxygen within the silo, yeasts and moulds flourish.

Infiltration of air into the silo during the storage period results in the growth of aerobic microorganisms which break down the organic matter to form compost-type material, unfit for consumption. This waste is commonly found on the sides and top of the silage face. Yeasts and bacteria oxidise the organic acids, alcohols and sugars, with eventual destruction of the cell wall polysaccharides and other more stable components. Eventually the proliferation of moulds will aid the deterioration process by the oxidation of lactic acid and water soluble carbohydrates and deamination of amino

acids (McDonald 1981).

Ohyama *et al* (1979) found that silages of a high dry matter content were particularly susceptible to aerobic deterioration. Whilst bacteria and moulds were found in the greatest quantities on low dry matter silages, yeast counts tended to be higher with silages of higher dry matter. Not only were yeast counts on opening the silo greatest for high dry matter silages, but after an earlier temperature rise compared to that of the low dry matter silages, yeast counts were the highest seven days later and these silages had large pH increases and water soluble carbohydrate losses.

Honig and Woolford (1979) concluded that the use of additives to stem aerobic deterioration is unnecessary. As there is no additive which will ensure that aerobic deterioration does not take place these adverse changes in the silo due to the presence of air are best overcome by efficient silo management.

1.2.5.4 Additives

The use of additives alters fermentation pathways and hence the end products formed, the direction of the change depending on the type of additive. There are two classes of additives: fermentation stimulants and fermentation inhibitors.

Lactic acid bacteria are fermentation stimulants and the addition of homofermentative lactic acid bacteria to a grass crop either alone or with enzymes added to produce extra substrate for the bacteria will increase the occurrence of a stable lactic acid fermentation in silage with the minimum loss of nutrients (Henderson and McDonald 1984). The addition of sugar, a fermentation stimulant usually in the form of molasses, is necessary in a crop of low water soluble carbohydrate content to ensure that there is adequate substrate for a rapid proliferation of lactic acid bacteria with concomitant fermentation. The resultant silage is lower in pH and has greater levels of fermentation acids than control silages (Thomas 1978, McDonald 1981). The use of enzymes, fermentation stimulants, as silage additives firstly increases the content of fermentable sugars and secondly improves the digestibility of the organic matter (McDonald *et al* 1991). The enzymes used are either cellulolytic or hemicellulolytic and are generally used in conjunction with an inoculum of lactic acid bacteria, added to convert the glucose to lactic acid only (Henderson *et al* 1987).

Both organic and inorganic acids are fermentation inhibitors as they lower the pH of the forage to a level at which plant and microbial enzymes are inhibited. Formic acid is the most commonly used acid treatment and acts by lowering the pH of the silage and inhibiting bacterial action. Increasing the concentration of formic acid applied results in decreasing levels of lactic and acetic acids and increasing concentrations of water soluble carbohydrates (Carpintero *et al* 1979).

Sulphuric acid has been used as a silage additive since the 1930s when Virtanen (1933) developed the AIV method of preserving crops using mineral acids. This strong acid lowers the pH of the silage rapidly and should inhibit undesirable fermentations. The use of sulphuric acid as an additive has been found to be associated with copper deficiency in livestock (O'Keily *et al* 1989). Thus sulphuric acid is generally fortified with copper. The use of sulphuric acid is not recommended where the grass cut for silage is contaminated with slurry or soil (Appleton 1991). Due to the reduced bacteriostatic effect of sulphuric acid (Carpintero *et al* 1979) enterobacteria from the slurry or soil are able to flourish resulting in an undesirable fermentation. One of the main objectives of Virtanen in using inorganic acids as silage additives was the prevention of proteolysis (McDonald 1981). This has not been found to be entirely successful as Carpintero *et al* (1979) found 45% of herbage protein to be degraded with the application of 4g sulphuric acid per kg macerated grass.

The performance of sulphuric acid compared to formic acid has been examined by O'Keily *et al* (1989) who reported that in terms of animal performance sulphuric acid was comparable to formic acid. Contrary to this, however, Kennedy (1990) concluded from a comprehensive series of trials that sulphuric acid was not as effective as formic acid. In four out of his five trials animal performance was increased by formic acid although the improvement was significant in only one case.

These experiments suggest that with grass of high nutritive value and which is easy to ensile there is little difference in animal performance from the use of either sulphuric acid or formic acid as an additive. Where the grass is difficult to ensile, however, as in the case of Kennedy (1990) in which animal performance with sulphuric acid was significantly less than that for formic acid, formic acid appears to be the superior additive.

Sulphuric acid has been used more successfully as a silage additive in conjunction with

formaldehyde. The latter is a fermentation inhibitor and is primarily used to ensure the protection of plant proteins against microbial degradation in both the rumen and the silo. When formaldehyde is used alone at high levels of application, above 8 l/t in a study by Wilkins *et al* (1974), fermentation was found to be negligible with the production of very small amounts of acids. At low levels, however, formaldehyde tends to encourage clostridial growth, illustrated by higher levels of ammonia and butyric acid in the formaldehyde treated silage compared with the control silage made with an other additive (Wilkins *et al* 1974).

1.2.6 Conclusion

In conclusion there are many factors which affect the fermentation pathways in silage, the most important being the chemical composition of the grass crop, the presence of air in the silo and the use of additives. The key to efficient silage making is achieving the preservation of herbage with a minimum loss of quality and quantity. In order to do this and to discourage adverse fermentation pathways, a rapid wilt of the crop to 250 g/kg dry matter is advisable with the application of additives as an insurance against poor fermentations.

1.3 CLASSIFICATION SCHEMES

Classification schemes have been constructed by many researchers in an attempt to simplify the diversity of silages by grouping together those with similar properties. Either well defined groups encompassing ranges of chemical components, or values of upper or lower limits of groups have been suggested as a means of classification.

On inspection of existing silage classification systems, three types or trends emerge which may for convenience be called simple, intermediate and complex. Both the simple and intermediate groups were devised in the 1950s and 1960s and differentiate between silages on only a few features. The complex group contains classification systems devised in the 1970s or 1980s and relies on a much wider range of chemical constituents. Thus over time the trend has been to develop classification schemes of increasing complexity and detail.

In an attempt to categorise this diverse range of silages, many workers have devised classification schemes based on silage quality. The term "silage quality" is generally

used to indicate the success of fermentation (McCullough 1978) or the extent to which the fermentation has proceeded in a desirable manner (Syrjala 1972). It should be emphasised that it does not denote the nutritive or feeding value of the silage.

1.3.1 Simple Classification Schemes

The first of the three types of classification systems, the simple schemes, classifies silage with respect to organoleptic criteria, namely silage colour, smell and texture. These criteria are easily assessed and are convenient as they require no laboratory facilities. This classification has one major disadvantage in that it is subjective and open to different interpretation, resulting in differences of opinion between assessors.

The DLG (German Agricultural Association) devised a classification scheme based on organoleptic criteria called the sensory test. Silages are given a value according to :

- | | |
|--|-------------|
| (a) Smell | 0-12 points |
| (b) Preservation of the structure of plant tissues | 0-5 points |
| (c) Colour | 0-3 points |

The total number of points determines to which classification group the silage is allocated, with 18-20 points being very good, 10-17 points satisfactory, 4-9 points bad to moderate and 0-3 points very bad (Konckamp 1960).

1.3.2 Intermediate Classification Schemes

The second type of classification systems, the intermediate schemes, classifies silages according to the amounts of the main fermentation end products which are present. This type of classification has the advantage over the simple schemes in that chemical assessments provide an unequivocal basis upon which to judge quality, although the definition of quality is still subjective.

The simplest of these classification schemes was devised by Nilsson *et al* (1956). In determining silage quality, they found that ammonia nitrogen content expressed as a percentage of total nitrogen and butyric acid content were good indicators. Low values of these two factors indicated good quality and high values, bad quality. On the basis of these two measurements, silages were assigned to one of five groups, ranging from very good to very bad (Table 1.1). Where these measurements were not consistent

with each other, the higher of the two values was taken as the decisive factor in assigning a silage to a group.

Four years later this system was slightly modified by Nilsson and Rydin (1960) and Rydin (1961). Again only two factors were used in this classification system, but instead of butyric acid content, the proportion of butyric acid in (butyric acid + lactic acid) was used. The proportion of total nitrogen present as ammonia nitrogen was used as per Nilsson *et al* (1956). The system proposed by Rydin (1961) defined six groups ranging in quality from excellent to very bad, whilst Nilsson and Rydin (1960) only separated the good and bad quality silages from the rest (Table 1.1).

Wieringa (1966) concluded that, in evaluating the success of preservation, the pH values as well as the fermentation end products can be used as criteria. Thus in his classification system, pH, butyric acid content and the proportion of total nitrogen as ammonia nitrogen were the discriminating factors in grouping the silages into good, medium or poor quality groups (Table 1.1). Ulvesli and Saue (1965) used the same criteria, but they defined good quality silage as having:

- (a) butyric acid content of less than 1 g/kg DM
- (b) NH₃-N of less than 80 g N/kg Total Nitrogen
- (c) maximum pH 4.2

Whilst proposing their own definition for satisfactory silage, Carpintero *et al* (1969), in a review of the literature, noted that several workers had attempted a crude classification of silage depending on the pH, amounts of ammonia nitrogen and butyric acid present. Their classification scheme was equally crude with a satisfactory silage having a pH less than or equal to 4.2, a butyric acid content of less than 1 g/kg DM and the proportion of volatile nitrogen in total nitrogen less than 110 g/kg DM (Table 1.1).

One final classification scheme based solely on these three parameters was suggested by Breirem *et al* (1954). Whilst having the same limits of pH less than 4.2 and butyric acid levels of 1 g/kg DM, ammonia nitrogen had to be between 50-80 g/kg TN to fall into his 'very good' quality group (Table 1.1).

Hellberg (1963) and Archibald *et al* (1954) both used four criteria for the classification of silage. In addition to ammonia nitrogen, butyric acid and lactic acid content, they

Table 1.1

INTERMEDIATE CLASSIFICATION SCHEMES

| pH | BUTYRIC ACID g/kg DM | LACTIC ACID g/kg DM | AMMONIA NITROGEN g/kg TN | CLASSIFICATION | REFERENCE |
|-----------|----------------------------|---------------------------|--------------------------------|----------------|-------------------------------|
| 3.9 - 4.8 | trace | 30.3 - 131.6 | 10.2 - 28.7 | good | Langston <i>et al</i> (1958) |
| 4.0 - 4.6 | 7.6 - 15.5 | 24.9 - 96.4 | 8.9 - 36.2 | intermediate | |
| 5.2 - 5.7 | increase | decrease | 32.2 - 98.2 | poor | |
| | 0 - 1.0 | | 0 - 125 | very good | Nilsson <i>et al</i> (1956) |
| | 1.1 - 2.0 | | 125 - 150 | good | |
| | 2.1 - 3.0 | | 151 - 175 | medium | |
| | 3.1 - 4.0 | | 176 - 200 | bad | |
| | > 4.0 | | > 200 | very bad | |
| < 4.5 | < 4.0 | 30.0 - 50.0 | < 50 | good | Archibald <i>et al</i> (1954) |
| < 4.5 | < 4.0 | > 15.0 | < 100 | good | Hellberg <i>et al</i> (1963) |
| > 5.1 | > 14.0 | < 5.0 | > 210 | poor | |
| < 4.2 | < 1.0 | | < 80 | good | Ulvessli <i>et al</i> (1965) |
| < 4.2 | < 2.0 | | < 80 | good | Wieringa (1966) |
| 4.3 - 4.5 | 3.0 - 5.0 | | 90 - 150 | medium | |
| > 4.5 | > 5.0 | | > 150 | poor | |
| < 4.2 | < 1.0 | | < 110 | satisfactory | Carpintero (1969) |
| 4.2 | < 1.0 | | 50 - 80 | very good | Breirem <i>et al</i> (1954) |
| | %BA of BA + LA | | NH ₃ - N (%TN) | | |
| | < 10.0 | | < 5.0 | excellent | Rydin (1961) |
| | 10.1 - 12.5 | | 5.1 - 12.5 | very good | |
| | 12.6 - 15.0 | | 12.6 - 25.0 | good | |
| | 15.1 - 17.5 | | 25.1 - 37.5 | satisfactory | |
| | 17.6 - 20.0 | | 37.6 - 50.0 | bad | |
| | > 20.1 | | > 50.1 | very bad | Nilsson and Ryden (1960) |
| | < 5 | | < 10 | good | |
| | > 50 | | > 20 | poor | |

included pH, both agreeing that for good quality silage the pH had to be less than 4.5. Similarities between the two systems went no further. Whilst Hellberg (1963) proposed that good quality silage had to have more than 15 g/kg DM of lactic acid and less than 4 g/kg DM of butyric acid, Archibald *et al* (1954) used a lactic acid content of 30-50 g/kg DM or greater and a butyric acid content of 20 g/kg DM or less (Table 1.1).

Butyric acid, lactic acid, ammonia nitrogen and pH are the basis for two further classification schemes (Brierem and Ulvesli 1954, Langston *et al* 1958). In addition to these four components, Breirem and Ulvesli included acetic acid as a factor in their classification. Thus they proposed that good quality silage has:-

- (i) pH less than 4.2
- (ii) lactic acid between 15 - 25 g/kg DM
- (iii) acetic acid between 5 - 8 g/kg DM
- (iv) butyric acid less than 1 g/kg DM
- (v) proportion of TN as NH₃-N between 50-80 g/kg TN

Langston *et al* (1958) again used these four criteria for the basis of their classification scheme. However, instead of acetic acid being included as another variable, they included bacterial spore counts. Initially, only good and poor silages were defined, but in some borderline silages, characteristics usually associated with good quality were found together with criteria characteristic of poor quality. Thus it was found necessary for comparative purposes to form an intermediate classification group (Table 1.1).

The above schemes all take into account ammonia which is the principal fermentation product of proteolytic clostridia. Two additional classification schemes which take account of butyric acid, the main fermentation end product of saccharolytic clostridia, but not ammonia were proposed by Fleig (1952) and Zimmer (1966).

The Fleig scheme awards points according to the proportion of lactic, acetic and butyric acids present: the higher the proportions of lactic and acetic acids to butyric acid, the higher the points scored and the better the quality of silage. Zimmer (1966) modified this scheme, placing less emphasis on butyric acid, as increasingly farm silages were found to contain less butyric acid.

A classification scheme which uses a combination of criteria from both the simple and

intermediate phases is that proposed by Heikonen *et al* (1979). In his paper he separates only good quality AIV silage from the rest. Both organoleptic criteria such as silage colour and odour are used along with further criteria e.g. pH, ammonia and sugars based upon the measurements in press juice. Thus for a good quality AIV silage:

- (i) the pH must be between 3.7 - 4.0
- (ii) the press juice must contain greater than 20 g/kg DM of sugar
- (iii) the press juice must contain less than 0.5g ammonia/l
- (iv) the press juice must contain no butyric acid
- (v) the silage must be light in colour
- (vi) the odour must be pleasantly acidic

Most of the intermediate classification schemes are applicable for silages of normal dry matters in the region of 160-230 g/kg DM. A major defect, however, of the intermediate classification schemes, however, is that they are relatively inflexible. They do not take into account different fermentation pathways e.g. those in high dry matter silages. For example, most of these schemes state that for a silage to be good quality it must have a lower pH than 4.2. This ignores the relationship between silage pH and dry matter content. A silage can be of excellent quality with a pH of 4.5, provided that the dry matter is sufficiently high, greater than 300 g/kg DM (Thomas and Fisher 1991).

1.3.3 Complex Classification Schemes

The later classification schemes are the most detailed and many chemical constituents are used as criteria in group distinction.

McDonald and Edwards (1976) were the first to propose a detailed silage classification scheme. They classified silages into five groups; lactate, butyrate, acetate, wilted and chemically restricted on the basis of pH, DM, buffering capacity, protein nitrogen, ammonia nitrogen, lactic acid, acetic acid, butyric acid, water-soluble carbohydrates, mannitol and ethanol. Of all the fermentation characteristics, residual WSC and nitrogen components were believed to be particularly relevant to the ruminant.

Lactate silages were characterised by having a low pH with values between 3.7 and 4.2 and containing high concentrations of lactic acid, usually 80 - 120 g/kg DM (Table

1.2). The nitrogenous components were mainly in a non-protein soluble form.

Acetate silages are produced when the silage is deficient in lactic acid bacteria, and it was thought the enterobacteriaceae dominate the fermentation. The main fermentation product of these silages is acetic acid with smaller amounts of lactic, propionic and butyric acids being formed. The pH is slightly higher than that of lactate silages (Table 1.2). Recent studies by Rook *et al* (1991), however, have indicated that in acetate silages lactic acid bacteria are the organisms responsible for acetate production.

In butyrate silages, which are characteristically badly fermented silages, a stable pH has not been obtained. Saccharolytic followed by proteolytic clostridia flourish, increasing pH further and producing ammonia. A butyrate silage typically has low lactic acid and WSC levels and a high content of ammonia (Table 1.2).

A fourth classification group is that of wilted silages. As fermentation becomes restricted at increasing dry matter levels, higher pH and WSC levels and lower levels of fermentation acids result (Table 1.2).

The final group in McDonald and Edwards' (1976) classification scheme is that of chemically restricted silages. In general, fermentation inhibitors e.g. acids or formaldehyde result in lower concentrations of fermentation acids and higher levels of WSC. Thus silages in this group are characterised by low levels of fermentation acids with high residual sugar levels (Table 1.2).

Wilkinson *et al* (1981), in an examination of the interrelationships between pattern of fermentation during ensilage and initial crop composition, devised a classification system based on fermentation quality. The silages they used were 50% grass silages, 30% legume silages and 20% from annual forage crops. In total 231 silages were used. The silages were classified by cluster analysis according to pH, WSC, lactic acid, acetic acid, propionic and butyric acids, and ammonia nitrogen. Seven groups A to G were defined, groups A to C being good quality and groups D to G poor quality (Table 1.2).

A modification of Wilkinson's classification was described by Thomas and Thomas (1985). They stated that whilst attempts to classify silages are necessarily oversimplistic, the analysis by Wilkinson *et al* (1981) serves to illustrate the wide range that can be encountered. Thomas and Thomas (1985) divided the silages into three

Table 1.2

COMPLEX CLASSIFICATION SCHEMES

| pH | BA g/kg DM | LA g/kg DM | NH ₃ -N g/kg TN | PA g/kg DM | AA g/kg DM | Class | Ref |
|-----|---------------|---------------|-------------------------------|---------------|---------------|---------------|-----|
| 3.9 | 1 | 102 | 78 | | | lactate | 1 |
| 5.2 | 35 | 1 | 246 | | | butyrate | |
| 4.8 | 2 | 34 | 128 | | | acetate | |
| 4.2 | 1 | 59 | 83 | | | wilted | |
| 5.1 | 1 | 26 | 30 | | | chem restr | |
| 4.8 | 0 | 62 | 7 | 0 | 38 | A good | 2 |
| 4.3 | 2 | 75 | 10 | 1 | 22 | B | |
| 4.3 | 1 | 52 | 9 | 1 | 46 | C | |
| 5.1 | 4 | 21 | 17 | 6 | 69 | D poor | |
| 5.4 | 32 | 39 | 31 | 5 | 24 | E | |
| 5.9 | 40 | 4 | 42 | 7 | 49 | F | |
| 6.8 | 25 | 0 | 56 | 14 | 61 | G | |

- References: 1 McDonald and Edwards (1976)
 2. Wilkinson *et al* (1981)

groups, well fermented, badly fermented and restricted, with subdivisions within all those groups, as follows:

- | | |
|-----------------|---|
| Well fermented | A high lactic, low acetic |
| | B medium lactic, high acetic |
| Badly fermented | C low lactic, high acetic |
| | D low lactic, high acetic, high butyric |
| | E low acetic, high butyric |
| Restricted | F restricted fermentation -wilted |
| | G - acid |
| | H - formaldehyde |

The complex classification schemes are superior to the intermediate classification schemes because they take into account the different fermentation patterns. Instead of the categories being based on a set level which defines quality, the categories in the complex classification tend to be representative of the silages produced from a particular fermentation pathway e.g. high dry matter and clostridial type fermentations.

These schemes classify silage according to fermentation pathways and give no indication of the nutritive value as they include no measurement of digestibility or intake potential. Classification schemes can, however, be used to practical advantage. They reduce the diverse range of silages to a known number of groups. A representative silage from each group can then be used as a control to aid feeding strategies. Thus indirectly the nutritive value of a silage can be assigned.

1.4 GENERAL THEORIES OF FOOD INTAKE

Once the great diversity of silages has been simplified, these groups can then be used as an aid to intake prediction. Before this can be done, however, an understanding of the fundamental processes affecting intake have to be understood.

Voluntary intake is defined as the amount of dry matter eaten during a given period of time when an animal has free access to food (Ulyatt 1973). If the factors causing an animal to start and stop eating could be understood, it would be possible to understand the control of voluntary food intake; unfortunately there is not yet sufficient knowledge to achieve this (Forbes 1986).

It is not the purpose of this discussion to examine the general theories of food intake in detail, but they will be mentioned briefly as an introduction to the theories of forage and silage intake.

1.4.1 Animal Factors

The voluntary food intake of an animal is affected by liveweight, pregnancy and lactation (Forbes 1986). The voluntary intake of growing cattle is strongly associated with liveweight (AFRC 1991). Whilst intake is related to metabolic liveweight ($LW^{0.75}$), an exponent of 0.8 was preferred for young animals (Taylor *et al* 1986).

Intake is also strongly associated with liveweight in lactating dairy cows, although liveweight change over time is often more useful than liveweight alone since it more accurately reflects the changes in body fat stores occurring over lactation whereas liveweight *per se* also reflects frame size differences between cows (AFRC 1991). Bines (1985) found that dry matter intake increases by about 1 kg/day per 50 kg increase in liveweight.

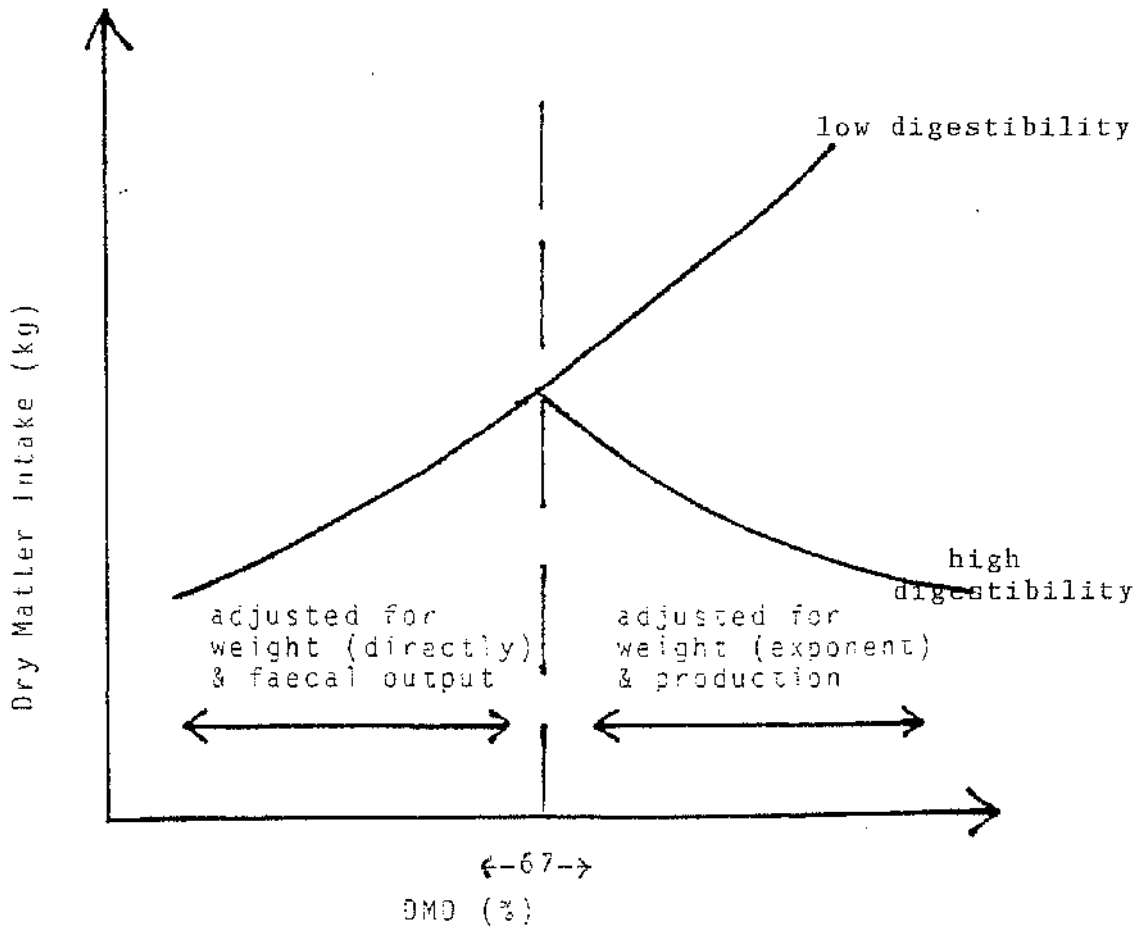
The stage of lactation has been found to have a greater effect on the intake of a lactating cow than does her milk yield at any particular time. Though intake was found to increase by 1 kg/day per 10 kg increase in milk yield, it is difficult to relate intake at any stage of lactation to the performance of the cow at the same time (Bines 1985). For example in early lactation when milk yield can be very high, intake is notably depressed.

In the early 1960s voluntary intake by ruminants was thought to be limited by the capacity of the rumen. This phenomenon which is sometimes known as gastric distension was illustrated by Campling and Balch (1961), Davies (1962) and Welch (1967).

A series of experiments by Conrad *et al* (1964) indicated that both physical and physiological factors were involved in determining voluntary intake in dairy cows. Their analysis showed that, when adjusted for body weight and faecal output, feed intake was positively correlated to the digestibility of dry matter below 0.67 and negatively correlated above 0.67 for cows yielding 17 kg of milk per day (Figure 1.1).

Figure 1.1

A Schematic Representation of the Relationship Between Voluntary Food Intake in the Cow and the Concentration of Energy in the Diet



Conrad, Pratt and Hibbs (1964)

From a series of multiple regression analyses, digestibility, faecal DM per day and bodyweight were found to account for most of the variation in feed intake between 0.50 and 0.66 ($r = 0.997$, $p < 0.01$), provided that the rations were mostly roughage. This agrees with Blaxter *et al* (1961) who showed that the rate of passage from and the amount of undigested materials in the digestive tract determined feed intake up to 0.66 DMD (digestibility of the dry matter). Above 0.66 the regression of feed intake on rate of passage of faecal DM was found to be negative and non-significant. As Conrad *et al* (1964) had noted that the average digestible DM intake remained constant and was presumably independent of per cent DM digested when the coefficient exceeded 0.66, it was therefore concluded that the volume of the digestive tract was not restricting the quantity of feed eaten. A highly significant correlation coefficient ($r = 0.81$) was obtained between digestible DM intake and 4% FCM (fat corrected milk yield). However, despite previous analyses illustrating that at low digestibilities variation in milk yield arose from variations in feed intake, at high levels of digestibility milk production was a determinant of concentrate feed intake. The intercept of the curves of intake for both low and high digestibilities is the point at which physical limitations on eating capacity vanished and metabolic control of intake became dominant. The point where physical and metabolic control diverge was not fixed at 0.67 DMD, but was found to vary depending on milk yield. The general conclusion drawn from this work is that intake of forages is primarily controlled by physical means, while the intake of more concentrated diets is controlled mainly by the energy requirements of the cow (Forbes 1986).

Many factors have been subsequently found to influence metabolic control of intake. Lipostatic regulation, the maintenance of body weight through the control of body fat content, was first suggested by Kennedy (1953) and later postulated to influence intake in ruminants by Bines *et al* (1969). More recent work by Garnsworthy and Jones (1987) clearly illustrated that thin cows had significantly higher intakes of DM, digestible DM and ME than fat cows. The control of voluntary intake by blood glucose levels, glucostatic control, was suggested by Mayer (1953) but subsequently was found to have no effect on intake in poultry (Richardson 1970) or ruminants (Muller and Colenbrander 1970). Experimental results illustrating that glucostatic control does not have an effect in ruminants can be explained by the mechanisms of digestion. The main end products of carbohydrate fermentation in the rumen are the three volatile fatty acids; acetic, propionic and butyric. It is these volatile fatty acids, not glucose, which are the major source of energy to the host animal, and are transported from the rumen

to the liver.

The thermostatic theory that animals maintain a relatively constant body temperature and that heat production is proportional to the weight of food eaten (Forbes 1986) has also been found to be consistent with voluntary intake, for example shearing stimulates intake in sheep (Ternmouth and Beattie 1971).

When certain chemicals have been added to the rumen or intestine either singly or in combinations, varying effects on intake have been reported. Chemostatic regulation involving volatile fatty acid infusion has been found to depress concentrate intake (McDonald *et al* 1981) with acetate having the greatest depressive effect (Baile and Forbes 1974) on intake mediated through receptors in the rumen wall (Baile 1971). Hormones play a part in the control of food intake in female animals, an important example being the depressive effect of oestrogen on pigs in oestrous (Friend 1973) and on ruminants during pregnancy (Forbes 1971).

Many of the effects which have been attributed to chemoreceptors could be due to osmotic effects rather than specific chemical actions on the rumen wall. This has been found during infusion of sodium salts of short chain fatty acids into the rumen of sheep causing a depression in intake (Ternmouth and Beattie 1971).

To summarise, voluntary food intake is affected by liveweight, pregnancy and lactation. Furthermore it can be said that in situations where the physical bulk of the diet does not limit intake, metabolic control plays an important role in the regulation of intake. The main factors involved are lipostatic, thermostatic, chemostatic and osmotic control. Most of these factors operate by a negative feedback mechanism and it is likely to be the sum of these signals that controls satiety and hunger (Forbes 1986).

1.5 FORAGE INTAKE

The control of forage intake, whilst influenced by the factors described in section 1.4, is primarily governed by the proportions of cell contents and cell wall in the forage which in turn determine digestibility.

Osbourne *et al* (1974) showed that voluntary intake was more highly correlated with the content of cell walls ($r = -0.83$) than with that of digestible organic matter i.e. cell

contents ($r=0.42$). Hence the high correlations between the digestibility of diets and their intake arise largely because of the high correlation that exists between cell wall contents and the digestibility within one forage species.

1.5.1 Stage of Growth of the Plant

The stage of growth of the plant has a great effect on voluntary intake of a forage, due to the ratio of cell contents to cell walls. At an early stage of growth where the proportion of cell contents is high the young leaves of temperate grasses are very digestible, with values of between 0.8 and 0.9. As senescence occurs digestibility falls to 0.7 (Forbes 1986). At an early stage of growth plant stems are highly digestible, but this falls to around 0.5 at maturity due to lignification (Forbes 1986). Thus combined with the changes in the proportion of stem:leaf as will subsequently be described in more detail highly digestible forages will result in higher intakes than will mature, less digestible crops (Minson 1982).

The cell walls consist of pectic substances, structural polysaccharides e.g. hemicellulose and cellulose plus lignin. As a plant matures, the proportion of cell wall increases from 35% to 60% at maturity. It is the increase in lignin and in particular its spatial distribution which accounts for most of this increase (Gill *et al* 1989).

The cell contents comprise the cell nucleus and cytoplasm and hence account for a major part of the proteins, peptides, nucleic acids, lipids, sugars and starches found in the whole plant. As the plant matures the cell contents decrease in proportion from 65% to 40% with a particularly marked decrease in the amount of protein (Gill *et al* 1989).

The factors affecting the proportion of cell wall to cell contents will now be examined as they indirectly affect forage intake.

1.5.2 Forage Species and Cultivars

Forage species differ in their resistance to breakdown in the rumen and thus their intake will differ. For example, legumes have a lower resistance to breakdown and are retained in the rumen for a shorter time than grasses (Ulyatt 1970). This lower resistance is explained by Ulyatt (1970) to be a consequence of the smaller proportion

of cell wall constituents and a greater proportion of cell contents. Therefore in comparison with grasses legumes are consumed in greater quantities.

Differences in forage voluntary intake have been found between cultivars. The largest difference of 8 g/kg metabolic weight was found between cultivars of *Phleum pratense* S.51 and S.352 (Walters 1971). This difference in intake was attributed to a difference in lignin content as the yield, leafiness, growth stage and content of digestible organic matter of the two grasses were similar.

1.5.3 Particle Size

Grinding or mincing forages prior to feeding has been found to affect voluntary intake. Where animals are fed predominantly forage based diets grinding the forage increases intake (Campling and Milne 1972). For example when a silage was minced and fed to wether sheep, intake was 19% greater for the minced compared to the normal silage (Thomas *et al* 1986). This was despite the depression in digestibility that is found to occur due to increased rate of passage in sheep when the particle size is between 5 and 10 mm.

The process of both grinding and mincing forages will break open the plant cells making the cell contents more available for digestion. In poor quality roughage which will contain a high proportion of cell walls the grinding and mincing will break down structural lignin bonds making the cell contents available for digestion. Thus intake is higher (Minson 1990). In good quality roughage, however, these lignin bonds will not be present, hence reducing particle size will not have such a marked effect.

1.5.4 Soil Fertility

A further factor, the fertility of the soil, has been found to affect forage intake. Despite the effect of increasing cell contents, fertiliser nitrogen has been found to have no consistent effect on the voluntary intake of either temperate or tropical forages (Minson 1990). Whilst some workers have found a positive response, others have found no response. For example, Minson (1990) found a mean increase of 0.3 g/kg metabolic liveweight when sheep were fed forage which had received a high rate of nitrogen fertiliser compared to a low rate. This increased the proportion of cell contents to cell wall, hence intake was greater. Where the nitrogen content of a forage

is low, i.e. below 10 g/kg intake can be depressed. This deficiency can be overcome, however, by the judicious use of fertiliser or alternatively by feeding a protein supplement (Milford and Minson 1965).

1.5.5 Climate

Climatic conditions have been found to affect forage intake indirectly through the proportion of cell wall to cell contents. Drought and frost will depress voluntary intake if they cause a loss of leaf, or if the frosted plant becomes infected with microorganisms (Minson 1990). A more direct effect on forage intake was light intensity: Hight *et al* (1968) found a fall of 9 g/kg metabolic liveweight when Lolium perenne was grown in the shade. This depression was attributed to a fall in soluble carbohydrates (cell contents) and a rise in lignin concentration (cell walls).

1.5.6 Conclusion

Thus the intake of forage has been found to be governed by the proportion of cell contents to cell walls. This ratio affects the digestibility of the forage. A high proportion of cell contents implies a highly digestible forage which will be consumed in large quantities, whilst a high proportion of cell walls (hemicellulose and lignin bonded together) implies a forage of low digestibility which will be eaten in small quantities.

There are several factors which affect this ratio of cell contents to cell walls and hence digestibility and forage intake. These include forage species and cultivars, soil fertility, climate and the most important factor - stage of growth of the plant. Whilst not influencing the ratio of cell contents to cell walls, particle size affects digestibility and hence forage intake.

1.6 CONTROL OF SILAGE INTAKE

It is well established that the voluntary intake of silage is less than that of the corresponding fresh forage. In a review of the literature Demarquilly (1973) showed a mean reduction of 0.33 (range 0.01 - 0.64) for sheep and furthermore this depression was found to be greater for sheep than for cattle (Demarquilly and Dulphy 1977). The extent of this reduction is much greater with grass than legume (Thomas *et al* 1985) or maize silages (Wilkinson *et al* 1976).

The reason for the reduction in intake of silage from fresh grass or hay of a similar digestibility is generally accepted to be due to the presence of fermentation end products in the silage (Minson 1990, Gill *et al* 1989). Due to the complexity of the fermentation and the large number of end products formed which are responsible for the silage composition, intake of a specific silage is likely to be a combined response to a number of variables. This has been concluded in response to single variable significant relationships only explaining part of the discrepancy in silage intake.

Though the reasons for the low intake of silage are not well understood, there are several factors which have been found to contribute to this depression. In addition to the fermentation end products these include dry matter content, fermentation pattern, additives and chop length. These factors will be discussed in turn below.

1.6.1 Dry Matter Content

Silage dry matter concentration has been found to be positively correlated with voluntary intake (Rook and Gill 1990, Wilkins *et al* 1971, Wilkins *et al* 1972). Furthermore Wilkins *et al* (1971) claimed that 74% of intake variation was due to dry matter content, total nitrogen, lactic acid as a percentage of total acids and Fleig index.

Wilting a grass crop prior to ensiling is a relatively straight forward method of increasing the dry matter of a crop. Wilted silage has in many trials been found to be consumed in greater quantities than the corresponding unwilted silage (Peoples 1982, Forbes and Jackson 1971, Zimmer and Wilkins 1984). The magnitude of this increase has, however, been found to vary considerably. In two experiments, Peoples (1982) found the DM intakes of wilted silage to be 33 g/kg DM and 183 g/kg DM greater than those of the corresponding unwilted silage. Forbes and Jackson (1971) found intake of a wilted silage of DM 350 g/kg to be 520 g/kg DM greater than that of the unwilted material of DM 190 g/kg .

In contrast to these large differences in intake, the Eurowilt studies (Zimmer and Wilkins 1984) illustrated that the intake of the higher DM silages (wilted to approximately 300 g/kg) was an average of 80 g/kg DM greater than that of the lower DM silages (approximately 170 g/kg) but this did not, however, take into account the alcohol content of the unwilted silages, which would reduce the difference. The

difference in intake was partly reduced due to the unwilted silage being of good quality due to treatment with formic acid.

Several studies have been conducted in order to study the effect of water content per se. While Dodsworth and Campbell (1953) found that wetting a silage overnight thus decreasing the dry matter content from 227 g/kg to 169 g/kg, depressed intake in sheep, a more comprehensive study by Moore et al (1960) contradicts this. The addition of water directly to the silage before feeding did not affect the amount of DM consumed. Similarly when water was added to hay to bring its moisture content up to that of wilted silage, there was no decrease in the consumption of hay DM. Finally there was no difference in the intake of wilted silage, dried to the same moisture content as hay.

It can be concluded from these observations that the moisture content of the silage per se does not affect the dry matter consumption of the silage. It is apparent, however, that the moisture content of the crop as it is ensiled does have a marked effect on the dry matter composition of the resulting silage.

1.6.2 Fermentation Pattern

The most generally accepted reason for the intake of silage being lower than that of the fresh forage or hay is the presence of the end products of silage fermentation. As many of these end products are acids, it seems probable that acidity has an effect on the voluntary intake of silage.

The acidity associated with extensively fermented silages has been found to affect intake detrimentally (Thomas and Thomas 1985). Ndwiga et al (1990) claim there is a relationship between the pH of the silage and voluntary consumption by animals. This relationship has been described in greater detail by Thomas et al (1961) and Harris et al (1966). In its simplest terms they suggested that the low intakes commonly encountered with high moisture silage may be due to an associated increased acidity.

In an attempt to overcome the acidity of silage, sodium bicarbonate, a neutralising base can be added. The effect of low intake due to the acidity of silage was illustrated in work carried out by McLeod et al (1970); when the pH of a silage was made less acidic with the addition of sodium bicarbonate, silage DM intake increased. Conversely

when lactic acid was added to lower the pH of the silage, silage DMI fell. Thomas and Wilkinson (1975) also found that an increase in silage DMI resulted from the use of sodium bicarbonate. Feeding a silage they found partial neutralisation also increased consumption of dry matter by 120 g/kg and organic matter by 70 g/kg DM. The mode of action of this partial neutralisation has been explained in several ways. McLeod *et al* (1970) forwarded the theory that intake responses from silage partially neutralised with sodium bicarbonate is a result of reduction in free acid content and not from an increase in sodium content. Other reported actions of added sodium bicarbonate as a neutralising agent include its effect on improving acid-base balance (Erdman *et al* 1988), increasing buffering capacity of rumen fluid (Orth and Kaufman 1966) leading to increased fibre digestion (Synder *et al* 1983) and the changes in molar proportions of volatile fatty acids in the rumen (Erdman *et al* 1988).

Harris *et al* (1966) suggested that the intake of silage over a prolonged feeding period may be limited by low pH, associated with a high content of organic acids. There has been a considerable number of experiments designed to investigate this relationship, the majority concerned with lactic acid.

Wilkins *et al* (1971), from studies using 70 silages fed to sheep, found that silage DM intake was positively correlated with lactic acid as a percent of total acids. The effect of lactic acid *per se*, however, appears to be less well defined. McLeod *et al* (1970) clearly illustrated that acidity was highly negatively correlated with silage DM intake. The relationships between titratable acidity, total content of organic acids and lactic acid with silage DM intake were $r = -0.996$, $p < 0.01$, $r = -0.991$, $p < 0.01$ and $r = -0.998$, $p < 0.001$ respectively. It should be noted that the correlation coefficient between silage DM intake and lactic acid was found to be the strongest. The relationship between DM intake and pH, however, was found to be positive ($r = 0.975$).

McLeod *et al* (1970) also evaluated this relationship between lactic acid and silage DM intake. They added lactic acid to silage to raise the concentration of acid from 54 g/kg DM to 113 g/kg DM, which lowered the pH from 5.4 to 3.8. The result of this was to decrease silage DM intake by 0.22.

Thomas *et al* (1980) fed silage to calves either without the addition of extra lactic acid (131 g/kg DM) or with the addition of 50 g/kg DM of lactic acid (173 g/kg DM). The addition of lactic acid was found to reduce silage intake significantly by 0.36 kg DM

per animal per day or 2.8g per kg liveweight per animal per day.

An experiment by Thomas *et al* (1961) illustrated that the addition of lactic acid had no effect on intake, when the acid was placed in the rumen. In an experiment by Morgan *et al* (1980) two well preserved silages of different lactic acid concentrations (165 g/kg DM and 34 g/kg DM) respectively were fed to sheep. Daily intakes were high (117 g/kg metabolic liveweight and 106 g/kg metabolic liveweight respectively) and the values did not differ significantly. A further factor which complicates this comparison is that the silage with the low lactic acid content had been wilted (365 g/kg as compared to 175 g/kg).

As wilting reduces the lactic acid content of a silage (Peoples 1982) part of the improvement in intake from wilting could result from the reduction in lactic acid content, as both Thomas *et al* (1980) and McLeod *et al* (1970) showed that lactic acid depresses intake. Wilkinson *et al* (1976) concluded that intake was restricted by the free-acid content of unwilted silages and that possibly there was a critical level of total acids below which the intake of silage is limited. They further concluded that lactic acid, which may be produced in large quantities as a result of excessive fermentation, may be of greater importance in limiting intake than a by-product of protein degradation e.g. ammonia.

The effect of a further organic acid, acetic acid on dry matter intake, has been investigated in a variety of trials. Wilkins *et al* (1971) reported a negative correlation between the acetic acid content of 70 silages and their voluntary consumption by sheep. This effect was subsequently investigated in greater detail by Hutchinson and Wilkins (1971). Three silages of low, medium and high levels of acetic acid (20 g/kg DM, 50 g/kg DM and 88 g/kg DM) were fed to wether sheep. Dry matter and pH were the same for each silage. Differences in dry matter intake were small and non-significant. The infusion of 69 g of acetic acid into the rumen of sheep, in a further experiment by Hutchinson and Wilkins (1971), resulted in severe depressions in intake, 11 g/kg metabolic liveweight. Several earlier experiments investigating the effect of acetic acid infusion into the rumen also illustrated its depressive effect in this situation. Both Ulyatt (1965) and Rook *et al* (1963) found significant effects in intake with the ruminal infusion of acetic acid. These experiments indicate that although acetate *per se* does not limit silage intake, large quantities of free acetic acid may bring about depressions in silage consumption, due to the possible effect of acid/base imbalance.

The addition of both lactic and acetic acids to a silage has been investigated. Thomas *et al* (1961) found that the addition of either lactic acid or acetic acid had no significant effect on the intake of silage. When 576 g of lactic acid and 252 g of acetic acid were added together, voluntary intake did not decrease significantly either. Edwards *et al* (1976) also found no significant differences in silage intake when lactic acid and acetic acid were added either singly or together and fed to sheep.

A study involving the addition of both lactic and acetic acids to silage prior to sham feeding to sheep was carried out by Buchanan-Smith (1989). The amounts of acid ranged from 0 g/kg of DM lactic acid and acetic acid to 53.2 g/kg of DM lactic acid and 35.4 g/kg DM of acetic acid. The voluntary intake of silage increased in a linear manner and was significant ($p < 0.05$). From this regression it was predicted that 10 g/kg DM of added lactic acid and acetic acids would enhance intake by 7.6 g DM whilst 10 g/kg acetic acid would depress intake by 20.4 g/kg DM. From these experiments Buchanan-Smith (1989) concluded that the odour or taste of acetic acid caused an adverse response in sheep to silage, however, this effect appeared to be neutralised by elevated levels of other constituents as well. Furthermore he went on to say that these observations support conclusions drawn from other feeding trials, namely that acetic acid levels in silage are negatively associated with silage intake.

Lactic and acetic acids have been found to be negatively correlated with silage DM intake by Rook and Gill (1990). Furthermore they postulated that the absolute levels of the volatile fatty acids were more important than their level in relation to other acids, with respect to voluntary intake.

The relationship between butyric acid and silage DM intake has not been as thoroughly documented as those between lactic and acetic acids and intake.

Wilkins *et al* (1978) found that butyric acid was not significantly related to intake of non-formaldehyde treated grass silage by sheep. They analysed the relationships between the existing content of butyric acid in silage, rather than adding varying levels of the acid. The highest value of butyric acid included in their investigation was 8.3 g/kg DM, which is quite low (MAFF 1990). Higher levels of butyric acid, added via intraruminal infusions, were found to depress significantly intake of silage by sheep (Ulyatt 1965). The effect on intake of mixing butyric acid into the silage would be an

area worth reviewing, but unfortunately no literature concerning this has been found.

The relationship between ammonia nitrogen and silage DM intake has been described by several authors. In most cases it is determined from linear regression of existing silage analyses. Wilkins *et al* (1971) found that ammonia nitrogen was negatively correlated with silage DM intake and accounted for 0.31 of the variation in intake of silage by sheep, while Wilkins *et al* (1978) also found it strongly negative accounting for 0.42 of the variance in non-formaldehyde treated grass silages fed to sheep.

Rook and Gill (1990) analysed three large sets of data and also found that the relationship between silage DM intake and ammonia nitrogen was strongly negative, accounting for between 0.16 and 0.44 of the variation in intake.

The addition of ammonia to silage with a resultant decrease in silage intake was reported by Buchanan-Smith (1989). Though not significant, there was a pronounced trend of decreasing silage DM intake with increasing ammonia content.

AFRC (1991) concluded from a review that there is no evidence that ammonia itself has a deleterious effect on intake. Rook and Gill (1990) also went on to state that there is no definitive evidence in the literature that ammonia nitrogen *per se* limits intake. They suggested that this may be an indirect effect due to the concentration of ammonia nitrogen with some other variable which is the causal agent.

1.6.3 Additives

The use of additives to control the fermentation has been discussed in section 1.2.5.4. This section will give a brief description of additive use on silage intake.

Several additives have been developed for commercial use in order to alleviate problems associated with undesirable fermentation. Formic acid treated silages generally result in a greater silage intake than untreated silages (Castle and Watson 1976), the reasons for this being that formic acid application results in decreasing concentrations of fermentation acids and increasing contents of water soluble carbohydrates in silages (McDonald 1981). When crops of dry matter 190-220 g/kg and rich in water soluble carbohydrates are ensiled, the performance of animals offered untreated silages does not tend to be significantly different from those treated with

formic acid (Hinks *et al* 1976).

The effects of formic acid on silage DM intake have been reviewed recently by Steen (1991). A significant increase of 0.82 kg DM/day in silage DM intake was calculated as the mean of 14 trials involving dairy cows. Not only was intake higher, but animal performance factors e.g. milk yield and liveweight gain were also higher for the formic acid treated silages over the control silages.

The effects of sulphuric acid on intake have been mentioned briefly in section 1.2.5.4. Steen (1989) compared the animal performance and intake of sulphuric acid treated silages with untreated control silages. Silage intake was marginally greater at 4%, whilst performance parameters e.g. milk protein yield were significantly less. The negative responses in animal performance to sulphuric acid treatment in these studies may have been related to the copper deficiency it causes (O'Keily *et al* 1989). However, in the experiments examined by Steen (1989) sulphuric acid had been fortified with copper.

Silage intake and animal performance have been found to be more variable when an inoculant is the additive. Kennedy *et al* (1989) found that an inoculant gave a better performance than no treatment or formic acid in only one out of four trials. Steen (1991), however, in a review of recent experiments involving data from eight trials, found that an inoculant significantly increased silage DM intake by a mean of 0.46 kg/day. Only in one of these eight experiments did an inoculant treated silage not have a greater intake than the untreated control. In this one trial milk yield was higher with an inoculant treated silage in comparison to an untreated silage; there was no difference in liveweight gain.

In grass of low sugar content (19g/kg DM), Steen (1989) showed that an inoculant treated silage gave a good level of performance from a relatively poor silage. Formic acid was found to give a similar performance from a better quality silage.

Experiments by Thomas *et al* (1991) illustrated that in well preserved silages the use of inoculants can result in an increase in animal performance. However, with a badly fermented silage, use of an inoculant resulted in a marked increase in liveweight gain equivalent to that of formic acid. In these experiments the increases in gain were not related to improvements in fermentation characteristics measured and hence the mode

of action of biological additives is not clear.

The above factors, *viz* DM content, fermentation pattern and additive use all directly affect the control of silage intake. A further factor which indirectly affects the intake of silage, and is mentioned briefly, is chop length.

1.6.4 Chop Length

The intake of silage can be increased by chopping the grass prior to ensiling which improves fermentation in the silo whilst chopping after ensilage increases the rate of passage through the digestive tract (Forbes 1986). As compared with that of chopped silage the voluntary intake of unchopped silage is lower due to the slower rate of particle reduction, consequently animal performance in growing cattle has been shown to be higher on finely chopped silage than on coarsely chopped material (Hastings 1974). McDonald (1981) attributed the greater animal performance with chopped silage to not only the increased intake of digestible organic matter but also to an increase in the efficiency of utilisation of the digestible organic matter.

There are interactions between silage chop length, concentrate intake and type of concentrate (Castle 1982). Sheep have been found to respond well to an increasing fineness of chop length but cattle appear to be much less responsive, due to the practice of feeding concentrates in the cattle experiments (Marsh 1979). For example, with steers given no supplementary concentrates Murdoch (1965) found that the DM intake was 220 g/kg DM greater with chopped than with lacerated silage. In contrast, with a dairy concentrate included in the ration, Dulphy and Demarquilly (1975) reported an increase of only 120 g/kg in DM intake when the silage was of 40 mm chop length compared to 100 mm. Castle *et al* (1979) also found that when silage was the sole food of dairy cows DM intake was increased by 330 g/kg DM as a result of chopping the silage to 9mm. When the same silage of the same chop length was supplemented with concentrates, the DM increase due to short chopping was 140 g/kg DM.

1.6.5 Conclusion

The voluntary intake of silage is generally less than that of the corresponding fresh forage or hay. This reduction in intake, which varies considerably, is generally

attributed to the presence of fermentation end products in the silage. Due to the complexity of the fermentation and the large number of end products formed, intake of a specific silage is likely to be a combined response to a number of variables, hence the large degree of variation in intake amongst silages. The concentration of fermentation end products e.g. lactic acid, acetic acid and ammonia, both in the silage and when infused intraruminally has been found to affect silage intake directly. Chop length has been found to affect silage intake indirectly, with fine chopped silage being consumed in greater quantities than unchopped silage. Chopping the grass before ensiling improves the fermentation of the silage, whilst chopping after ensilage improves the rate of passage through the digestive tract.

1.7 SUPPLEMENTATION

In many cases the feeding of forage as a sole feed provides insufficient energy to achieve the required level of animal production. Thus it is common practice to feed concentrates; up to 70% of the total diet of dairy cows is sometimes fed as concentrates (Gill *et al* 1989). Supplementation increases the total ME energy intake, but where an increased proportion of ME is derived from concentrates, improvements in the efficiency of utilisation of ME for growth and fattening may occur (Gill *et al* 1989). Supplementation may be either in the form of mixed compounds or in the form of individual components, straights.

1.7.1 Substitution Rate

Very few energy supplements have a purely additive effect on forage intake (Gill *et al* 1989), the feeding of concentrates generally depressing intake. When forage is supplemented with a fixed level of concentrate due to the animal eating either to maintain a constant energy level (Thomas 1987) or within its capacity to consume indigestible bulk (Forbes 1986) forage intake decreases. Additionally starch-based supplements tend to decrease rumen pH and fibre digestion and as a consequence forage intake. The probable reason for concentrates substituting for forage rather than supplementing it is the depressed cellulose digestion of the diet (Vadiveloo and Holmes 1979). The fermentation of supplementary soluble carbohydrates reduces the cellulolytic activity of the rumen microorganisms and leads to a slower passage of roughage through the digestive tract and a reduction in forage intake. This decrease is termed the substitution rate or replacement rate and is defined as the decrease in the

intake of silage dry matter per kilogram increase in concentrate dry matter.

Stone *et al* (1964) and Campling and Murdoch (1966) quantified the relationship between concentrate amount and silage intake. They suggested that when 33-85% of the dry matter intake was concentrate, the voluntary intake of silage was depressed, but where small quantities of concentrate were fed silage intake tended to be unaffected.

A variation in substitution rate is found by different authors (Table 1.3). Steen (1978) quotes the mean substitution rate of ten studies to be 0.31, whilst Thomas (1980) gives 0.50 as the mean substitution rate for sixteen studies. Gordon (1984) calculated a mean substitution rate of 0.26 kg silage DM/kg concentrate DM, which is considerably lower than other values. His trials were conducted with a wide range of concentrate levels, from 3.6 kg/day to 9.4 kg/day and the low levels of concentrate fed would lower the substitution rate. This illustrates the wide range in substitution rate that can occur due to combinations of animal, forage and supplement factors.

There are three main factors affecting the substitution rate in ruminants: the forage, the supplement and the animal. Though they are interdependent, they will be dealt with separately.

1.7.1.1 Animal Factors

The effects of animal factors on intake in general have been previously mentioned in section 1.4.1. Some of these affect the substitution rate, hence will be discussed below. The most widely documented animal effect associated with substitution rate is the stage of lactation. The majority of trials conclude that the rate of substitution declines as lactation progresses (Bines 1985, Phipps *et al* 1987).

Phipps *et al* (1987) suggested that the substitution rate (SR) at different stages of lactation for good and average qualities of silage could be described by different equations. For a good quality silage:

$$SR = 0.972 - 0.051X - 0.0016XP^2$$

and for an average quality silage:

$$SR = 0.516 - 0.015X$$

where X = week of lactation.

Table 1.3

COMPARISON OF SUBSTITUTE RATES

| Author | Substitution Rate |
|----------------------------------|-------------------|
| Gordon (1984) | 0.26 |
| Steen (1978) | 0.31 |
| Castle <i>et al</i> (1981) | 0.35, 0.45 |
| Mayne and Gordon (1980) | 0.40 |
| Steen and Gordon (1980) | 0.40 |
| Castle <i>et al</i> (1966) | 0.46 |
| Thomas (1980) | 0.50 |
| Castle and Watson (1975), (1976) | 0.51 |

Contrary to this Ostergaard (1979) found that the substitution rate increased as lactation progressed. This occurred even at fixed levels of concentrate feeding, as opposed to increasing concentrate fed with increasing milk yield. Some workers state that the yield-status of dairy cows affects substitution rate. Whilst Thomas (1980) noted a trend for high yielding animals to display higher substitution rates, Gordon (1984) found no difference; both high and low yielding groups of cows gave the same substitution rate.

1.7.1.2 Characteristics of the Forage

The quality of the forage fed is a major determinant of the substitution rate. As the quality of the roughage increases, so does the substitution rate (Bines 1979). The substitution rate declines as the intake characteristics of the forage decline, be this digestibility, or fermentation quality (Blaxter and Wilson 1963, Wilkins 1974). This is exemplified in a review by Bines (1985) who found that poor hay had the lowest substitution rate at 0.17 and spring grass had the highest substitution rate at 1.00 (Table 1.4).

The response of supplements with silage is different from that with hay or grass. With silage, the effect of digestibility on substitution rate is modified by the effects of fermentation acids.

The digestibility of the forage has an influence on the substitution rate, with the greatest depression occurring in highly digestible silages (Leaver 1973, Moisey and Leaver 1984, Campling and Murdoch 1966).

On the other hand, Thomas (1987) analysed the data from Bines (1985) and did not find a relationship between digestibility and substitution rate. Likewise Kristensen (1983) concluded from a series of nine experiments, with a range of substitution rates from 0.65 to 0.85, that there was no relationship between digestibility and substitution rate.

Blaxter (1980) and Osbourn (1980) hypothesised that one feed substitutes for another in proportion to their digestibility, but taking account of intake and the effects on the microbial activity of the rumen. Thomas *et al* (1981) did not find this to be the case

Table 1.4**COMPARISON OF SUBSTITUTION RATES FOR DIFFERENT SILAGES**

| | Substitution Rate |
|-------------------|--------------------------|
| poor hay | 0.17 |
| poor grass silage | 0.32 |
| lucerne hay | 0.44 |
| grazing | 0.55 |
| dried grass | 0.55 |
| zero grazing | 0.6 - 0.7 |
| medium grass hay | 0.63 |
| maize silage | 0.63 |
| good grass silage | 0.68 |
| lucerne wafers | 0.78 |
| spring grass | 1.00 |
| Mean | 0.58 |

(Bincs 1985)

when comparing high and low digestibility ryegrass and clover silages (Table 1.5).

The grass silage had the lowest substitution rate, with the high D grass silage having the lowest substitution rate, as well as the lowest intake, due to poor fermentation characteristics (Table 1.5). Thomas *et al* (1981) concluded that across species and in silages where fermentation quality affected intake, substitution rate is more related to the intake of the silage as a sole feed rather than to its digestibility. The higher the intake potential of the silage, the greater will be the depression in silage intake when concentrate is fed.

1.7.2 Level of Supplementation

It has been proposed that both the level and type of supplement can influence the decline in silage intake (Thomas 1987). As the amount of concentrate fed increases, the DM intake of silage decreases curvilinearly (Osbourne 1980). Numerous authors have found that increasing the proportion of concentrates in the diet increases the substitution rate in a curvilinear manner (Ostergaard 1979, Thomas *et al* 1986, Leaver 1973, Butler 1976). The results of trials by Gordon (1984), however, did not illustrate this relationship. Gordon (1984) suggested that this could be due to the use of a much narrower range of concentrate inputs than in the trials where a curvilinear relationship was found.

Phipps *et al* (1987) found that when dairy cows were fed either good or average quality silage of DOM 600 g/kg DM and 680 g/kg DM, as concentrate intake increased, the substitution rate decreased. This occurred at daily concentrate intakes of between 4.3 and 8.4 kg. The effect of feeding a high level of concentrate on substitution rate, however, is complicated by the frequency of feeding. It is difficult for cows to consume in excess of about 8 kg concentrate DM in two feeds (Thomas 1987).

1.7.3 Type of Supplement

"The approach of using specific supplements is a valid one, but the balance of protein, sugar and starch to achieve the maximum response will undoubtedly depend on the composition of the silage, and it is undoubtedly this problem of specifying the appropriate supplementary nutrients for silage of varying composition which remains

Table 1.5**THE INFLUENCE OF DIGESTIBILITY OF SILAGE ON
SUBSTITUTION RATE**

| | Perennial Ryegrass | | Red Clover | |
|--------------------|---------------------------|------------|-------------------|------------|
| | High | Low | High | Low |
| Digestibility | | | | |
| D Value | 67 | 56 | 65 | 60 |
| Silage Intake | 216 | 233 | 274 | 269 |
| (g/kg as solefeed) | | | | |
| Substitution Rate | 0.60 | 0.73 | 0.88 | 0.82 |

the most immediate and important challenge" (Thomas and Thomas 1988).

1.7.3.1 Protein

An animal fed silage is particularly dependent on microbial protein for its supply of amino acids due to the relatively low UDP (undigestible protein) content of silage, despite its having high crude protein and digestible crude protein levels. As the yield of microbial protein per unit of organic matter apparently digested in the rumen is lower on silage than on hay diets (Armstrong (1980), it is likely that cattle fed silage based diets will show a response to supplementary UDP.

Kirby (1981) proposed that there are four methods through which responses to supplementary protein may be mediated:

(i) increased ration digestibility

Thomas et al (1980) fed entire male 117 kg calves silage supplemented with fishmeal. This increased the digestibility of DM, OM and GE.

(ii) improved yield of microbial protein

Oldham and Smith (1982) suggested that a benefit of fishmeal in the diet could be a slow release of ammonia nitrogen in the rumen. Addition of small amounts of amino acids to diets containing urea as the sole nitrogen source increased rumen microbial protein yields (Maeng and Baldwin 1976).

(iii) improved supply of UDP

Beever et al (1982) found that the increase in total amino acid flow to the duodenum was due to an increased out flow rate of UDP when maize silage was fed with a high level of fishmeal inclusion.

(iv) increased supply of essential amino acids from UDP

Improved performance of animals fed supplementary protein may be due to an increased supply of essential amino acids reaching the small intestine. A greater daily LWG was found with fishmeal when compared to soyabean meal supplementation partly because of an increased supply of methionine, lysine and threonine (Hennessey et al 1981).

There are two types of protein supplements which are commonly fed to cattle: vegetable protein straights (e.g. soya bean meal, ground nut meal, sunflowerseed meal) and animal straights (e.g. fishmeal). The latter is a very good source of UDP as the value for the degradability of dietary protein is 0.29 compared to 0.39 for soya bean meal and 0.81-0.63 for other vegetable proteins (Armstrong 1976).

Fishmeal is a most commonly used source of protein for supplementation due to its being a good source of UDP. Its effect on silage dry matter intake and liveweight gain has been found to vary considerably due to the level of inclusion and to the silage type.

An alternative source of protein frequently used in protein supplementation is soyabean meal. It is regarded by McDonald *et al* (1981) as one of the best sources of protein available to animals due to its containing all the indispensable amino acids. The concentrations of cystine and methionine are, however, sub-optimal (McDonald *et al* 1981).

Groundnut meal is not used as frequently as either fishmeal or soyabean meal for supplementation. It is a poorer source of protein than soyabean meal because of its higher degradability (McDonald *et al* 1981).

The response to protein supplementation is primarily governed by an animal's requirements for protein.

Since increased production can result from protein supplementation of both grazing animals and those offered silage it would appear that protein supply may limit production on some forages. Part of this limitation is due to the effect of low protein diets in limiting intake, but even where protein supplementation has no effect on intake a production response may still be observed (Gill *et al* 1989).

Two types of ruminants are known to have high protein requirements: high-yielding dairy cows and young growing cattle, especially when on silage-based diets. In this case the discrepancy between supply and requirement is exacerbated where, due to extensive proteolysis during harvesting and conservation, the supply of amino acids is often below expectations (Gill *et al* 1989).

In addition to an animal's requirements for protein, the nitrogen content of the silage fed also affects the response to protein supplementation. Castle and Watson (1969) found the response to protein supplementation, in terms of milk yield, greater for silages low in protein compared to high protein silages. Silage dry matter intake has also been found to be positively correlated with protein content (Wilkins *et al* 1971, England and Gill 1985).

1.7.3.2 Protein Level

Not only does the source of additional protein in ruminant diets affect intake, but the level of protein supplementation has also been found to influence silage consumption (Table 1.6).

Increasing the protein concentration in a concentrate fed to dairy cows has been found to increase both silage dry matter intake and milk yield (Gordon 1979, Castle 1982, Castle and Watson 1976). Both mean yield and peak yields increased when the crude protein of the concentrate fed increased from 95 to 209 g/kg freshweight (Gordon 1979).

Castle and Watson (1976) found an additional milk yield of 0.49 kg milk per 0.1 kg additional digestible crude protein (DCP), though according to MAFF (1971) the total intake of DCP was adequate without the additional protein.

Whilst young growing animals achieve a greater daily liveweight gain with protein supplementation due to their higher protein requirements, mature store cattle can be fattened at sufficiently high rates on good quality silage alone. Thus relatively little work on protein supplementation of silage diets for fattening cattle has been carried out (Kirby 1981).

Feeding a high protein concentrate has been found in many cases to result in a higher silage DM intake and a lower substitution rate than feeding a lower protein concentrate (Reeve *et al* 1986, England and Gill 1985, Gill and Castle 1983, Mayne and Gordon 1984, Gill and England 1984).

Gill and Castle (1983) fed concentrate, at a crude protein content of either 128 g/kg DM or 202 g/kg DM, with a good quality silage to dairy cows. In two concurrent

Table 1.6

**EFFECT OF INCREASING THE LEVEL OF FISHMEAL
SUPPLEMENTATION ON TOTAL DMI AND LWG**

| Fishmeal Inclusion (g) | Total DMI (g/kg DM/day) | Total DLWG (kg) |
|---|--|----------------------------------|
| 0 | 2.48 | 0.25 |
| 50 | 2.65 | 0.35 |
| 75 | 2.79 | 0.43 |
| 100 | 2.78 | 0.46 |

experiments the silage DM intake was higher for the high protein content than for the low protein content concentrate, though this effect was non-significant ($p>0.05$).

In comparison with the experiments by Gill and Castle (1983), those of Reeve *et al* (1986) illustrate the same effect at a much higher protein level. Feeding a concentrate of crude protein content 403 g/kg DM compared to 214 g/kg DM to dairy cows resulted in a higher, though non-significant, silage DM intake. Feeding 6 kg of a soya/fishmeal based concentrate compared to 3 kg depressed silage DM intake but increased the effect of the protein level.

| | 3 kg | 6 kg | 3 kg | 6 kg |
|-------------------|------|------|------|------|
| Crude Protein | 214 | 214 | 403 | 403 |
| Silage DM intake | 13.1 | 11.0 | 13.9 | 12.3 |
| Substitution Rate | | 0.59 | | 0.37 |

(Reeve *et al* 1986)

Mayne and Gordon (1984) also investigated the effect of different amounts of low and high protein concentrates, both barley based and sugarbeet pulp based. Increasing the protein content increased the silage DM intake for either 7 kg or 10 kg of the barley based concentrate. Increasing the protein content whilst feeding 10 kg of the sugarbeet pulp based concentrate had no effect on silage DM intake. As with previous experiments, all effects were non-significant.

England and Gill (1985) found that the crude protein content of the concentrate significantly ($p<0.01$) affected the silage DM intake in four month old British Friesian castrates. These protein contents at 122 g/kg DM, 138 g/kg DM and 148 g/kg DM are considerably less than those of other experiments described. The effect of increasing the protein content from 122 g/kg DM to 138 g/kg DM had a greater increase in intake per g/kg CP; 1.7 g silage DM per kg CP compared to 0.05 g silage DM per kg CP for the change from a crude protein level of 138 g/kg DM to 148 g/kg DM.

Both Thomas *et al* (1980) and Gill *et al* (1987), contrary to the above experiments, found that fishmeal inclusion to give a high crude protein concentrate depressed silage DM intake.

In the above trials, silage intake increased on the inclusion of fishmeal in the diet where the nitrogen content of the silage was low and decreased where the nitrogen content

was high. For example, the total nitrogen content of the silage fed by Gill and England (1984) was 15.5 g/kg DM and that fed by Thomas *et al* (1980) was 18.7 g/kg DM. Thus the inclusion of fishmeal in the diets of silages with a low total nitrogen content will overcome the limitations of a low protein supply (Thomas and Gill 1988). Both where silage alone is fed and where concentrates are also fed, silage intake is positively related to nitrogen content (Wilkins *et al* 1971, England and Gill 1985, Mayne and Gordon 1984).

From these experiments it can be concluded that the level of protein supplementation affects silage intake; the higher the crude protein content the greater the silage intake. This effect appears to be dependent on the nitrogen status of the silage, with a response to protein supplementation occurring only in low nitrogen silages.

1.7.3.3 Starch

Starch based concentrates e.g. barley are frequently used as supplements to silage. They are fed primarily for the energy they provide as silage alone cannot always provide sufficient energy for high levels of production.

By increasing total ME intake through the use of concentrate supplements, where an increased proportion of ME is derived from the concentrates, improvements in the efficiency of utilisation of ME for growth and fattening may occur (Gill *et al* 1989). In a trial quoted by Thomas and Gill (1988) steers fed a barley supplemented diet required 9% less ME to achieve the same energy gain as those given silage as a sole feed. Though this occurred when silage intake was restricted, the increase in efficiency was thought to be associated with a lower proportion of the energy being derived from the cell walls (Thomas and Gill 1988).

Very few energy supplements have a purely additive effect on forage intake. Starch based supplements tend to decrease rumen pH and fibre digestion and as a consequence forage intake (Osborn 1980). This reduction in silage DM intake is attributed to a reduction in the ruminal digestibility of the silage because of the effect the cereal starch has on the rumen microorganisms (Castle 1982). High starch compounds e.g. barley have a particularly marked effect on silage intake and in four comparisons the mean reduction in silage DM intake was 0.51 kg per kg barley DM, over a range of 3.3 to 6.0 kg barley per day per cow (Castle and Watson 1976).

Substitution rates for starch based concentrates were 0.43 and 0.44 kg silage per kg barley DM as reported by Castle *et al* (1981).

A high substitution rate where barley was the sole supplement to silage was found by Etala and Lampila (1978). From 13 trials with 296 Ayrshire dairy cows fed an average of 2.1 kg barley per day, they calculated the substitution rate to be 0.64.

Cereal based supplements have been found to have marked effects on the digestibility of chemical components and energy. For example, Kaiser *et al* (1983) found that feeding a concentrate with a high starch content to calves increased dry matter, organic matter and energy digestibility but reduced nitrogen and cellulose digestibility.

Depression in the digestibility of crude fibre was reported by Burroughs *et al* (1950) who found that the addition of 1.8 kg of starch fed to steers reduced the digestibility of crude fibre by 47%, from 60% to 13%. Likewise the addition of maize/potato starch to hay reduced the digestibility of cellulose (Head 1953). Contrary to this, however, Arias *et al* (1951) found that small amounts of starch had a stimulating effect on cellulose digestion. Furthermore, in agreement with Head (1953), Kaiser *et al* (1983) found that maize starch depressed the digestibility of the nitrogen fractions, as did potato starch to a greater extent.

Starch has been found to affect nitrogen retention when fed as a supplement, both positively and negatively. Whilst Kaiser *et al* (1983) found nitrogen retention in g/day to be reduced by starch supplementation, Burroughs *et al* (1950) found urinary nitrogen to decrease, and hence nitrogen retention to increase; in contrast Head (1953) stated that nitrogen retention was not decreased.

According to Kaiser *et al* (1983) one of the effects of starch supplementation was that, despite reducing silage DMI, total DMI was not influenced. This implies that the substitution rate is 1.0. In practice, however, a substitution rate of 1.0 rarely occurs, except in young, highly digestible spring grass (Bines 1985). The inevitable consequence of supplementation with starch based concentrates, however, is that they do replace rather than supplement silage (Thomas and Gill 1988) thus reducing silage dry matter intake but at the same time increasing total dry matter intake.

1.7.3.4 Fibre

In recent years there has been a trend towards the replacement of starch based supplements with fibre based supplements; the basis of this lies in the belief that fibrous concentrates have a lower substitution rate than starch based ones. The results from trials by Thomas *et al* (1986) with silage and Sutton *et al* (1987) with hay indicated that for concentrate inputs of between 6 and 12 kg DM per day no differences in substitution rate existed. However, both authors found that forage intake was higher with the fibre based supplement.

There are three types of fibre based supplements which are typically used as supplements to silage; hay, dried grass cubes and sugar beet pulp. Each of these will be examined in turn with comparison where applicable.

The first of these supplements, hay, is sometimes fed with silage. In a series of eight trials where silage was supplemented with hay the mean reduction in silage intake was 0.84 kg DM per kg hay DM. This reduction occurred with hays of DOMD values ranging from 580 to 700 g/kg and with silages ranging from 598 to 683 g/kg (Castle 1982). Forage intake was non-significantly higher and as a result Castle concluded that long hay, regardless of its DOMD, was of little value as a silage supplement.

Ettala and Lampila (1978) also found hay to have a high replacement value when used as a supplement to silage; silage DM intake was found to be reduced by an average of 1.15 kg/kg hay DM.

The physical form of the hay supplement has been found to affect both intake and liveweight gain. In a further experiment comparing high quality hay in either the long or the ground and cubed form, the total intake of silage plus hay was increased by grinding and cubing the hay (Castle 1982).

Additionally, the physical form of the hay supplement was found to influence the liveweight gains of the experimental animals in a trial by Gill *et al* (1981). At 6.5 g DM/kg, pelleted hay produced a similar LWG to dried grass but both were markedly higher than chopped hay.

The second fibrous supplement to be examined is dried grass pellets or cubes. Dried

grass cubes were found to reduce silage intake by 0.36 kg DM/kg DM (Castle 1982) and compared favourably in substitution rate with other fibrous supplements. When fed with silage at either 2, 3 or 4 kg/10 kg milk, on average the intakes of silage DM and total DM were significantly higher in the treatments containing dried grass as compared to barley ($p < 0.01$) (Castle and Watson 1975). Additionally whilst the highest total DM intake was achieved when dried grass was fed at 4 kg/10 kg milk, the greatest silage intake was achieved whilst feeding 3 kg/10 kg milk.

The final fibrous silage supplement to be considered here is sugar beet pulp. Dried sugar beet pulp is high in fibre (200 g/kg DM) which makes it a desirable concentrate as the fibre is as digestible as any other readily available carbohydrates and has good chemical and physical properties for the rumen microorganisms (McDonald 1981). Sugar beet pulp nutrients are as well utilised as those of corn up to the 60% level in a ruminant ration (Bhattacharya and Sleiman 1971). The majority of feeding trials which have been conducted involving sugar beet pulp provide a comparison with a starch based concentrate. Bhattacharya and Sleiman (1971), in a comparison of sugar beet pulp with corn, found that, when fed alone, sugar beet pulp was not as palatable and hence intake fell.

1.7.3.5 Comparison of Starch Versus Fibre Concentrates

In an attempt to clarify the reasoning behind the tendency to feed fibre based rather than starch based concentrates, a comparison of silage intake and substitution rate for the two concentrate types was undertaken.

In a comparison of barley and sugar beet pulp supplements of similar feeding value, Castle *et al* (1981) stated that for practical purposes the two are interchangeable on an equal weight basis.

Dried grass cubes have also been used in a comparison of fibrous and starchy supplements. In direct comparison with barley, dried grass cubes with a crude protein of 23% increased milk yield significantly more than barley did. Castle and Watson (1975) concluded that it was the protein supplied by the dried grass that increased milk production and that, with a high digestibility silage, a high quality dried grass supplement was superior to barley for milk production.

Contrary to this, however, Thomas *et al* (1986) found silage intake to be higher when a sugar beet pulp based concentrate was fed rather than a barley based one. Despite this no difference in substitution rate between the two diets at 0.37 kg silage DM/kg concentrate DM was found.

The results of some trials indicate that fibrous compounds induce higher silage dry matter intakes and milk yields, whilst others indicate that fibrous supplements have a lower substitution rate, eg Castle (1982).

There has been some controversy on the effect of the use of starch based or fibre based concentrates on the substitution rate. Unlike Castle *et al* (1981), who found no difference in intake and substitution rate when comparing sugar beet and barley concentrates, Mayne and Gordon (1984) found significantly lower intakes with the sugar beet concentrate than with the barley based starchy concentrate.

A range of responses from -0.2 to +1.0 kg silage DM (mean 0.37, sd 0.48) was found when a fibre based concentrate replaced a starch based supplement. This occurred with a range of 5.8 to 10.9 kg concentrate DM (mean 8.0, sd 2.3) (Thomas and Thomas 1988), highlighting how variable the substitution of starch with fibre is.

Thus, in conclusion, there is controversy over whether it is preferable to feed fibrous or starchy supplements, but the trend appears to be towards a preference for fibrous supplements. This is due to the silage DM intake generally being greater when a fibrous concentrate is fed.

1.7.3.6 Bicarbonate

The reasoning behind the feeding of bicarbonate has been discussed previously in section 1.6.2. To summarise; bicarbonate is added either to the silage or to the concentrate in an attempt to alleviate the rumen acidity associated with the end products of fermentation.

As early as the mid sixties, supplementation with alkaline salts was found to increase DMI, the extent of the response depending on the method of ingestion (McCarrick *et al* 1966). They found that the addition of sodium bicarbonate either in the drinking water or by dosing gave the highest DMI compared with intake of the silage either

with the addition of molasses or ammonium bisulphate.

Dosing cows with sodium bicarbonate at a rate of 100 g/animal/day was found to increase silage DMI (Orth and Kaufman 1966). This level increased silage DMI by 102 g/kg DM. Dairy cows fed silage plus a concentrate containing bicarbonate at 150g/day had an elevated silage DMI of 90 g/kg DM over control cows. When the level of bicarbonate rose to 300 g/day/animal silage DMI decreased to that of the control animals (Farhan and Thomas 1978).

Additionally, Edwards and Poole (1983) found that cows fed sodium bicarbonate had higher silage DMI. They also found that bicarbonate was associated with higher milk yields, smaller weight losses and better service records.

Not all experiments, however, have recorded increases in intake. Lancaster and Wilson (1975) and Wilkins (1974) found that the addition of sodium bicarbonate had no effect on intake. The former fed a high moisture silage to sheep with sodium bicarbonate included in the diet at 25 g/kg wet weight. The latter found that partial neutralisation of whole crop barley silage did not increase intake in sheep.

Bicarbonate addition did not increase silage DMI in an experiment by Farhan and Thomas (1978). They added bicarbonate to the food at either 8 g/kg or 16 g/kg fresh weight. This diet was fed to dry cows resulting in a depression in silage DMI of 0.6 kg and 1.3 kg daily for each of the two diets. These are large depressions in intake and they illustrate that in this case the greater the inclusion of sodium bicarbonate the greater was the depression in intake.

Erdman (1988), in summarising reports on neutralisation of silages, suggested that much of the variation in response could be accounted for by the initial and final pH of the silage used, with greater intake responses shown in low pH silages. Buffering capacity could also be a factor responsible for some of the variation.

From analysis of several experiments Erdman (1988) found that the optimum pH for maximum silage intake in dairy cows was 5.71; intake was distributed normally about this value. In one of the experiments used in this analysis silage dry matter intake increased by 1.0 kg/day ($p < 0.01$) when the pH of the corn silage was increased from 3.64 to 5.44. Partial neutralisation of alfalfa haylage from 4.62 to 5.45 prior to

feeding had no effect on alfalfa dry matter intake. Erdman (1988) suggested that the discrepancy in these results for the above trial may be related to the initial pH of the forages.

The same trend of normally distributed silage dry matter intake about a mean of 5.6 has been described by Shaver *et al* (1984). This optimum pH was achieved by feeding silage ranging in pH from 3.72 to 8.05 to Holstein heifers.

Thus, according to Erdman (1988) and Shaver *et al* (1984), lower pH silages will be consumed in smaller quantities than higher pH silages.

After a series of experiments using different neutralising agents Ndwigwa *et al* (1990) suggested that reduction in silage acidity alone may not be solely responsible for the observed changes in intake of partially neutralised silage. It is possible, however, that the alteration of the acid-base balance and the buffering capacity of the silage may be responsible for the changes in silage DMI. Ndwigwa *et al* (1990) illustrated that the base used for neutralising the silage affects silage intake. They found that neutralisation with sodium bicarbonate increased intake by 137 g/kg DM over untreated silage, sodium carbonate decreased silage intake by 30 g/kg DM and sodium hydroxide decreased intake by 5 g/kg DM. This also illustrates that sodium does not appear to be responsible for the changes in silage DM intake.

Farhan and Thomas (1978), however, concluded that the conflicting results of silage intake being affected by bicarbonate neutralisation may be explained by the differences in the quantity of bicarbonate offered. This would be due to inclusion of high levels of bicarbonate reducing palatability and consequently food intake.

In an attempt to explain the anomalies found (Table 1.7), Thomas and Chamberlain (1982) suggested that responses to bicarbonate may be affected by a variety of factors including the species and age of the animals, the type of silage and the level of supplementation. It was further suggested that responses are most likely to occur in animals suffering acid stress, but that condition is not an inevitable consequence of the consumption of low pH silage. pH is a poor indicator of neutralising value and the consumption of low pH silage is a potential disturbance to the animal's acid/ base balance.

Table 1.7

THE EFFECT OF BICARBONATE ADDITION

| | Silage DMI Increase | Bicarb Addition |
|--|------------------------|--------------------|
| Thomas and Wilkinson (1975) - beef cattle | 120 g/kg | not known |
| McLeod <i>et al</i> (1970) - sheep | 97 g/kg | 16 g/kg freshwt |
| - 5 month cattle | 207 g/kg | 16 g/kg freshwt |
| Orth and Kaufman (1966) - dairy cows | 102 g/kg | 100 g/animal |
| | 90 g/kg | 150 g/animal |
| | 0 g/kg | 300 g/animal |
| Edwards and Poole (1983) - dairy cows | yes | not known |
| Lancaster and Wilson (1975) - sheep | 0 g/kg | 25 g/kg wet wt |
| Wilkins (1974a) - sheep | 0 g/kg | not known |
| Farhan and Thomas (1978) - dry cows | 0.6 kg/day | 8 g kg freshwt |
| | 1.3 kg/day | 16 g/kg freshwt |
| Erdman (1988) - dairy cows | 1.0 kg/day | not known |

1.8 PREDICTION OF INTAKE

In order to optimise the utilisation of feed it is useful to be able to predict the level of voluntary intake of a feed or feeds by farm animals or to predict the optimum formulation of a ration to meet the animals' requirements under conditions of ad libitum feeding. In particular, prediction of silage consumption is a valuable aid in the planning of forage conservation, and is most useful for groups of animals rather than single cows because of the variation individual animals exhibit. Due to the complex interactions between the ruminant and the diet, prediction of intake is often difficult, but despite this numerous methods have been used to determine the voluntary intake in cattle.

There are two different methods of predicting the voluntary intake of forage; in a controlled situation where single plant variables or animal factors are measured as predictors of intake, or where mathematical equations which combine these individual factors are used.

1.8.1 Prediction From Single Plant or Animal Factors

Single measurements of either plant variables e.g. the proportion of leaf, or animal variables e.g. liveweight have been used to predict intake. Some of these factors will be examined subsequently.

1.8.1.1 Plant Factors

The prediction of voluntary intake from plant variables is normally based on measurements from either the physical or chemical composition of the forage. From experimental work the correlations between plant variables and voluntary intake can be determined and used in mathematical models and equations.

As forages grow and mature they pass through a succession of growth stages which are accompanied by increases in yield and proportion of stem and flowering head, a decrease in the proportion of leaf and a fall in voluntary intake. The fall in voluntary intake is caused by three factors: an increase in the proportion of stem, which is eaten in smaller quantities than leaf due to the lower resistance of the leaf to chewing (Laredo and Minson 1973), the increase in the fibre content of both the leaf and stem

fractions and nutritional deficiencies in the mature forages (Wilson and McCarrick 1967) (Table 1.8).

Once the voluntary intake of a forage has been measured it can be used for prediction purposes. For example Rao *et al* (1987) found that voluntary intake was positively correlated with the proportion of leaf in a dried, separated sample of forage. The correlation coefficient was found to be 0.87 for legumes and 0.66 for grasses, the difference between these correlation coefficients being due to leaf being eaten in greater quantities than stem of similar chemical composition or dry matter digestibility. One flaw of this method of prediction was that the method used was found to be sensitive to the way the forage was grown and to the characteristics of the leaf separator. For example, the amount of water the crop receives affects the proportion of stem to leaf and hence the voluntary intake and correlation coefficient.

The grinding energy (i.e. the electrical energy required to pulverise 5g of an oven dried forage through a 1mm screen in a laboratory mill) or fibrousness index has been shown by Laredo and Minson (1973) to be negatively correlated to intake; the higher the energy required to grind the sample, the lower the voluntary intake. This is primarily due to the ease of food mastication affecting the rate of passage, hence intake. In a study of 30 leaf and stem samples of five tropical grasses, they found a negative correlation where $r = -0.81$.

Attempts have been made to predict forage intake using Near- Infrared Reflectance Spectroscopy (NIR). Though the regression equations have resulted in low error levels, in practice they only work when the forage species used is the same as that in the calibration. It has not been possible to predict intake of uncalibrated types of forage with any degree of accuracy (Minson *et al* 1983). A possible solution, however, could be the use of IADF (indigestible acid detergent fibre) or a combination of chemical constituent analyses with multiple regression equations.

Chemical analyses of forages have been used to predict voluntary intake with a high degree of success. Regression equations have been calculated involving pepsin soluble DM or OM, solubility in water, crude protein, NDF (Mertens 1987), ADF, MADF, crude fibre, lignin, cellulose solubility in cupriethylenediamine and DM solubility in 1N sulphuric acid. The best correlation was the relationship between voluntary intake and ADF in temperate grass where $r = -0.90$ (Jones and Walters 1975) and the worst was

Table 1.8

**COMPOSITION PHYSICAL CHARACTER AND
VOLUNTARY INTAKE OF TROPICAL GRASSES**

| | Fraction | | | |
|---|----------|--------|----------|--------|
| | Leaf | | Stem | |
| | Immature | Mature | Immature | Mature |
| Lignin (g/kg DM) | 27 | 37 | 35 | 51 |
| Voluntary Intake (g/kg metabolic LW) | 69 | 51 | 49 | 35 |

(Laredo and Minson 1973)

that between voluntary intake and pepsin soluble DM or OM in tropical grasses where $r=0.26$ (Laredo and Minson 1975).

The voluntary intake of silage is less than that of the corresponding fresh or dried forage due to the end products of fermentation (section 1.6.2). Thus prediction of silage is more complicated than prediction of fresh or dried forages.

Many single variable regression equations for predicting the voluntary intake of silage have been developed. McLeod *et al* (1970) determined the relationship between silage DM intake and pH, organic acid content and lactic acid. This has been discussed previously in section 1.6.2.

One of the most accurate single factor predictions to predict silage intake by lambs is that by Henderson *et al* (1984). From a wide range of parameters, including digestibility and the chemical constituents of both grass and silage they concluded that the parameter with the greatest positive effect on intake was the content of residual water-soluble carbohydrates in the silage.

$$\begin{array}{ll} \text{Silage DM Intake} = 716 + 3.67 \text{ silage WSC} & (r=0.69) \\ \text{(g DM per day)} & \text{(g/kg DM)} \end{array}$$

This prediction was improved by including ethanol as a negative factor and grass water-soluble carbohydrates and protein nitrogen as positive factors.

Single variable regression equations for predicting the voluntary intake of silage in dairy cows from the dry matter, protein nitrogen, ADF, pH, lactic acid, acetic acid and crude protein have been determined by Jones *et al* (1980). From this work they concluded that no single factor alone was accurate in predicting voluntary intake. No more than 0.25 to 0.29 of the variation could be attributed to one variable. To this end, from the results of the multiple regression equations, they suggested that dry matter crude protein and ADF were the most reliable factors. The r^2 was increased from 0.25 with dry matter to 0.54 with the inclusion of crude protein and ADF. A more accurate prediction was obtained ($r^2 = 0.71$) with the use of four variables, cell wall constituents, ADF, ADL and crude protein.

1.8.1.2 Animal Factors

Rook *et al* (1991) suggested that animal characteristics were more important than silage characteristics in determining silage intake by dairy cows. This may reflect the relatively narrow range of generally well fermented silages used.

The simplest animal variable used in intake prediction is liveweight. Kleiber (1961) illustrated that between species comparison of the voluntary food intake of adult animals is related to liveweight to the power 0.73 (metabolic liveweight). Within species the level of intake may also be related to metabolic liveweight, as determined using sheep by Blaxter *et al* (1961).

$$\text{Intake} = \text{constant} \times \text{weight}^{0.70}$$

The quality of food offered to animals will affect their voluntary intake. Smaller food particles disappear from the rumen faster than larger ones, so that the ease with which a feed can be masticated will affect rate of passage and thus food intake. Thornton and Minson (1973) calculated that the mean retention time of organic matter in the rumen (h) was negatively correlated with the intake of digestible organic matter (g/day), $r = -0.96$, by the following equation:

$$\begin{array}{ccc} \text{DOMI} = 1276 - 50.7 \times h & & \\ \text{(g/day)} & & \text{(min)} \end{array}$$

In conclusion, several animal factors e.g. live weight, species, age, sex and lactating or not have been used to predict voluntary intake, with limited success, due to the complexity and interrelationships of the processes involved.

1.8.2 Mathematical Methods of Intake Prediction

More complex methods of intake prediction, compared to prediction from single plant or animal factors, are based on multiple regression equations and models. These will be examined in turn.

Several multiple regression equations have been used to predict voluntary intake. These encompass many of the correlations calculated with plant variables or animal factors in controlled situations. The simplest equation is that by MAFF (1971) for dairy cows which takes liveweight (kg) and milk yield (l) into account and has been widely

used for advisory purposes:

$$\text{TDMI} = 0.025 \text{ liveweight} + 0.1 \text{ milk yield}$$

(kg) (l)

The complexity and factors used in prediction have varied enormously. Neal *et al* (1984) used data from the Grassland Research Institute from six experiments between 1974 and 1982 and compared intakes from seven different prediction equations with actual intakes. From comparison of the mean square prediction errors they concluded that the equation by Vadiveloo and Holmes (1979) was the most accurate for all cows and for adult cows, and that by Lewis (1981) was the best for heifers. The mean square prediction error is a measure of how the actual and predicted intakes compare, the smaller the value the closer the predicted intake is to the actual intake. The equation by Vadiveloo and Holmes (1979) takes into account metabolic liveweight ($LW^{0.75}$), concentrate intake (C), milk yield (MY) and week of lactation (WL) in the following way:

$$\text{Total DM Intake} = 0.076 + 0.404C + 0.013 LW - 0.129WL + 4.120 \log WL + 0.140MY$$

(kg/day) (kg/day) (kg) (week of lactation) (kg/day)

That by Lewis (1981), however, is more complex, taking account of concentrate intake and silage intake where

$$\text{Silage Intake} = 0.103 \text{ SDM} + 0.0516 \text{ DOMD} - 0.05 \text{ NH}_3\text{-N} + 45.0$$

(gDM/kg $W^{0.75}$) (g/kg) (g/kg) (g/kg TN)

thus

$$\text{SDMI} = 1.086 \text{ SI} - 0.00247 C^{0.75} \times \text{SI} - 0.00337 (C^{0.75}) - 10.9$$

(gDM/kg $W^{0.75}$) (gDM/kg $W^{0.75}$) (gDM/kg $W^{0.75}$) (gDM/kg $W^{0.75}$)

A study by Rook *et al* (1991) attempted to produce accurate prediction models for voluntary intake of silage for dairy cows. They found that the most important variables affecting silage DMI were; silage ammonia nitrogen, concentrate DMI, silage DOMD and liveweight and the best multiple regression for the mean silage DMI accounted for proportionally 0.649 of the variation. After lengthy research, however, Rook *et al* (1991) suggested that there is little to be gained from further refinement of the functional form of the models and that most progress may be made by the construction of a number of models for specific food and management practices.

Despite the relative accuracy of predicting intake with multiple regression equations,

mathematical modelling has become increasingly popular. Models enable a greater number of factors and interrelationships to be considered, hence intake prediction now tends to be computer orientated.

A mathematical model used to predict voluntary intake in fattening, pregnant and mature sheep was devised by Forbes (1977) (Figure 1.2). It was developed to see whether the various patterns of food intake observed long-term in fattening, pregnant and mature sheep could be accounted for by changes in physical and metabolic factors known to affect voluntary intake in short term experiments. In this model the voluntary intake by sheep is related directly to ME requirements, unless physical or endocrine limitations intervene. The underlying relationship is that between dietary quality and voluntary intake, with positive correlations between content of available energy and the weight of food eaten with a poor and medium quality forage, and negative correlations with high quality roughage or cereal diets.

These voluntary intake predictions do show reasonable agreement with observed intake measurements. One drawback of this model, however, is that it does not take account of growth. The inclusion of this factor should ultimately improve prediction of voluntary intake.

A model which has been used for advisory purposes to predict voluntary intake is that by Jarrige *et al* (1986). It is based on

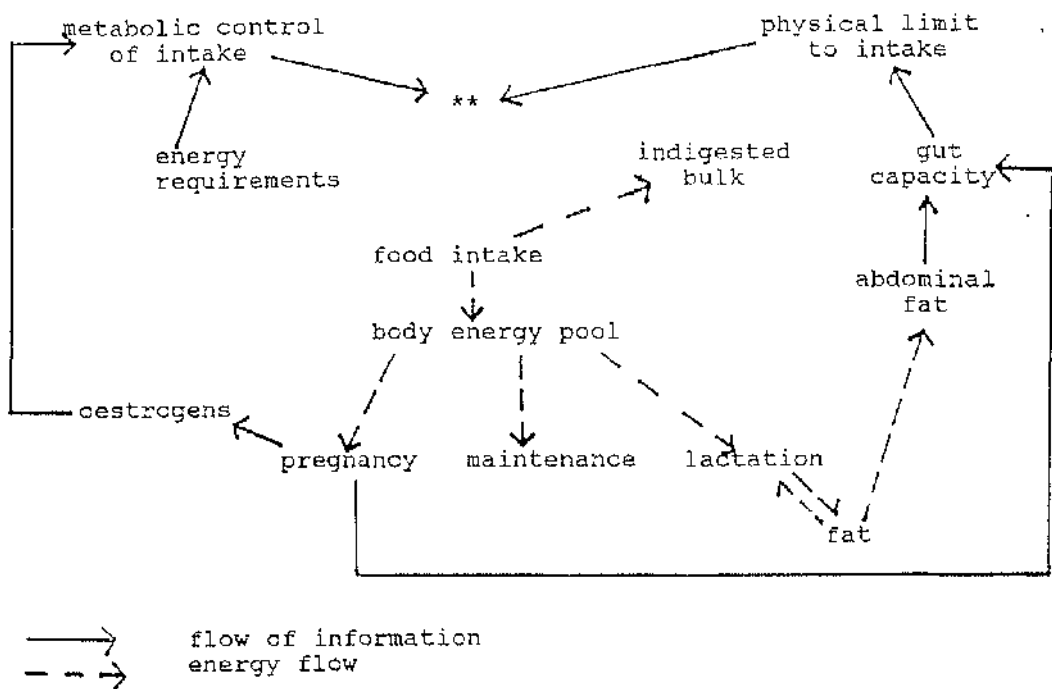
- (i) an animal's ingestion capacity for a standard feed;
- (ii) ingestibility of a feed relative to that of a standard feed (grass) by a standard animal (wether); and
- (iii) substitution rate for concentrate/roughage

This model was designed for use where physical factors were dominant in the control of intake. In order to achieve the objectives, a system based on the "fill unit" was developed. By definition 1 kg DM of the reference pasture grass has a "fill value" of 1FU. A FU for sheep (FUS) is different from a FU for cattle (FUC). The reference grass selected was an average of 50 samples cut at grazing stage of first growth and comprised ryegrass, natural pastures, timothy and fescues. It had a crude protein of 150 g/kg DM, crude fibre of 250 g/kg DM and OMD of 0.77.

Though this model enables the voluntary DMI for an animal to be defined by its

Figure 1.2

Schematic Diagram of the Model Used to Predict Voluntary Food Intake of Sheep



from Forbes (1977)

physical state e.g. sex, age, stage of growth or lactation and its energy and nutrient requirements, it is influenced by the food characteristics. The model is based entirely on physical control, but ideally should also encompass metabolic control.

In conclusion, prediction of intake has been found to differ greatly in accuracy. It has been found to be more applicable for groups of animals compared to single animals due to individual animal variation. Essentially there are two methods of prediction, using either single factors or multiple variables. On account of the increasing complexity of prediction methods, there is now a tendency towards the use of computers.

1.9 SUMMARY

Silage is the end product of a series of chemical reactions. These are mediated by bacteria which are naturally present on the crop; lactic acid bacteria which produce a good quality silage and enterobacteria and clostridia which are responsible for poor quality silage.

The type of fermentation that will proceed is determined by a number of factors which include the chemical composition of the grass e.g. water soluble carbohydrate content and dry matter, the presence/ absence of air and the use of additives.

From the fermentation patterns that occur, a wide range of silages of different chemical compositions is produced. Many attempts have been made to simplify this wide range by classification into a number of groups according to chemical composition.

The first type of classification system was based on mainly organoleptic criteria and hence were very subjective. Later schemes grouped silages according to a small number of chemical constituents whilst the most recent classification schemes are based on a large number of chemical constituents. The most recent classification schemes tend to group silages according to their fermentation pathway.

The voluntary intake of food is controlled both by physical factors e.g. gastric distension and physiological factors e.g. glucostatic, lipostatic, thermostatic, chemostatic and osmotic mechanisms. The dominant mechanism of intake regulation is primarily determined by the physical bulk of the diet; where the diet is fibrous and

bulky physical regulation tends to dominate, but where the diet is of a high energy concentration physiological factors tend to govern intake.

With forages, a number of factors helps to regulate their intake, the main factor being the ratio of cell contents to cell wall constituents. An important factor influencing the cell contents to cell wall ratio, and ultimately forage intake, is the stage of growth of the plant. The forage species, cultivar, soil fertility and climate also affect the proportion of cell contents to cell wall and hence forage intake.

Other factors governing forage intake include dry matter content, chop length, concentrate supplementation, fermentation pattern and additive use, with the last two factors being specific to silage.

Very few energy supplements have a purely additive effect on forage intake, most depress intake. This decrease in the intake of silage dry matter per kilogram increase in concentrate dry matter is termed the substitution rate.

Three factors control the substitution rate in ruminants; the animal, the forage and the supplement.

The most important forage factor affecting the substitution rate is the quality of the forage mediated through its digestibility. Poor quality forages or those of low digestibility tend to have low substitution rates, whilst good quality highly digestible forages have high substitution rates.

The stage of lactation is a further factor governing the substitution rate, though there is some disagreement on the exact nature of the effect.

The characteristics of the supplement fed with a silage play a major role in affecting the substitution rate and regulation of forage intake. Even within a concentrate type e.g. protein, the source and level can have a great effect on forage intake. For example, on comparison of low and high protein levels the latter were generally found to be superior in terms of an increase in silage intake.

There has been a tendency in recent years towards the replacement of starch based concentrates by fibre based supplements in the belief that fibrous concentrates have a

lower substitution rate than starch based ones. Whilst some indications are that fibrous concentrates do give a lower substitution rate, there is, however, evidence to the contrary.

The inclusion of bicarbonate in concentrate is generally believed to increase silage intake due to partial neutralisation of the silage. Evidence illustrating both an increase in silage intake and no effect has been produced. Thus the conflicting results obtained with bicarbonate concentrates may be attributed to differences in the quantity used, but the exact mechanism remains unclear.

Prediction of intake enables the optimal utilisation of feed. Prediction of the quantity of silage which will be consumed has been attempted from single plant or animal factors e.g. the proportion of leaf and liveweight, with a limited degree of success. More accurate prediction equations have been formulated using several variables, either animal or plant. Complex mathematical models have been devised to predict forage intake but despite encompassing a greater number of factors they are not necessarily more accurate than multiple regression equations.

Thus a wide variety of forage factors when combined with various concentrate types results in a complex interaction with different intake potentials. Experimental work reported in this thesis was carried out to discover whether it was possible to classify silages of widely differing types into a number of groups on the basis of their chemical composition. Subsequently the interaction between a silage representative of each of these groups and concentrate type with respect to intake was to be investigated.

CHAPTER 2

2.1 INTRODUCTION

The aim of this investigation was to discover whether it was possible to classify grass silages into a low number of distinct groups on the basis of their chemical composition. Both 'research silages' which had been made and analysed at research institutes and 'advisory silages' which were farm silages sent to the Scottish Advisory Service for analysis were examined.

Such a classification was attempted by Wilkinson *et al* (1981) who combined 231 silages into seven groups by a multivariate statistical method, cluster analysis. All of the silages in their study had been made at one research institute and in laboratory silos and so the data set was not necessarily representative of the variety of British commercial or farm silages.

In this investigation a group classification of research silages was carried out using a much larger and more varied data set than that of Wilkinson *et al* (1981). The statistical method used was the same as that used by these authors, namely cluster analysis. The feasibility of such a group classification system was first examined on a small data subset, and the nature and number of the chemical constituents required for classification was then identified; it was hoped that these could ideally be quantified to produce mean values characteristic of each group.

Subsequently a large data set of advisory silage analyses was studied with the aim of classifying them into groups which could be related to those of the research silage classification.

2.2 RESEARCH SILAGES

2.2.1 Materials

Data on the chemical composition of 885 silages were obtained from various research institutes in the United Kingdom: East of Scotland College of Agriculture (ESCA), Hurley, Hillsborough, Newcastle, Shinfield, Greenmount, North of Scotland College of

Agriculture (NOSCA), ADAS, Rowett Research Institute and ICI. The silages had been chemically analysed for various constituents although the available data were unfortunately not always consistent across the sites. The variety of components measured including their range of values is given in Table 2.1.

In general most of the results of the silage analysis had been quoted on an oven dry matter basis, despite the fact that in many cases toluene dry matter had been determined. For comparative purposes, all measurements were converted to a toluene dry matter basis. As there is no precise relationship between oven dry matter content and loss of volatiles on drying, a correction factor adopted by the ADAS nutrition chemists was used (Givens 1986, equation 1). This empirical single fixed correction factor of 19 g/kg is added to the oven DM to allow for the loss of volatiles on oven drying and allows silage analyses to be expressed on a toluene dry matter basis. It should be noted that this correction was based on measurements taken on only 300 silage samples and it is not known whether it would prove to be a good estimate over a very wide range of moisture contents.

As the silage analysis data were received, they were recorded in four data files for convenience and ease of data handling:

1. ESCA - 341 silages
2. Hurley, Hillsborough, Newcastle, Greenmount, Shinfield, NOSCA - 167 silages
3. Rowett Research Institute, ADAS, NOSCA - 170 silages
4. ICI - 207 silages

2.2.1.1 First Data File

The first data file from ESCA was the most extensive both in terms of numbers of silages and range of analyses. These analyses, which were collated from experiments concerned with silage deterioration, intake, digestibility, chemical changes during ensiling, wilting and additive use, tended to be given on a toluene dry matter basis. ESCA generally measured the amount of water soluble carbohydrates in the silage. Additionally data on the fermentation acids were almost always complete, with ethanol contents nearly always being quoted.

Energy values and Digestibility (D) values were seldom given. Likewise cellulose had

Table 2.1

DESCRIPTION AND RANGE OF DATA CHEMICAL VARIABLES

| Constituent | Min | Mean | Max | sd | Median |
|------------------------------------|-------|-------|--------|-------|--------|
| oven dry matter g/kg | 143.0 | 224.3 | 697.0 | 70.5 | 211.0 |
| toluene dry matter g/kg | 110.0 | 236.0 | 728.0 | 79.4 | 218.0 |
| pH | 3.49 | 4.19 | 6.10 | 0.42 | 4.10 |
| watersoluble carbohydrates g/kg DM | 0.0 | 47.4 | 278.0 | 56.3 | 22.0 |
| total nitrogen g/kg DM | 14.0 | 28.8 | 115.4 | 10.3 | 26.3 |
| protein nitrogen g/kg TN | 154.0 | 385.4 | 925.0 | 106.2 | 377.0 |
| ammonia nitrogen g/kg TN | 9.0 | 121.2 | 426.7 | 70.8 | 101.7 |
| volatile nitrogen g/kg DM | 0.60 | 77.3 | 323.0 | 53.5 | 70.1 |
| soluble nitrogen g/kg DM | 131.0 | 395.2 | 712.7 | 203.9 | 501.2 |
| organic matter g/kg DM | 827.0 | 906.7 | 939.0 | 21.5 | 911.0 |
| acetic acid g/kg DM | 0.80 | 40.21 | 218.0 | 311.5 | 28.85 |
| propionic acid g/kg DM | 0.00 | 3.42 | 70.00 | 5.64 | 1.46 |
| butyric acid g/kg DM | 0.00 | 7.23 | 108.0 | 12.43 | 2.00 |
| lactic acid g/kg DM | 0.00 | 73.62 | 202.30 | 39.30 | 74.08 |
| ethanol g/kg DM | 0.00 | 12.16 | 51.60 | 8.73 | 10.00 |
| formic acid g/kg DM | 0.00 | 5.36 | 69.80 | 10.49 | 1.60 |
| total volatile fatty acids g/kg DM | 0.00 | 68.06 | 324.00 | 52.53 | 51.00 |
| total acids g/kg DM | 11.3 | 106.5 | 229.3 | 50.2 | 107.4 |
| MAD fibre g/kg DM | 201.0 | 330.6 | 542.0 | 52.1 | 334.0 |
| cellulose g/kg DM | 194.0 | 316.7 | 579.0 | 96.2 | 285.5 |

been determined in only a small number of cases, with Modified Acid Detergent fibre (MAD fibre) content being measured more frequently.

Whether a silage was wilted or not was given in the silage data, as was the presence and type of any additive used. Approximately half of the silages obtained from ESCA had been made without additives, the rest having been preserved with various additives including acids, formaldehyde, inoculants and enzymes, or varying mixtures of these.

2.2.1.2 Second Data File

The second data file contained an amalgamation of small data sets from Hurley, Hillsborough, Newcastle, Greenmount, Shinfield and NOSCA. The majority of the data came from silages made from first cut perennial ryegrass with about half the silages having been wilted. A notable feature of these silages was that the majority had been made using an additive. A mixture of formic acid and formaldehyde tended to be the most usual additive with only the occasional occurrence of an inoculant.

The chemical constituents measured for the silages in this data file varied considerably, depending upon the source of the data and the objective of the experiment for which the data were procured. None of these institutes had measured water soluble carbohydrate levels, and data from Shinfield, Newcastle and NOSCA had been expressed on an oven dry matter basis.

D values were given for all data except those from Shinfield where the analysis appeared to have concentrated on the different fibre fractions and no energy values, either gross energy or D value, were available.

2.2.1.3 Third Data File

The third data file consisted of data from ADAS, Rowett Research Institute and NOSCA. A distinctive feature of this file was that the silages from the different sources tended to have been analysed for the same chemical constituents.

Whilst being quoted on a toluene dry matter basis, these analyses were not very detailed with respect to fermentation acids. Lactic acid was reported in all analyses whilst acetic acid had been determined in approximately half of the silage samples.

Neither propionic acid nor butyric acid values were given. Whilst water soluble carbohydrates were absent from the analysis as were ammonia nitrogen values and MAD fibre, both gross energy and D values were given for all the silage samples. All of the D values given in this data file were determined in vivo.

2.2.1.4 Fourth Data File

The fourth and final data file consisted solely of data from ICI. These had been obtained from experiments on the effects on animal growth of the level of concentrate feeding. The silage analysis tended to be quite detailed and to include the complete determination of fermentation acids. Very few of these silages had been analysed for water soluble carbohydrates, and ethanol was measured in only about half the samples. Additives had been applied in almost all of the silages, the main additive being a mixture of sulphuric acid and formalin. An inoculant had been used on the small number of remaining silages. In contrast to the widespread additive use, very few of the silages had been wilted prior to ensilage. As in previous data files, a large number of the analyses had been quoted on an oven dry matter basis. Conversion to a toluene dry matter basis was relatively straight forward as in most cases both toluene and oven dry matters were given. The proportion of oven dry matter to toluene dry matter was used as a constant to convert the ammounts of the constituents quoted on an oven dry matter basis.

The DOMD value was quoted for a large number of the silages, and was generally an estimate, calculated according to the following equation (Givens 1986 eqn 2):

$$\text{DOMD(g/kg TDM)} = 945.1 - 9.12 \text{ MADF(g/kg TDM)}$$

| | |
|--|--|
| (digestible organic matter in the dry matter g/kg DM) | (modified acid detergent fibre g/kg DM) |
|--|--|

This prediction equation either requires a knowledge of the toluene dry matter content of silages or alternatively the toluene dry matter can be calculated from an oven dry matter as per Givens (1986, equation 1). The MAD fibre content can, however, be measured on an oven dry matter basis and converted to a toluene dry matter basis, thus enabling the D value to be estimated. Gross energy values were not given for any of the silages analysed in this file.

2.2.2 Analysis of Research Silages

2.2.2.1 Identification of Analysis Methods

With such a large data set, the initial attempts to group the silages were restricted to a smaller, more manageable subset until such time as the direction and method of analysis became clear. Hence the initial investigation was carried out on a subset of 166 silages from the first data file (ESCA).

For ease of comprehension and interpretation the initial analysis was limited to six parameters with toluene dry matter, pH, protein nitrogen, ammonia nitrogen, butyric acid and lactic acid being selected as criteria which might serve to group the silages. These constituents were chosen as many of them are fermentation end products in the ensilage process and it seemed likely that they might be particularly suitable for silage classification. The preliminary analysis was carried out on a number of silages which had all of these parameters present.

2.2.2.1.1 *Statistical Methods*

All data were analysed using the Genstat V Statistical package, Release 1.3, Lawes Agricultural Trust, Rothamsted Experimental Station 1988.

There are two basic steps which were taken in separating the silage data into distinct groups: the division and identification of the best number of groups and the selection of the chemical constituents necessary to achieve this grouping. Due to the large number of variables in the data, a multivariate statistical method -cluster analysis - was used to divide the data into a number of mutually exclusive groups or classes. The basic function of cluster analysis is to find the 'natural groupings' of individuals of similar properties within a set of individuals having certain known properties (Chatfield and Collins 1980).

Cluster analysis of the silage analysis data should enable:

- (i) the optimum number of distinct groups to be determined;
- and
- (ii) the mean values of the chemical constituents in each of the groups to be found.

Cluster analysis seeks to group units in tightly bound, well spaced out groups by maximising the total Euclidean distance between the classes which is equivalent to minimising the total distances within each class. The Euclidean distance between two points in three dimensions is equal to the square root of $x^2 + y^2 + z^2$ where x , y and z are functions of the constituents of the silages.

The process of searching for the optimal classification proceeds as follows. Starting from some initial arbitrary classification of the silages into the required number of groups, silages are repeatedly transferred from one group to another so long as such transfers improve the classification by maximising the between- group sums of squares and minimising the within-group sums of squares. When no further transfers can be found to improve the groupings, two silages from different classes are exchanged. Transfer of silages to other groups and exchanges of pairs of units alternate until no improvement in groupings is found.

The best number of groups into which silages can be classified can be found by examination of a single number, the criterion value, for each group. This is a measure of group dispersion and is calculated from the sums of squares of values in that group. The greater the criterion value, the more dispersed are the group members. Appendix 1 contains a simplified example of this technique.

In addition to determining the best classification of data into a given number of groups, the criterion value also allows the best number of groups into which the data divide to be established. This is done by examining the percentage differences between criterion values on reduction of number of groups. The smallest percentage difference produces the best group number. The principles of this procedure are again illustrated in Appendix 1.

Once the optimum groupings have been found in this way, Canonical Variate Analysis (CVA) is used to produce a 2- dimensional representation of the groups. This is discussed more fully in section 2.2.3.3.

2.2.2.1.2 Analysis of Data Subset with Six Parameters

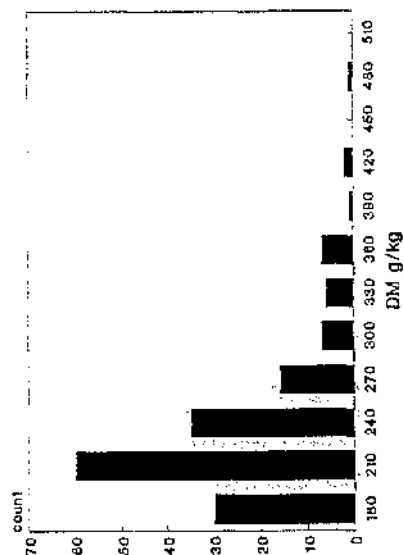
The first step in the analysis was the production of a histogram of each of the six parameters selected for the initial investigation in order to give a qualitative impression

of the range of each parameter in the 166 silages in the subset under investigation (Figure 2.1). This illustrated that for most of the constituents the mean value tended to be closer to the minimum value than to the maximum value; this is apparent from Table 2.1. To facilitate the analysis, natural logarithms were taken of the values of all the data on chemical composition except for pH which is already on a logarithmic scale. The reason for this procedure was not to transform the data closer to a normal distribution, which is not necessary for cluster analysis, but to make the variances of the variables more similar, and thus give more equal weight in the analysis to each of the chemical constituents. On this subset of data, cluster analysis was used to group the silages into five, four, three and two groups. In the Genstat package the units of a data set can be classified by optimising some suitably chosen criterion directly from the data, the criterion selected depending on the type of data. The criterion need not rely on multi-normality or equal within-class dispersion. As indicated above, in the present analysis the sums of squares of the co-ordinates is calculated to yield a criterion value which is a measure of how concentrated the groups are.

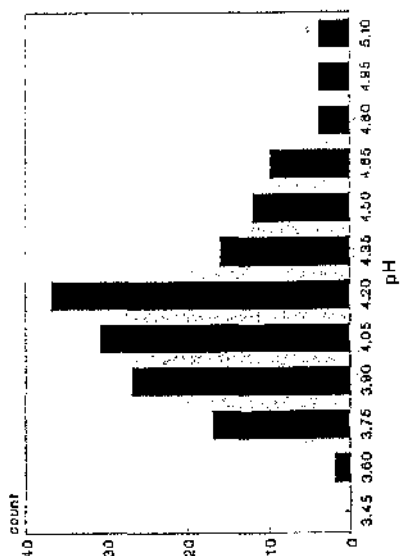
Using this method, the 166 silages under investigation were successively divided into 5, 4, 3 and 2 groups. Comparison of the dispersion of the various silage groups was made by looking at the percentage difference in the criterion value between groups. The smaller the percentage difference on the reduction of group numbers, the more concentrated the groups are relative to other solutions (see Appendix 1). Thus with this data subset and these six parameters, 4 groups was found to be the best number (Table 2.2).

In trying to obtain a general impression of the data, the question of whether there was any relationship among the initial six parameters, (toluene dry matter, pH, protein nitrogen, ammonia nitrogen, butyric acid and lactic acid) was investigated. Calculation of a correlation matrix for those parameters using the Genstat package indicated whether there was a positive or negative interaction between pairs of parameters and the strength of the association. It was found that lactic acid was negatively correlated to all of the other five parameters, i.e. where lactic acid levels are high the values for the other parameters were low. The highest positive correlations occurred between butyric acid and ammonia nitrogen ($r=0.55$), pH and ammonia nitrogen ($r=0.51$) and pH and butyric acid ($r=0.47$) (Table 2.3) i.e. where ammonia nitrogen values were low, both butyric acid levels and pH also tended to be low.

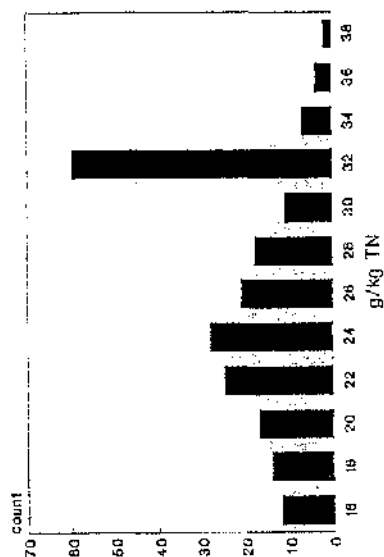
HISTOGRAM OF DRY MATTER IN DATA SUBSET



HISTOGRAM OF pH IN DATA SUBSET



HISTOGRAM OF PROTEIN NITROGEN IN DATA SUBSET



HISTOGRAM OF AMMONIA NITROGEN IN DATA SUBSET

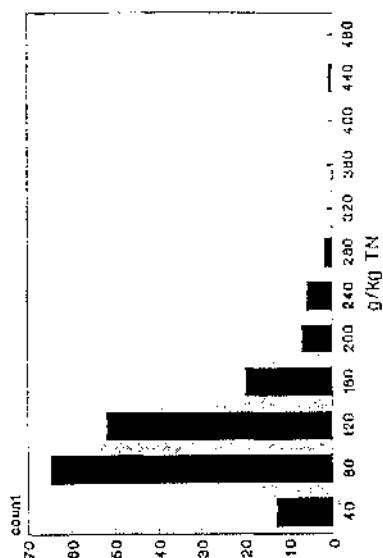
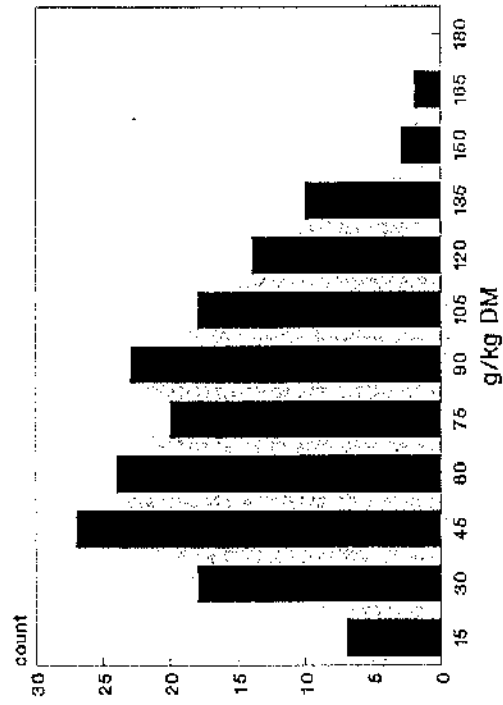


Figure 2.1

Figure 2.1 cont.

HISTOGRAM OF LACTIC ACID IN DATA SUBSET



HISTOGRAM OF BUTYRIC ACID IN DATA SUBSET

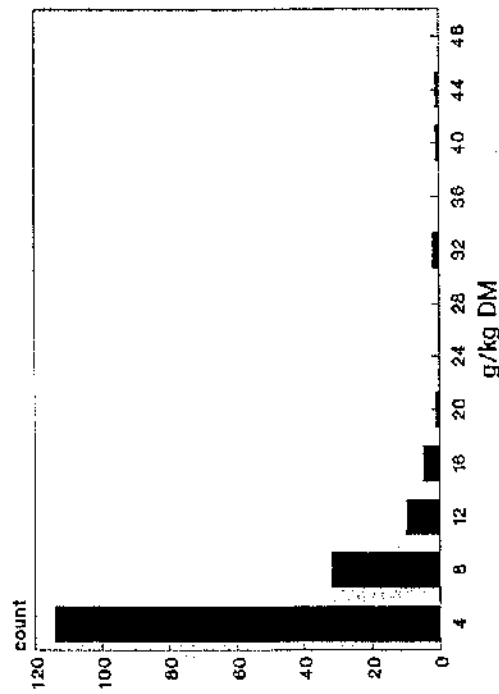


Table 2.2

**COMPARISON OF CRITERION VALUES
FOR SIX AND THREE PARAMETERS**

| | Six Parameters | | Three Parameters | |
|----------|------------------|-----------------------------|------------------|-----------------------------|
| | Criterion Values | % Difference Between Groups | Criterion Values | % Difference Between Groups |
| 5 groups | 136.99 | | 109.16 | |
| 4 groups | 148.17 | 7.55 | 129.46 | 15.68 |
| 3 groups | 177.09 | 16.33 | 147.02 | 11.94 |
| 2 groups | 218.74 | 19.04 | 186.51 | 21.77 |

Table 2.3

CORRELATION MATRIX OF DATA SUBSET USING SIX PARAMETERS

| | | | | | | |
|------------------|--------|--------|--------|--------|--------|-------|
| pH | 1.000 | | | | | |
| Dry Matter | 0.237 | 1.000 | | | | |
| Protein Nitrogen | 0.3111 | -0.044 | 1.000 | | | |
| Ammonia Nitrogen | 0.505 | -0.085 | 0.030 | 1.000 | | |
| Butyric Acid | 0.469 | -0.062 | 0.176 | 0.551 | 1.000 | |
| Lactic Acid | -0.553 | -0.225 | -0.056 | -0.126 | -0.289 | 1.000 |
| | pH | DM | PN | NH3-N | BA | LA |

2.2.2.1.3 *Analysis of Data Subset with Three Parameters*

The data subset was then restricted to only three of the six variates - butyric acid, lactic acid and ammonia nitrogen - for further cluster analysis. These three parameters were selected as they are important end products in fermentation pathways; this approach is substantiated by the high correlation coefficients between these parameters in the above correlation matrix. The purpose of this restricted analysis was to establish whether the same groupings of silages would result or, if they were different, to examine how significant the differences between the groups would be.

The most noticeable feature of the results of the analysis with three variates was that the percentage differences of the criterion values in two out of three cases were greater than those of six variates, indicating greater group dispersion (Table 2.2). As the criterion value percentage difference was lowest for reduction from four to three groups the optimum number of groups using three variables was three. This percentage difference at 11.94 is larger than that of four groups with six parameters at 7.55, and hence the latter was felt to give a tighter group pattern. It should be noted, however, that direct comparison of the criterion values produced from six or three parameters is not possible as the greater the number of parameters the greater is the criterion value.

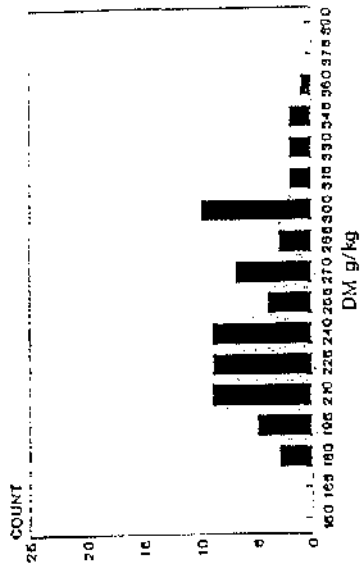
2.2.2.1.4 *Further Analysis of the Data Subset with Six Parameters*

Histograms were produced of each variate for each of the above four groups. This did not provide an unambiguous separation but indicated that one group tended to be high in one component whilst another had low levels of that component (Figure 2.2). For example the pH of the silages in Group 1 tended to be low compared to that of Group 4, and the silages in Group 3 had lower levels of butyric acid than those in the other groups. Though there tended to be considerable overlapping between groups for some of the constituents, a comparison of the six histograms for each of the four groups shows that each group has a number of distinctive features: for example Group 4 has high pH levels and large amounts of ammonia nitrogen and butyric acid.

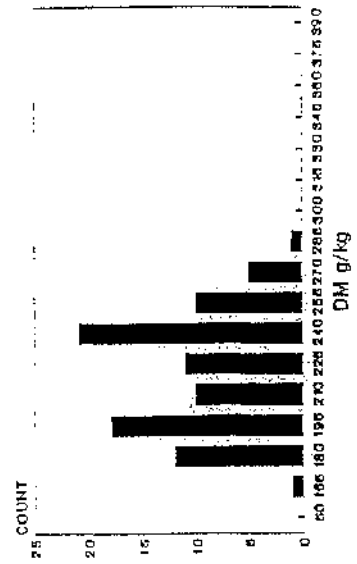
Despite all values being on a logarithmic scale, there was still considerable variation in the numerical values of the data and in the range over which the values of each component were spread. For example the spread of values for propionic acid was from 0 to 70 g/kg, whilst for dry matter the maximum value was 728 g/kg (Table 2.1). It

Figure 2.2a

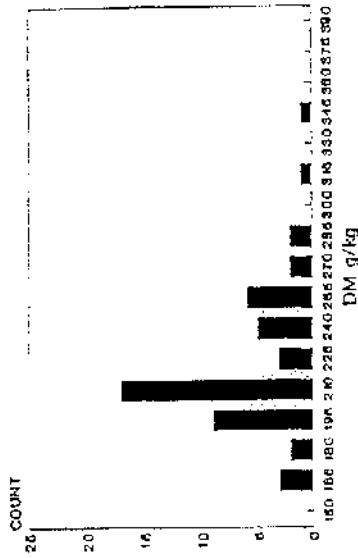
HISTOGRAM OF DRY MATTER
GROUP 2



HISTOGRAM OF DRY MATTER
GROUP 4



HISTOGRAM OF DRY MATTER
GROUP 1



HISTOGRAM OF DRY MATTER
GROUP 3

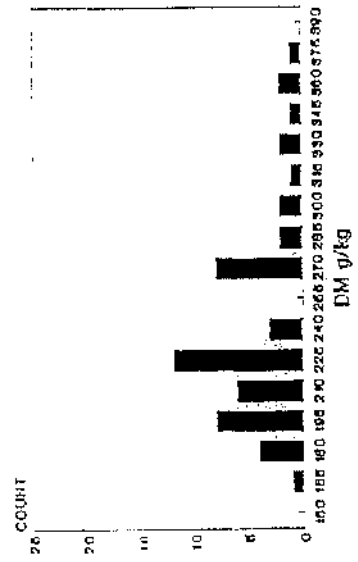


Figure 2.2b

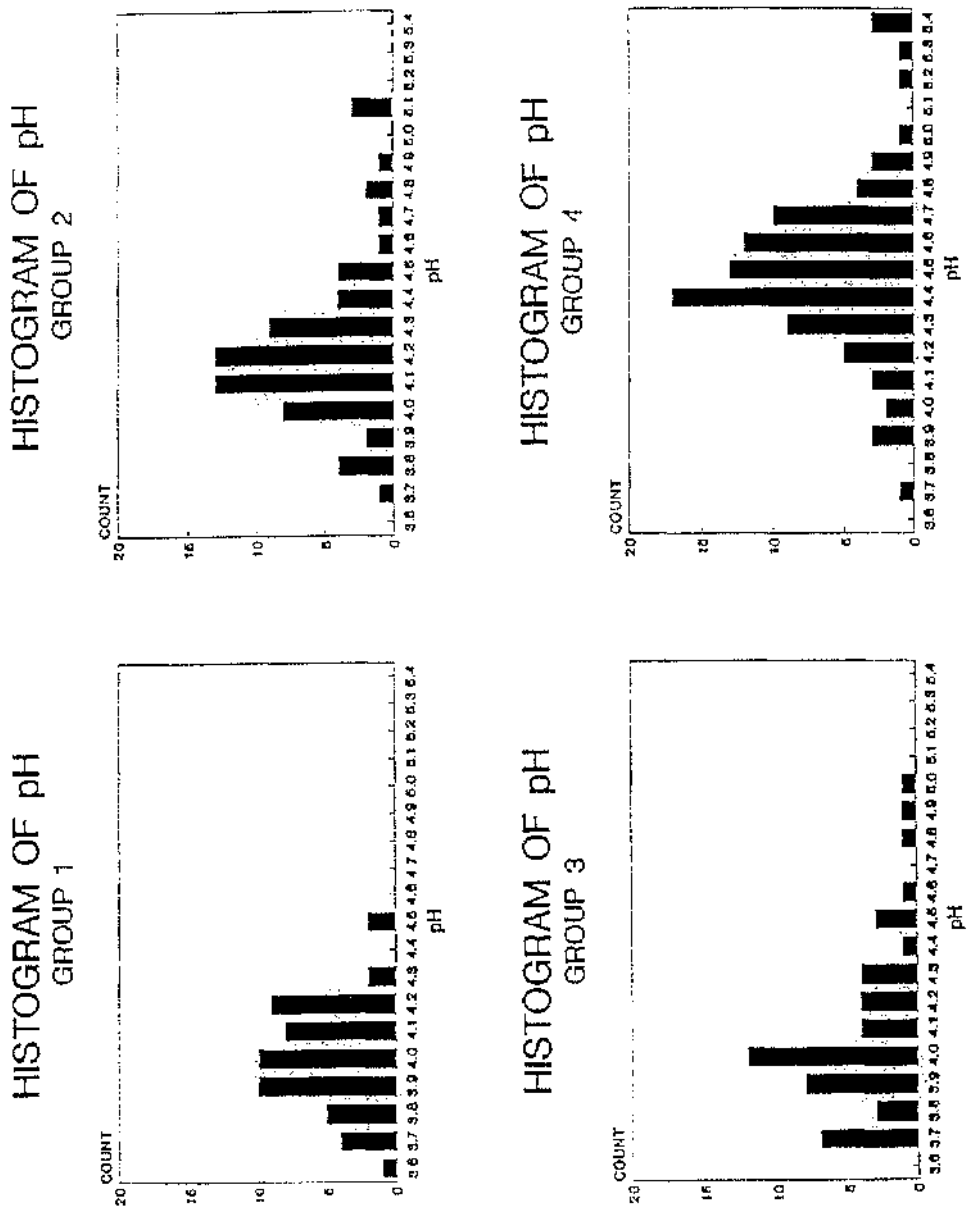
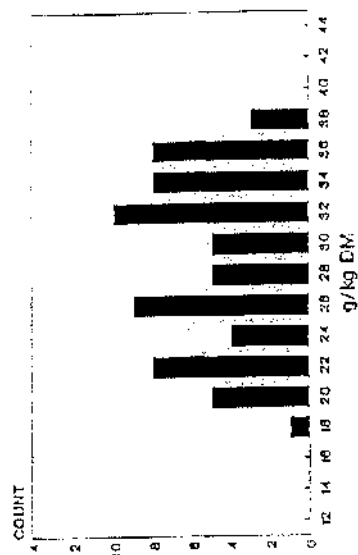
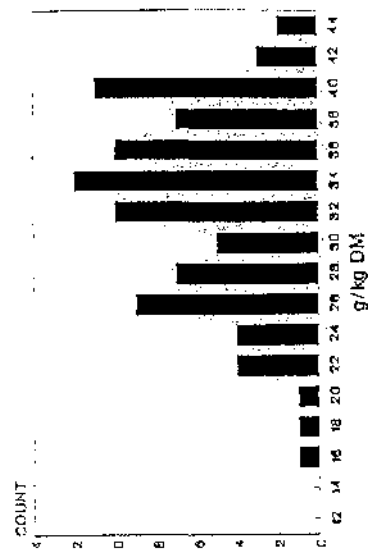


Figure 2.2c

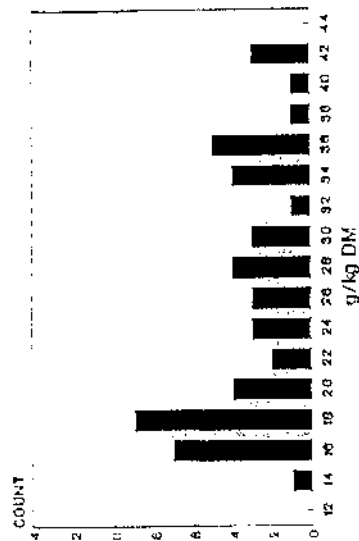
HISTOGRAM OF TOTAL NITROGEN
GROUP 2



HISTOGRAM OF TOTAL NITROGEN
GROUP 4



HISTOGRAM OF TOTAL NITROGEN
GROUP 1



HISTOGRAM OF TOTAL NITROGEN
GROUP 3

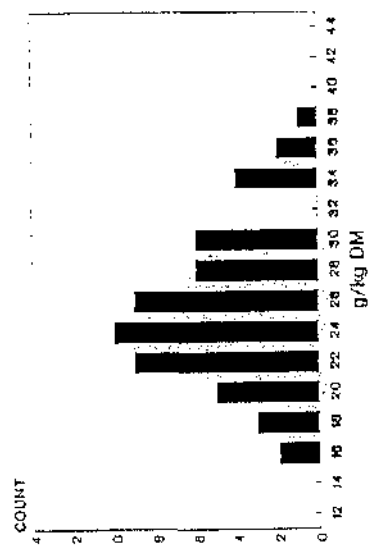
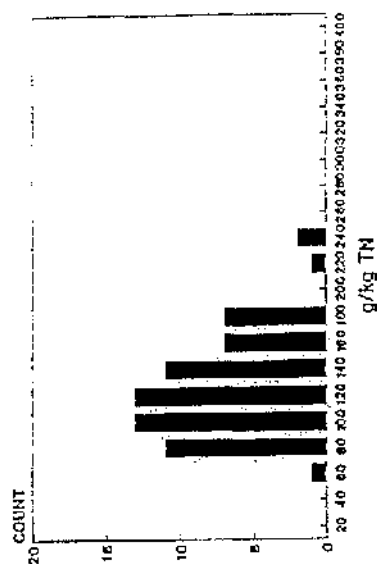
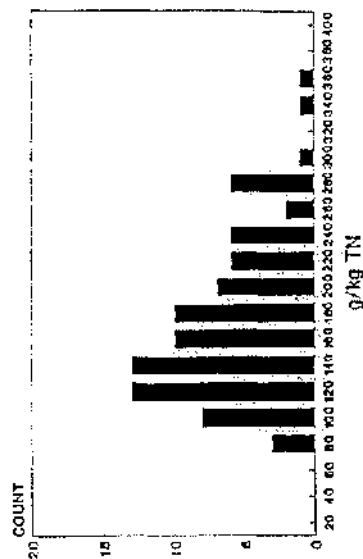


Figure 2.2d

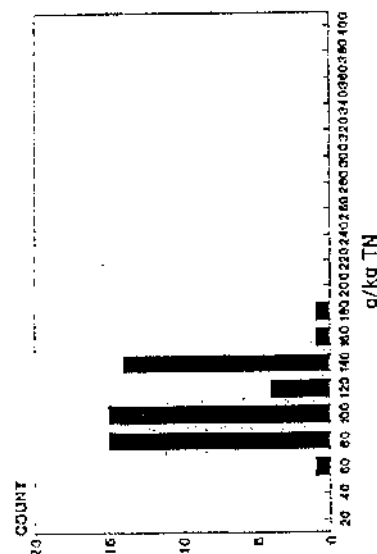
HISTOGRAM OF AMMONIA NITROGEN
GROUP 2



HISTOGRAM OF AMMONIA NITROGEN
GROUP 4



HISTOGRAM OF AMMONIA NITROGEN
GROUP 1



HISTOGRAM OF AMMONIA NITROGEN
GROUP 3

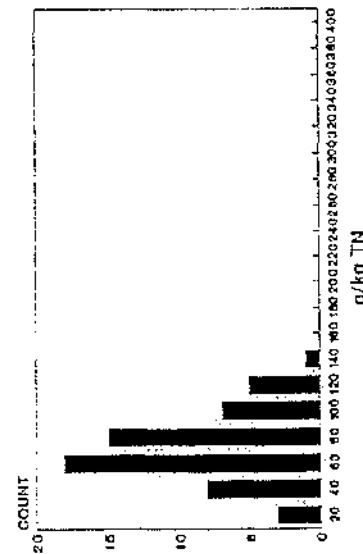


Figure 2.2c

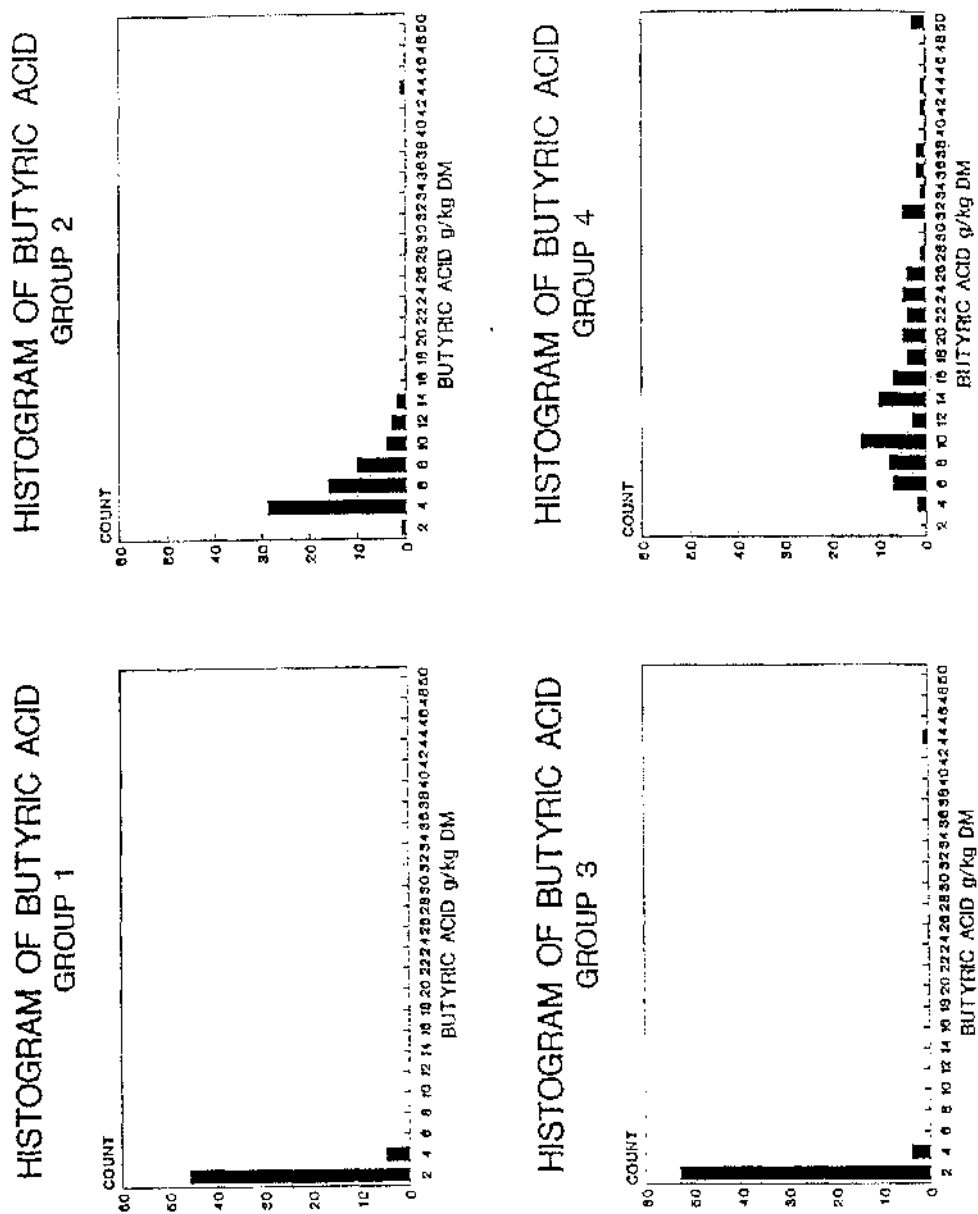
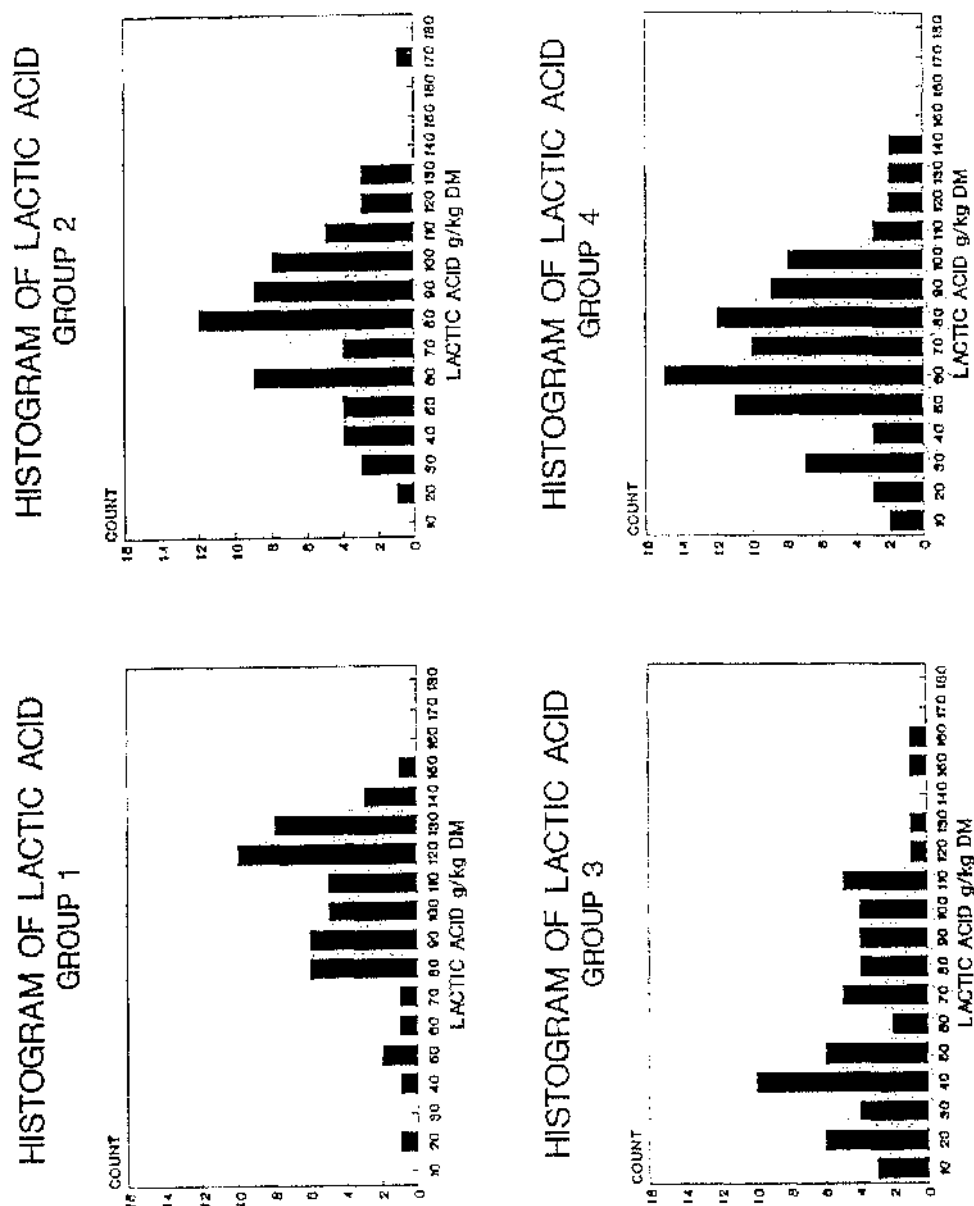


Figure 2.2f



thus appeared possible that a better classification might be achieved with data having a common mean of zero.

To test this possibility, the same data subset was reanalysed into five, four, three and two groups. A common mean of zero assumes that only one scale of measurements with the same range (e.g. -1 to +1) is necessary for all chemical constituents, and it is the relative position of the points on the scale, not their actual values that is important. This classification did not appear, however, to be any improvement on the previous one. Though two out of the three percentage differences between criterion values with a common mean were smaller, there was no clear pattern of group division and reformation when reducing the number of groups (Table 2.4 c.f. Table 2.2). Table 2.5 illustrated the large amount of movement of silages when the group number is reduced from five to four, four to three and three to two respectively. For example, on reduction from five to four groups, Group 1 was split from five groups into three with 25 data points going to Group 1, 24 to Group 2 and 6 to Group 3. On group number reduction the five groups are not split in a logical way to form four groups. This can be seen by the five groups containing 55, 40, 7, 23 and 41 silages and the four groups containing 63, 62, 13 and 28 silages. Tabulation of the reduction from four to three groups and three to two groups similarly shows that considerable group reorganisation takes place. This movement of data points tended to suggest that the nuclei were volatile and that data points were not strongly associated with one group. In view of this it was felt that better classifications were obtained without a common mean of zero.

2.2.3 Development of Analysis

By this time an outline of the methods of group discrimination had been developed. The introductory data exploration had shown that classification was feasible and so the data set was increased to include all the silages in one of the four complete data files, that obtained from ESCA.

2.2.3.1 Analysis of the First Data File of Research Silages

Eleven variables, *viz* toluene dry matter, pH, water soluble carbohydrates, total nitrogen, protein nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid, lactic acid and ethanol were selected as discriminating factors in group classification.

Table 2.4

GROUP CRITERION VALUES WITH A COMMON MEAN

| | Six Parameters | |
|----------|-----------------|-----------------------------|
| | Criterion Value | % Difference Between Groups |
| 5 groups | 511.81 | |
| 4 groups | 578.77 | 11.57 |
| 3 groups | 688.25 | 15.91 |
| 2 groups | 756.84 | 9.06 |

Table 2.5

**TABULATION OF GROUP POSITION OF SILAGES
ON REDUCTION OF NUMBER OF GROUPS**

| 4 groups: | Group 1 | Group 2 | Group 3 | Group 4 | Total no. in each group |
|------------------------------------|--------------------|--------------------|--------------------|--------------------|------------------------------------|
| 5 groups: | | | | | |
| Group 1 | 25 | 24 | 6 | 0 | 55 |
| Group 2 | 2 | 37 | 1 | 0 | 40 |
| Group 3 | 0 | 0 | 6 | 1 | 7 |
| Group 4 | 1 | 1 | 0 | 21 | 23 |
| Group 5 | 35 | 0 | 0 | 6 | 41 |
| Total no. in each group | 63 | 62 | 13 | 28 | 166 |

| 3 groups: | Group 1 | Group 2 | Group 3 | Total no. in each group |
|------------------------------------|--------------------|--------------------|--------------------|------------------------------------|
| 4 groups: | | | | |
| Group 1 | 50 | 13 | 0 | 63 |
| Group 2 | 0 | 62 | 0 | 62 |
| Group 3 | 0 | 7 | 6 | 13 |
| Group 4 | 27 | 0 | 1 | 28 |
| Total no. in each group | 77 | 82 | 7 | 166 |

| 2 groups: | Group 1 | Group 2 | Total no. in each group |
|------------------------------------|--------------------|--------------------|------------------------------------|
| 3 groups: | | | |
| Group 1 | 77 | 0 | 77 |
| Group 2 | 0 | 82 | 82 |
| Group 3 | 2 | 5 | 7 |
| Total no. in each group | 79 | 87 | 166 |

Using these eleven variates, the silages were initially grouped into twelve groups, decreasing to six. Twelve groups were arbitrarily chosen as being a large enough number from which to start the analysis.

The percentage differences in the criterion values indicated that the data were best described by either seven or six groups (Table 2.6). As the percentage difference between these two groupings was only 0.06, the smaller number of groups -six -was selected for further investigation. Interpretation of a smaller number of groups, without compromising on the distinctiveness of these groups, would clearly be easier. These six groups were plotted graphically, plotting the sum of the second vector loading multiplied by the logarithm of the chemical constituent by the sum of the first vector loading multiplied by the logarithm of the chemical constituent and turned out to be quite separate (Figure 2.3 (i)).

2.2.3.2 Identification of the Constituents Characteristic of Each Classification

To aid interpretation to determine which chemical constituents best characterises the six groups, the technique of Canonical Variate Analysis (CVA) was used. This permits the identification of factors responsible for the differences between groups and gives functions of the constituents that can be used to discriminate between them.

The importance of each variable in group classification may be established by examination of the vector loading. This is a linear function of the values of each variable and is calculated for each variable for $n-1$ dimensions, where n is the number of groups for that classification. The smaller the loading, the less important is the associated variable in group discrimination in this dimension.

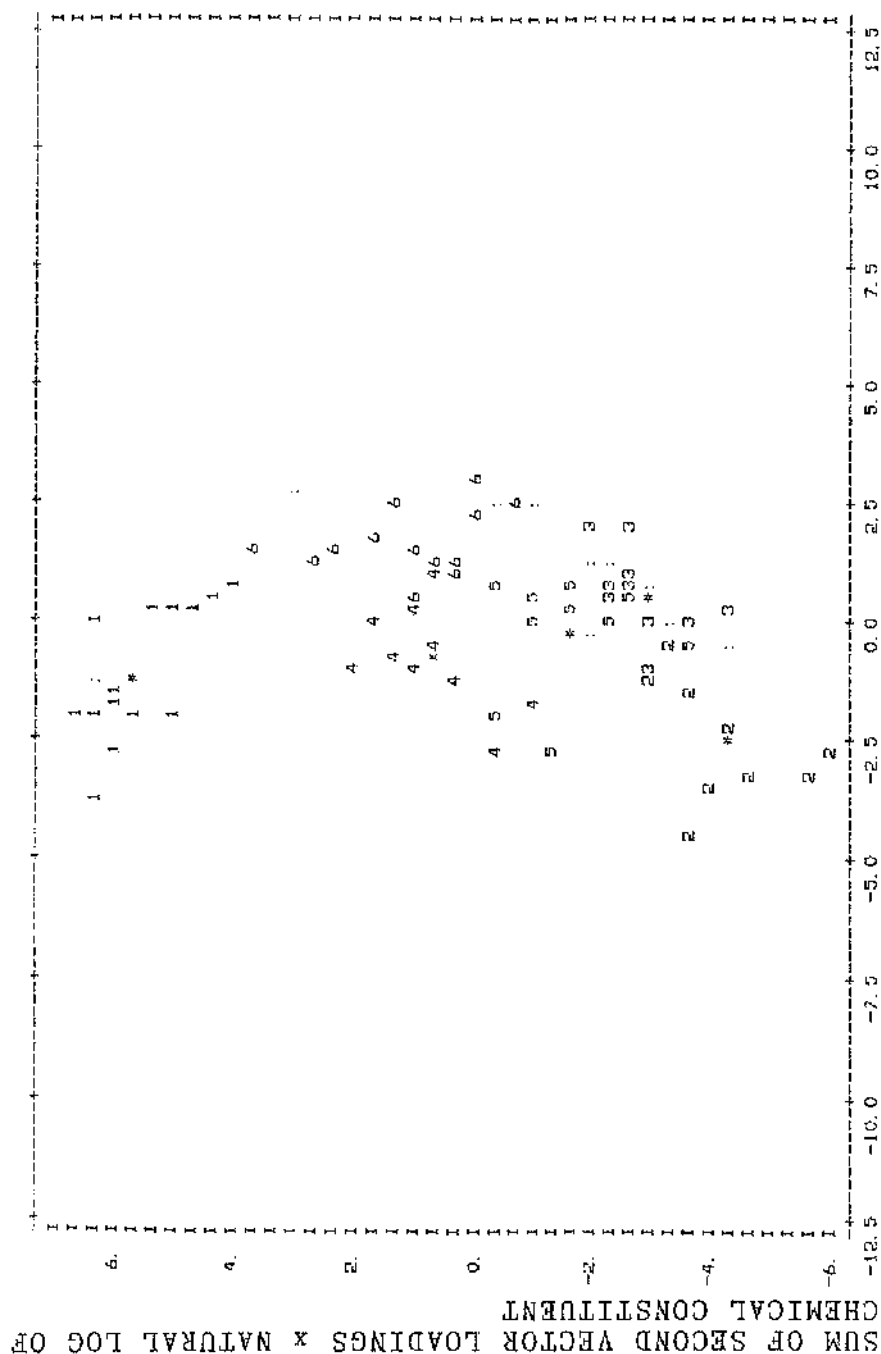
It seemed likely that some of the eleven chemical constituents used in the above analysis were not significant in group discrimination. In fact it was found that pH, acetic acid, propionic acid and ethanol had low first and second vector loadings (Table 2.7). It was thus possible that an acceptable classification could be produced by omitting these four variables and using only seven.

Classification was therefore carried out with seven variables (toluene dry matter, water soluble carbohydrates, total nitrogen, protein nitrogen, ammonia nitrogen, butyric acid and lactic acid). Despite the fact that total nitrogen, protein nitrogen and butyric acid

Table 2.6
CRITERION VALUES FOR ESCA DATA FILE (11 PARAMETERS)

| | Criterion Value | % Difference Between Groups |
|-----------|-----------------|--------------------------------|
| 11 groups | 97.51 | |
| 10 groups | 109.43 | 10.90 |
| 9 groups | 134.89 | 18.88 |
| 8 groups | 142.64 | 5.43 |
| 7 groups | 147.09 | 3.03 |
| 6 groups | 151.78 | 3.09 |
| 5 groups | 165.18 | 8.11 |
| 4 groups | 193.99 | 14.89 |

CLASSIFICATION OF FIRST DATA FILE INTO SIX GROUPS



SUM OF FIRST VECTOR LOADINGS x NATURAL LOG OF CHEMICAL CONSTITUENT

Figure 2.3(i)

Table 2.7

VECTOR LOADINGS FOR ELEVEN CONSTITUENTS

| | First Vector Loading | Second Vector Loading |
|--------|---------------------------------|----------------------------------|
| tol DM | -1.0376 | -1.4840 |
| pH | 0.2303 | 0.0902 |
| WSC | -1.6340 | 0.3247 |
| TN | 0.1367 | -2.3478 |
| PN | -0.0762 | 3.1252 |
| NH3-N | 0.6090 | 1.3921 |
| AA | 0.4300 | 0.2186 |
| PA | 0.2553 | 0.6288 |
| BA | 0.1280 | -1.0499 |
| LA | -0.1394 | 2.1946 |
| ETH | -0.0082 | 0.2683 |

had very low first vector loadings, they had considerably higher second vector loadings, and as such were felt to be important. This selection of components again produced an optimum grouping into six groups.

The question which had then to be considered was how well the classification based on seven variables compared to that of eleven variables. The answer was provided by computer tabulation showing the position of silages in each group for eleven variates against those of seven variates (Table 2.8). The table showed that most and in some cases all of the silages in one classification corresponded to those of the other classification. The larger numbers show where groups are the same or nearly the same in the two classifications. For example all eleven silages from Group 1 with seven variates are members of Group 1 with eleven variates. The additional five silages in Group 1 from eleven variates come from Group 2 with seven variates. Thus Group 1 (11 variates) is an amalgamation of Group 1 and part of Group 2 (7 variates). For this reason seven variates appeared to be as good as eleven.

It should be noted that, if silages were assigned directly to the desired number of groups (e.g. six during cluster analysis), an allocation of the silages occurs which is different from that resulting from the silages being assigned to a large number of groups (e.g. eleven) and then the number of groups being progressively reduced by one until the desired number is reached. Tabulation (Table 2.9) of the six groups formed from eleven and six groups directly from cluster analysis showed, however, that the nucleus of groups tended to remain the same for the two classifications. The nucleus of Group 1 common to both classification schemes is 11 silages, whilst that of Group 2 is 15 silages, Group 3 18 silages and Group 4 14 silages. Unfortunately the nuclei of Groups 5 and 6 were not common to both schemes; Group 5 by direct assignation to six groups was just a small part of Group 5 (by reduction from 11 to 6 groups), and Group 6 (by reduction from 11 to 6 groups) was divided across the three groups from the direct assignation to six groups (Table 2.9).

When starting to assign silages to groups, each silage was initially given an arbitrary group number. The silages in these groups were then repeatedly exchanged and transferred until the best group pattern was achieved. When silages are assigned to six groups by reduction from eleven groups, they have been grouped with other like silages five times. Thus rigid nuclei of similar silages will have been formed, which will be present at each step in group reduction. Compared to classification of silages

Table 2.8

**GROUP POSITION OF SILAGES THAT BELONG TO SIX GROUPS
WITH 11 VARIATES COMPARED TO SIX GROUPS WITH 7 VARIATES**

| | | 11 Variates | | | | | |
|-------------------|---|-------------|----|---|---|----|---|
| | | Groups | 1 | 2 | 3 | 4 | 5 |
| 7 Variates | 1 | 11 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 5 | 10 | 0 | 0 | 0 | 0 |
| | 3 | 0 | 10 | 9 | 0 | 0 | 1 |
| | 4 | 0 | 0 | 9 | 6 | 0 | 0 |
| | 5 | 0 | 1 | 0 | 0 | 10 | 4 |
| | 6 | 0 | 0 | 2 | 3 | 0 | 7 |

Table 2.9

**COMPARISON OF POSITION OF GROUP NUMBERS IN AT SIX
GROUPS AND SIX FROM ELEVEN GROUPS**

| Group nos for 6 groups formed from 11 | Group Nos Directly in at 6 Groups | | | | | |
|--|-----------------------------------|----|----|----|---|----|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 11 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 15 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 18 | 0 | 0 | 2 |
| 4 | 0 | 0 | 1 | 14 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 4 | 11 |
| 6 | 0 | 0 | 1 | 6 | 0 | 5 |

assigned directly to six groups from an arbitrary group number, the former method was preferred in subsequent work, as it was found to group silages in groups with distinctive nuclei able to withstand movement of silages.

2.2.3.3.1 *Interpretation of Graphs*

To ease interpretation and ascertain that the groups calculated by cluster analysis are distinct and well separated, a technique was used to display the silages graphically with each silage identified by its group number. Visual examination then illustrated whether all of the silages in a given group are close to each other, with few outlying values and separated from all other groups. In order to position a silage on the graph, values must be assigned to both the x and y co-ordinates. The x co-ordinate used was the sum of the natural logarithms of the chemical constituents for that silage multiplied by their first vector loading and the y co-ordinate was obtained by multiplying this sum by the second vector loading.

Once the graphs have been drawn, their interpretation and comparison tends to be subjective. There is no statistical test which can be used to compare the group distribution in these graphs. This has therefore to be done visually and different people could interpret the graphs in a slightly different manner.

2.2.3.3.2 *Analysis of the Second and Subsequent Data Files*

The second data file was classified in the same way as the first: by cluster analysis into six groups by reduction from eleven groups, with respect to the eight constituents toluene dry matter, pH, total nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid and lactic acid. Despite three of these (pH, acetic acid and propionic acid) having been shown previously on the small data set for this file to contribute very little to group discrimination, they were subsequently found to be useful parameters in group classification, by the size of their vector loadings, when a more extensive and varied set of silage analyses was used. Ethanol and protein nitrogen, which both had small vector loadings, were found not to be important factors in classification, and hence were omitted from subsequent analysis. In addition water soluble carbohydrates had to be omitted to increase the number of silages used for analysis as a large proportion of silages had not been tested for sugars.

These same eight variates (toluene dry matter, pH, total nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid and lactic acid) were then used to group the third and fourth data files into six groups, this having previously been shown to be the optimum number. The silages assigned to each group were then plotted on a graph. For comparison the first data file was reclassified using the above eight parameters and then the silages were plotted on a separate graph. Both graphs showed visually distinct groups.

2.2.3.4 Analysis of the Complete Data Set

2.2.3.4.1 *Verification of the Number of Groups*

Combinations of the first two data files were then grouped and graphed, and finally the four data files were combined into one for analysis. Despite this large data file containing 714 silages, only a proportion of these (263) were able to be used in subsequent analyses due to only this number having been chemically analysed for all of the required constituents. This data file was classified into six groups and the individual silages plotted on a graph. Visual examination showed that, whilst the first four groups appeared to be spatially separate, Groups 5 and 6 were not as tightly knit as the others (Figure 2.3 (ii)). In this figure Group 5 was very small and looked as if it could form part of Group 6. Also Group 3 seemed to have a natural split between Groups 1 and 6. It therefore seemed possible that four groups would produce a more distinct classification system and this did indeed prove to be the case (Figure 2.4).

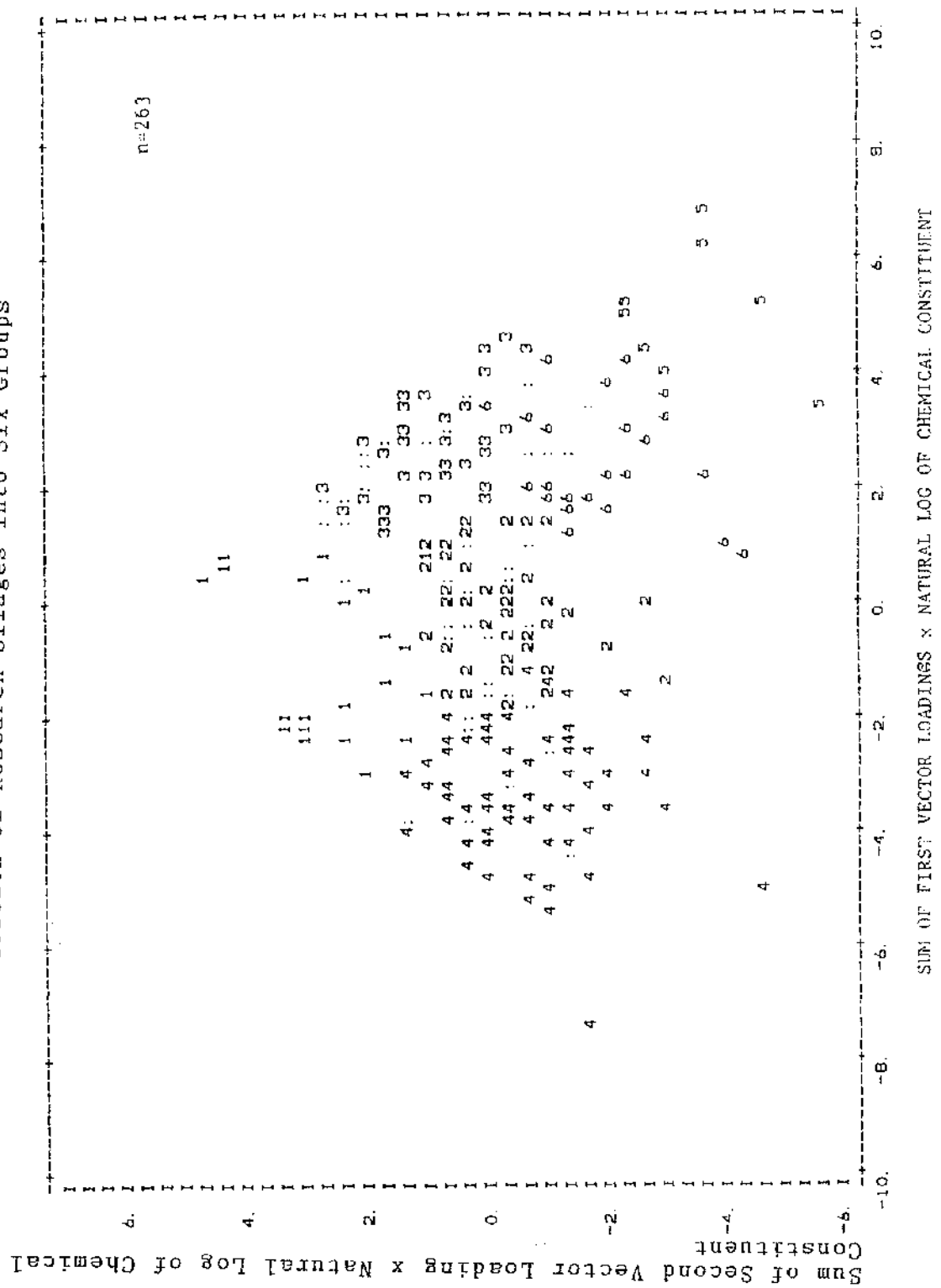
It therefore seemed likely that this classification would be the best available. Further investigation was carried out, however, to determine whether three groups would be better than four, with the data being classified into three groups by reduction from eleven. It was found that the criterion value percentage difference was only 1% smaller for four groups than for three; but when the silages allocated to the three group classification were examined graphically, it appeared that the resultant groups were too generalised and covered too large a range of values (Figure 2.5). It was therefore concluded that four groups provided a better classification than three.

2.2.3.4.2 *Verification of Parameters Used*

The use of alternative combinations of constituents to clarify groups appeared to be an

Figure 2.3 (ii)

Classification of Research Silages into Six Groups



Group Classification

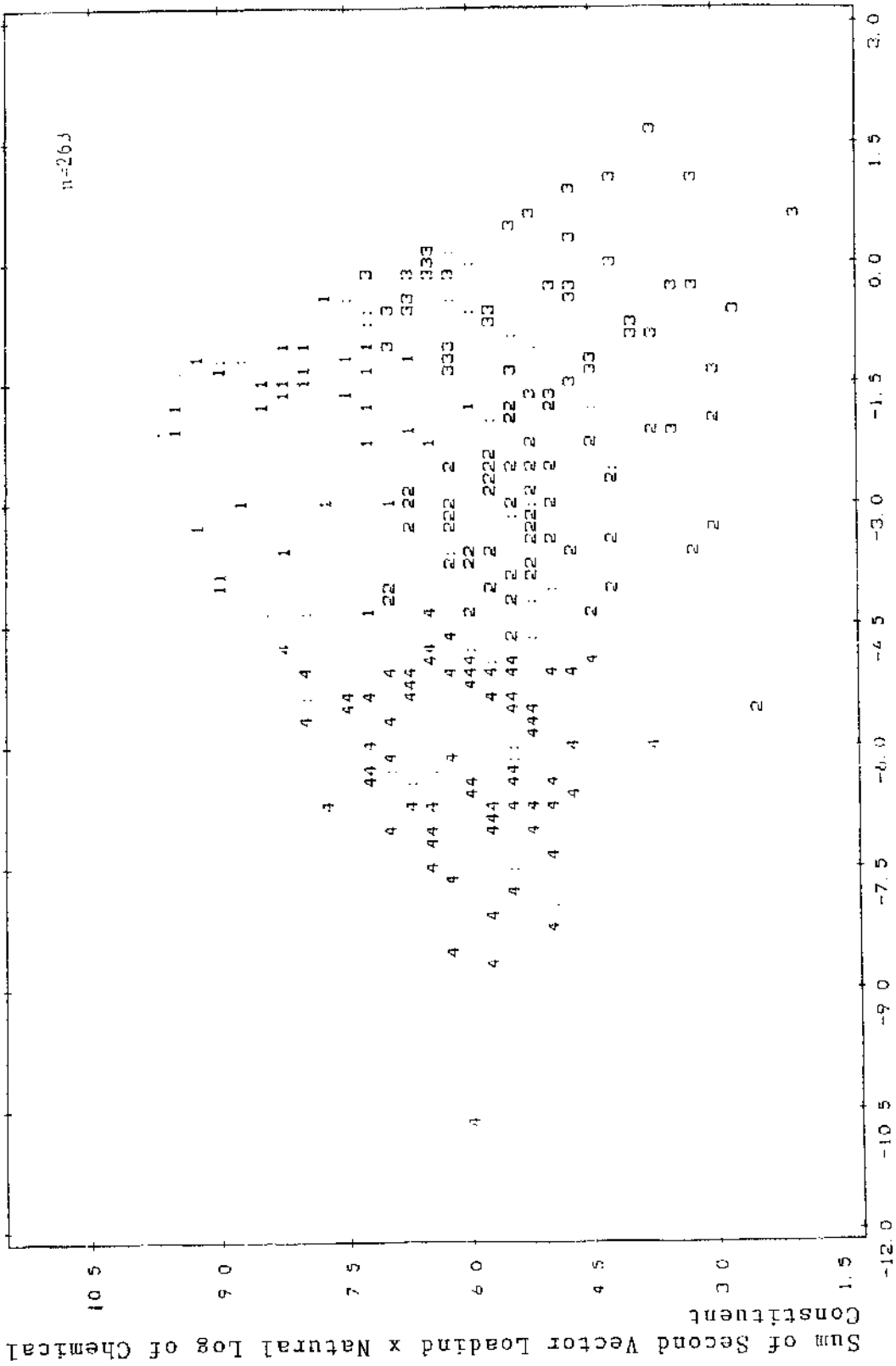
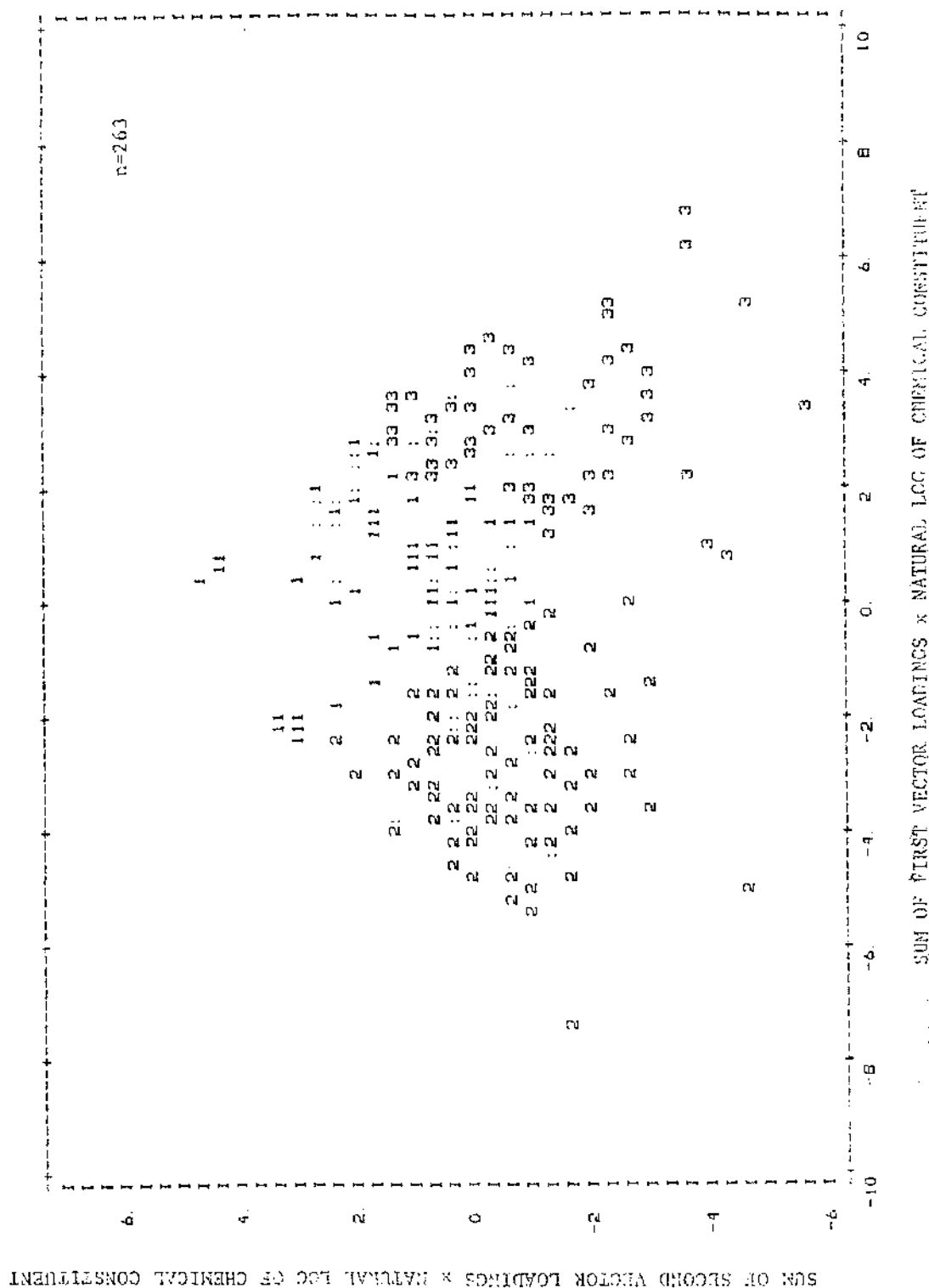


Figure 2.4

SUM OF FIRST VECTOR LOADINGS X NATURAL LOG OF CHEMICAL CONSTITUENT

CLASSIFICATION OF RESEARCH SILAGES INTO THREE GROUPS



area worth investigating. It seemed possible that MAD fibre, whilst not a fermentation end product, might be an important factor for classification and thus it was included in further analysis. In the same analysis total volatile fatty acids (TVFA) were substituted for acetic, propionic and butyric acids to determine whether one measurement instead of three might produce equally acceptable groupings.

The data were then re-analysed by cluster analysis and four groups formed (Figure 2.6). The inclusion of MAD fibre did not influence the groupings to any great extent as the vector loading for this constituent was very small and so it was discarded as a significant factor. From the vector loadings TVFAs were found to be making a more significant contribution to group discrimination, but they did not appear to be as sensitive a characteristic factor as the three individual acids. Hence this variable was also discarded in favour of acetic, propionic and butyric acids. Furthermore, the criterion value percentage difference was over 17% greater with the inclusion of MAD fibre and the substitution of TVFAs for individual acids. This substantiates the conclusion drawn from a subjective examination of Figure 2.6, namely that MAD fibre does not aid group classification and individual volatile fatty acids are better than TVFA in group classification.

From an examination of the mean values for each group, it was evident that the values for toluene dry matter and total nitrogen did not differ greatly and hence it seemed possible that they were not important in group discrimination (Table 2.10).

Removal of these two parameters produced groups less tightly knit, with many outlying values (Figure 2.7). Thus toluene dry matter and total nitrogen appeared to be making a significant contribution to the classification and were retained as variables.

2.3 FINAL CLASSIFICATION

2.3.1 Description of the Final Solution

The optimum classification was that found previously before the substitution and inclusion of additional chemical constituents namely, four groups with toluene dry matter, pH, total nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid and lactic acid as the discriminatory variables (Figure 2.4).

CLASSIFICATION INCLUDING MADF AND SUBSTITUTING TOTAL VOLATILE FATTY ACIDS

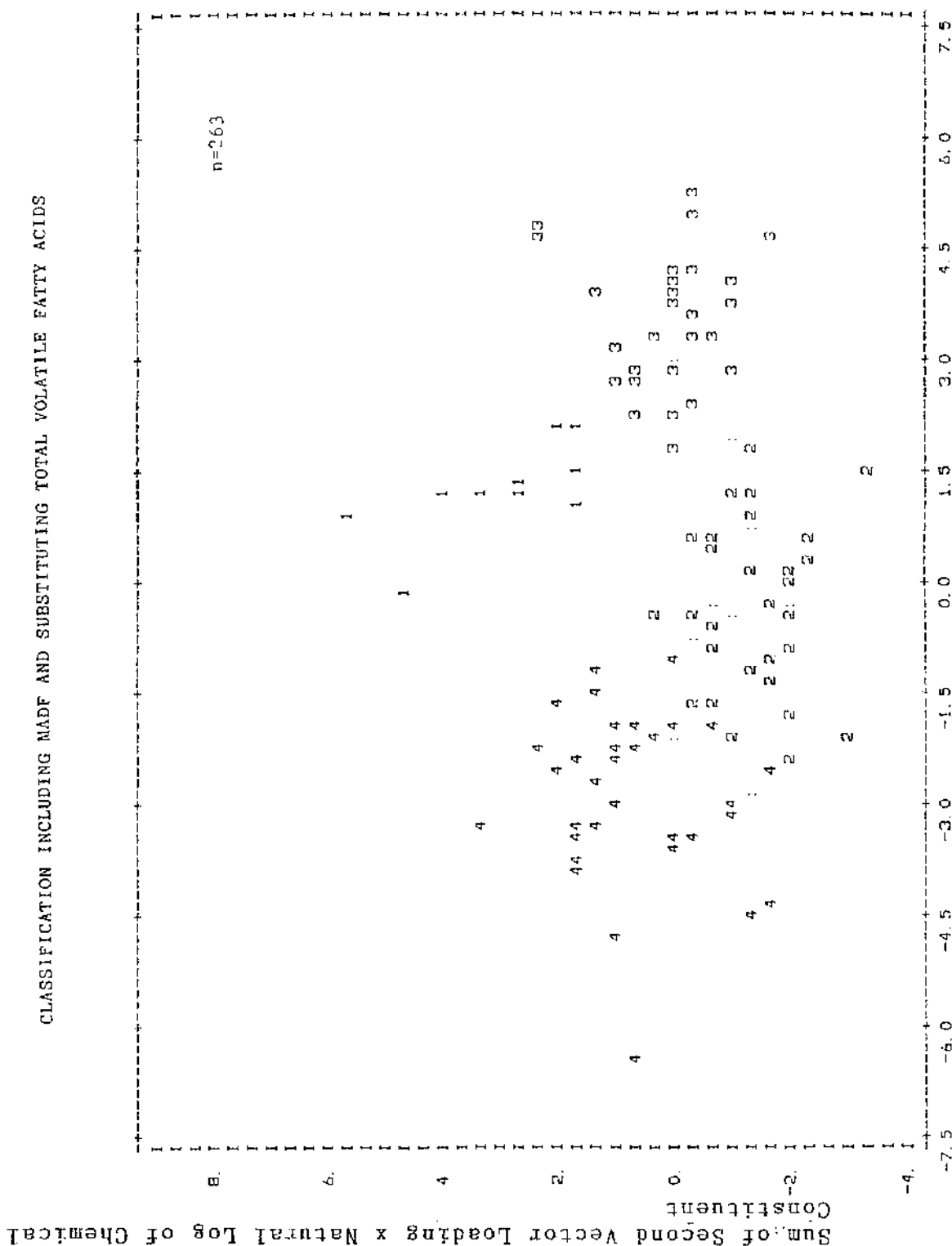


Figure 2.6

SUM OF FIRST VECTOR LOADINGS x NATURAL LOG OF CHEMICAL CONSTITUENT

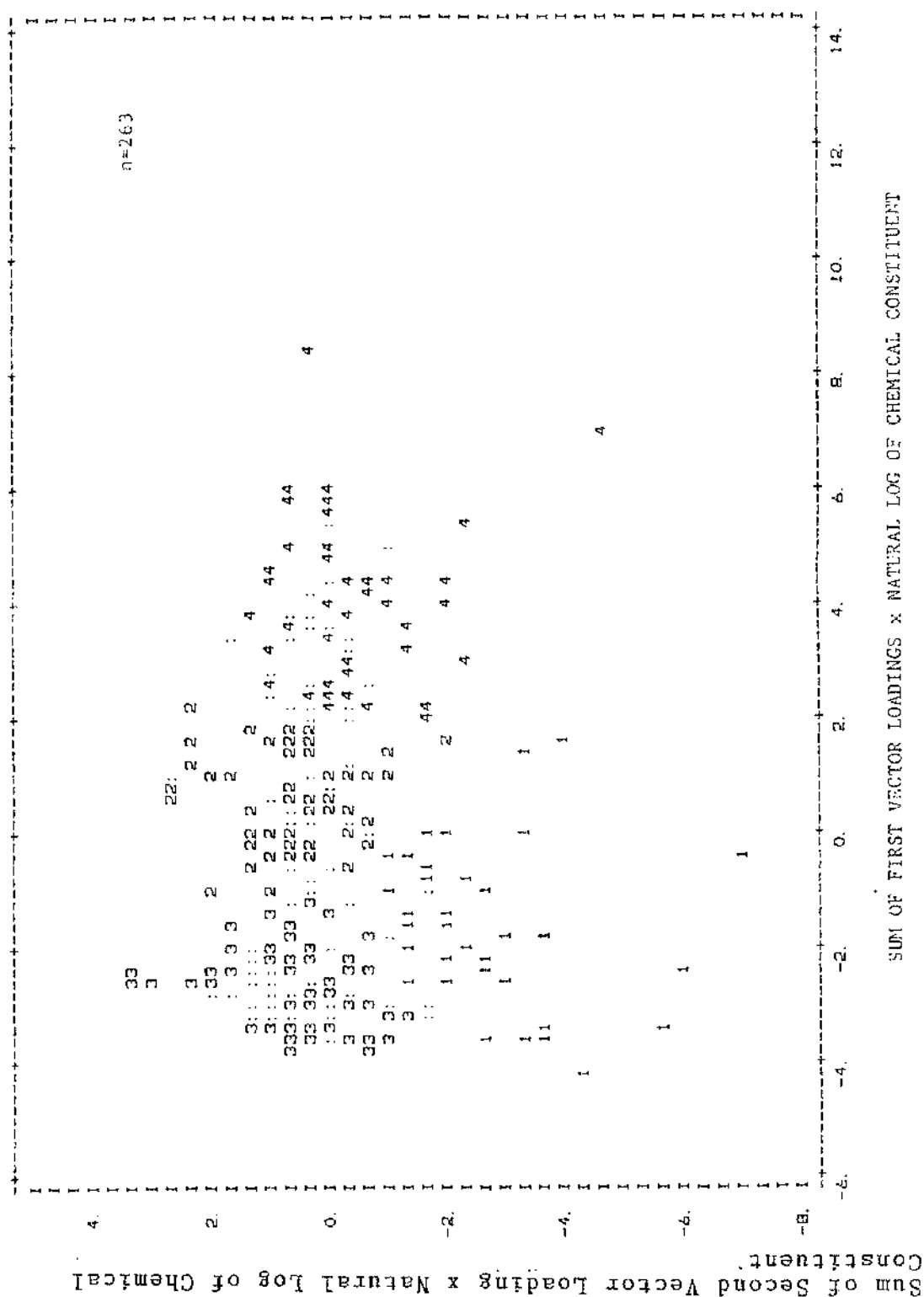
Table 2.10

TOLUENE DRY MATTER AND TOTAL NITROGEN VALUES

| Group | tDM (ln) | TN (ln) |
|--------------|---------------------|--------------------|
| 1 | 5.362 | 3.204 |
| 2 | 5.495 | 3.353 |
| 3 | 5.494 | 3.281 |
| 4 | 5.363 | 3.471 |

Figure 2.7

CLASSIFICATION WITHOUT TOTAL NITROGEN AND DRY MATTER



The four groups were distinct and characterised as follows:

GROUP 1- lowest pH, highest lactic acid,

a good homofermentative fermentation

GROUP 2- moderate amounts of lactic acid and

acetic acid

GROUP 3- lowest ammonia nitrogen and acetic acid,

a restricted fermentation

GROUP 4- highest pH, ammonia nitrogen, acetic,

propionic and butyric acids, a poor fermentation (Figure 2.4)

Groups with these characteristics had been produced from the data files whether run individually, or in different combinations of these files, as well as when all data files were combined and run together (Table 2.11). For each chemical constituent and for each group, both the minimum and maximum values allowed are given. Though the ranges of the chemical constituents overlap between groups, the mean value indicates how the groups compare. For example the range of pH for silages in Group 1 is 3.60 to 4.50 and that of Group 4 is 3.68 to 5.70. Despite considerable overlapping the mean pH for Group 1 is 3.98 as compared to 4.48 for Group 4. Thus the silages in Group 1 tend to be more acidic than those of Group 4. It should be noted, however, that it is the combination of the chemical constituents that distinguishes one group from another, not just a single parameter.

The maximum values in Table 2.11 do not correspond with those in Table 2.1 as the number of silages used in the final solution was a subset of the data originally collected, due to the inability of the analysis methods selected, to use missing values.

Vector loadings are calculated in three dimensions, thus when the groups were plotted in three dimensions, x, y and z, most of the between-group variation (79%) was found to be in the x- axis. The y-axis accounted for most of the remaining variation in groups (18%). The z-axis did not contribute much towards group discrimination (3%), hence was disregarded (Table 2.12).

2.3.1.1. Graphical Examination

On examination of the final solution graph and the group mean values, it can be seen that there is considerable overlapping in pH between groups (Figure 2.4, Table 2.11).

Table 2.11

CHEMICAL COMPOSITION OF SILAGES OF THE FINAL GROUPS

| | | Min | Mean | Max | |
|----------------|----------------------------|-------|-------|-------|------|
| Group 1 | tDM g/kg | 154.0 | 213.2 | 340.0 | n=51 |
| | pH | 3.60 | 3.98 | 4.50 | |
| | TN g/kg DM | 13.0 | 24.6 | 42.4 | |
| | NH ₃ -N g/kg TN | 58.0 | 96.6 | 165.8 | |
| | AA g/kg DM | 18.3 | 51.3 | 219.0 | |
| | PA g/kg DM | 0.0 | 2.4 | 8.1 | |
| | BA g/kg DM | 0.0 | 1.5 | 5.0 | |
| | LA g/kg DM | 20.0 | 95.5 | 182.9 | |
| Group 2 | tDM g/kg | 170.0 | 243.5 | 349.0 | n=66 |
| | pH | 3.70 | 4.22 | 5.10 | |
| | TN g/kg DM | 17.5 | 28.6 | 38.8 | |
| | NH ₃ -N g/kg TN | 52.0 | 112.8 | 220.1 | |
| | AA g/kg DM | 7.9 | 28.0 | 62.0 | |
| | PA g/kg DM | 0.0 | 2.5 | 6.4 | |
| | BA g/kg DM | 2.7 | 6.0 | 45.0 | |
| | LA g/kg DM | 16.0 | 70.7 | 166.0 | |
| Group 3 | tDM g/kg | 165.0 | 243.2 | 515.9 | n=57 |
| | pH | 3.61 | 4.09 | 5.09 | |
| | TN g/kg DM | 15.7 | 25.0 | 38.0 | |
| | NH ₃ g/kg TN | 9.3 | 37.5 | 134.0 | |
| | AA g/kg DM | 1.8 | 12.6 | 27.9 | |
| | PA g/kg DM | 0.0 | 1.5 | 4.0 | |
| | BA g/kg DM | 0.0 | 1.6 | 4.0 | |
| | LA g/kg DM | 0.0 | 58.1 | 141.0 | |
| Group 4 | tDM g/kg | 166.0 | 213.4 | 275.8 | n=89 |
| | pH | 3.68 | 4.48 | 5.70 | |
| | TN g/kg DM | 16.9 | 32.2 | 47.6 | |
| | NH ₃ -N g/kg TN | 70.8 | 159.7 | 409.1 | |
| | AA g/kg DM | 32.0 | 79.5 | 193.1 | |
| | PA g/kg DM | 2.9 | 7.8 | 48.0 | |
| | BA g/kg DM | 4.0 | 15.8 | 109.0 | |
| | LA g/kg DM | 0.0 | 58.1 | 141.0 | |

Table 2.12

VECTOR LOADINGS

| | 1 | 2 | 3 |
|-------------|---------|---------|---------|
| tDM | 0.6408 | -0.0063 | -0.0773 |
| pH | -0.1422 | 0.5996 | -0.7529 |
| TN | 0.2732 | -1.5545 | -0.4959 |
| NH3-N | -0.3364 | 0.8961 | 1.4396 |
| AA | -0.5859 | 1.3645 | 0.2783 |
| PA | -0.7853 | 0.4633 | -1.0744 |
| BA | -1.2582 | -1.4217 | -0.5417 |
| LA | -0.1121 | 0.3208 | 0.9443 |
| % variation | 79.05 | 17.94 | 3.00 |

Closer examination shows that Groups 1 and 3 have the lowest pH, with Group 2 having a slightly higher pH. Group 4 has the highest pH, and is clearly separated to the left of the graph. Thus in its simplest form this classification could broadly be described as a measure of acidity, with pH decreasing along the x-axis.

2.3.1.2 Water Soluble Carbohydrate Content

The occurrence and amounts of water soluble carbohydrates were found for each of the four groups where data were available (Table 2.13). For the silages in each group that had been analysed for sugars, a mean value was calculated. Ideally water soluble carbohydrates would have been used for group discrimination, but as this was not possible comparison of the mean values was examined. The group of silages where fermentation had been restricted, Group 3 had the highest mean sugar content. Group 2 had the next highest mean sugar content, but with a very large range of values and the good fermentation Group 1 was third. A probable reason for the water soluble carbohydrate content of Group 2 being higher than that of Group 1 would be the greater additive use on Group 2 silages; 72% compared to 45%. Group 4, the poor quality silages, had the lowest mean sugar content. This is to be expected as the clostridia and yeasts would dominate the fermentation, utilising all available substrate sugars.

2.3.1.3 Acid Content

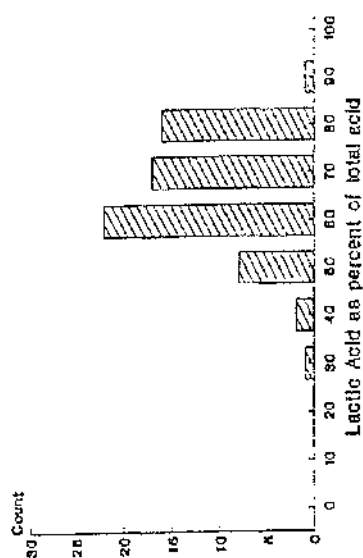
To investigate the relationship between the differing amounts of acids present in each of the four groups, the four acids, acetic, propionic, butyric and lactic, were converted to a percentage total acids basis (Figure 2.8). This was done to see whether the basis of expression had an effect on their relative order: perhaps when expressed on a percentage total acid basis, Group 1 would have the highest levels of lactic acid and Group 4 the lowest. All of the acids were in the same relative order when on this scale as on a g/kg DM basis except in the case of lactic acid. Group 3, which had the lowest amount of lactic acid on a DM basis, had the highest when expressed on a percentage total acids basis (Figure 2.9). This is also in contrast to the actual amounts of TVFAs on a g/kg DM basis, where Group 3 had the lowest levels at 34.2 g/kgDM, followed by Group 1 at 54.9 g/kgDM, Group 2 at 87.5 g/kgDM and Group 4 at 170.7 g/kgDM.

Table 2.13

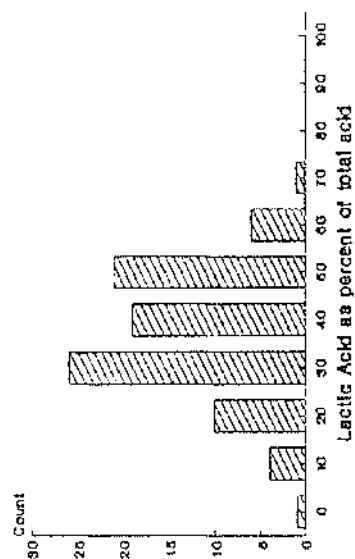
**MEAN GROUP WATER SOLUBLE CARBOHYDRATE CONTENTS
(g/kg DM)**

| Group | 1 | 2 | 3 | 4 |
|---------------|----------|----------|----------|----------|
| no. of values | 40 | 21 | 31 | 31 |
| mean | 13.3 | 40.9 | 96.3 | 6.8 |
| sed | 13.8 | 39.8 | 63.7 | 5.1 |

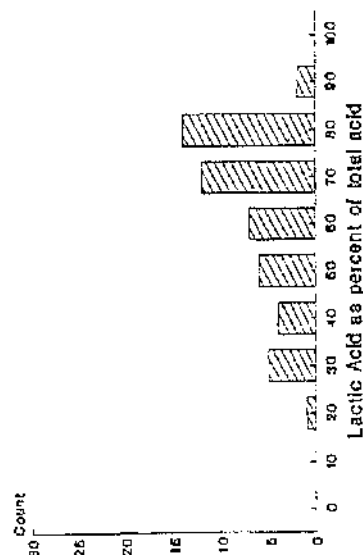
HISTOGRAM OF LACTIC ACID
GROUP 2



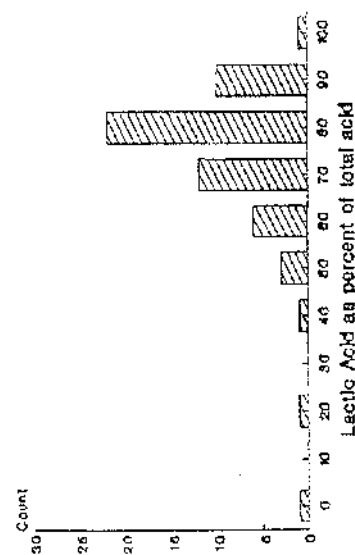
HISTOGRAM OF LACTIC ACID
GROUP 4



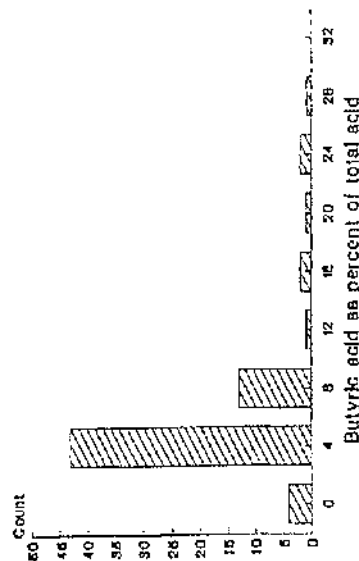
HISTOGRAM OF LACTIC ACID
GROUP 1



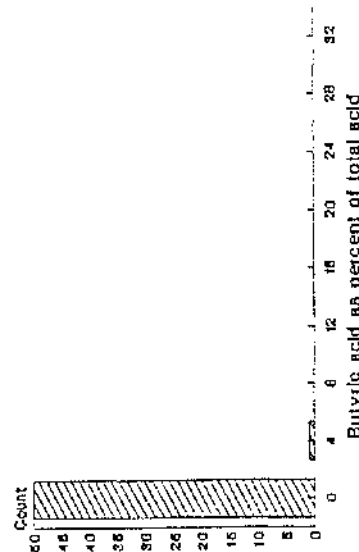
HISTOGRAM OF LACTIC ACID
GROUP 3



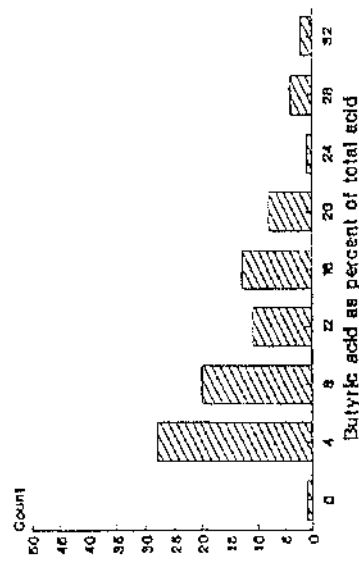
HISTOGRAM OF BUTYRIC ACID
GROUP 2



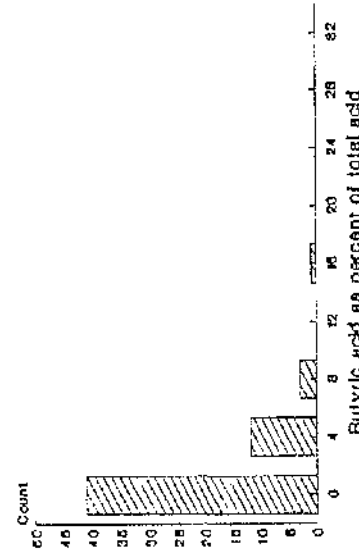
HISTOGRAM OF BUTYRIC ACID
GROUP 1



HISTOGRAM OF BUTYRIC ACID
GROUP 4



HISTOGRAM OF BUTYRIC ACID
GROUP 3



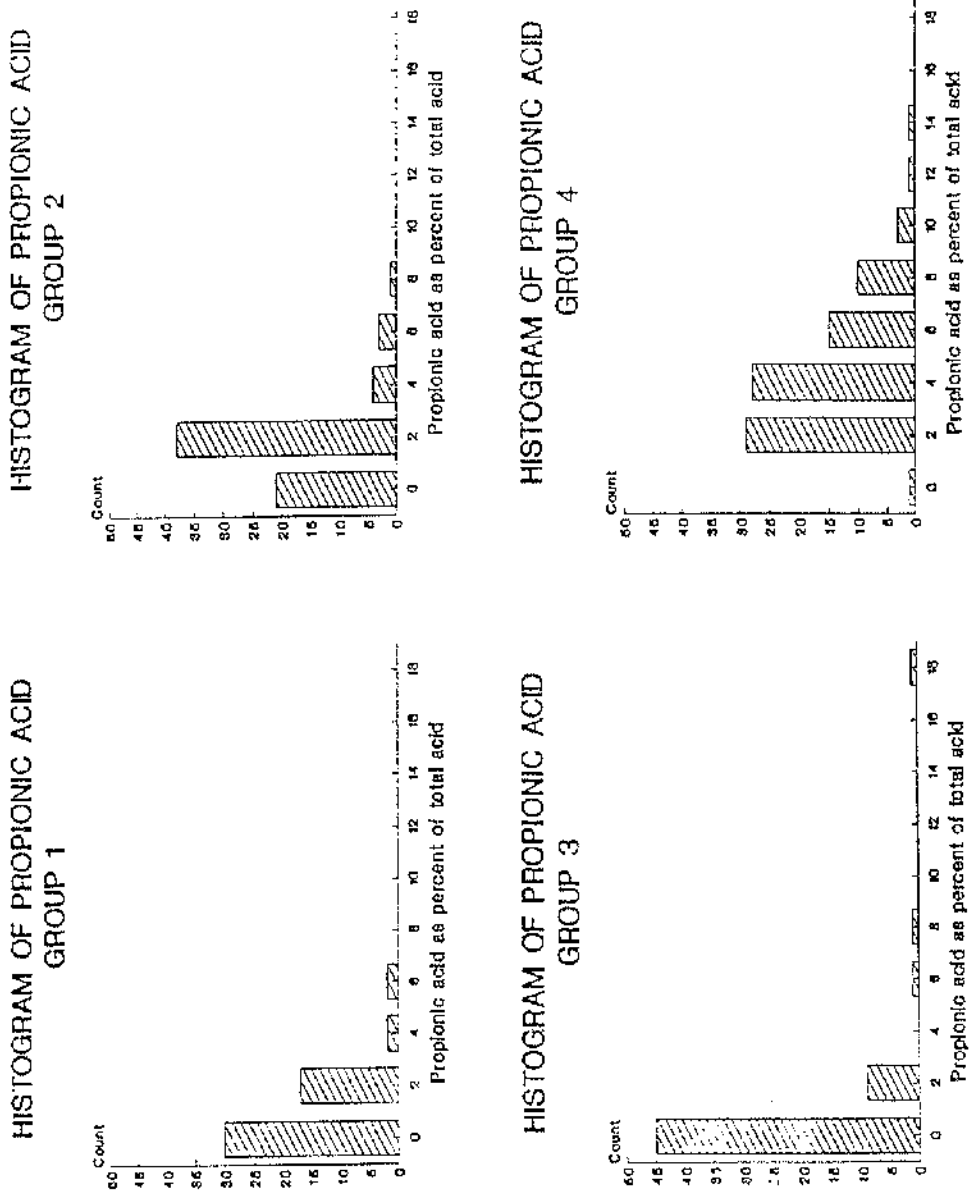
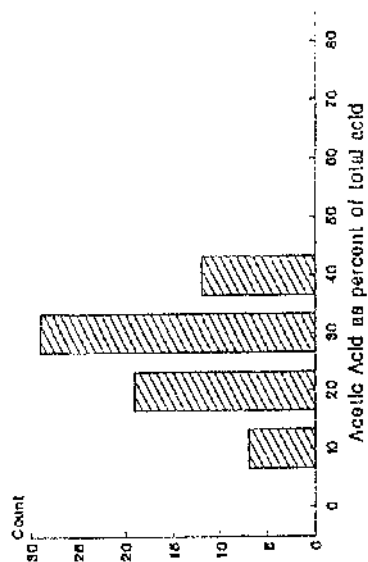
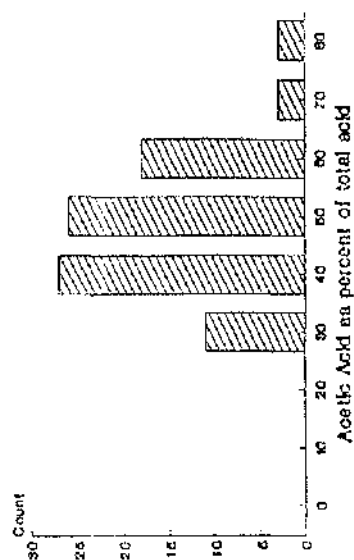


Figure 2.8 c

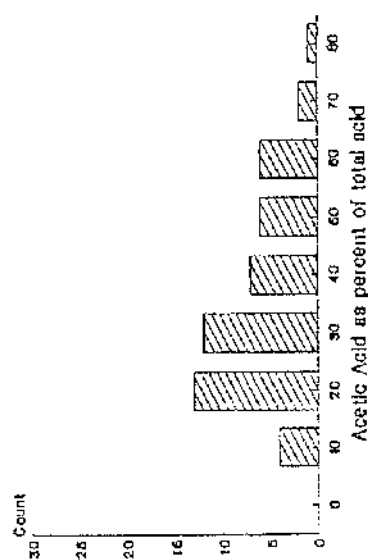
HISTOGRAM OF ACETIC ACID GROUP 2



HISTOGRAM OF ACETIC ACID GROUP 4



HISTOGRAM OF ACETIC ACID GROUP 1



HISTOGRAM OF ACETIC ACID GROUP 3

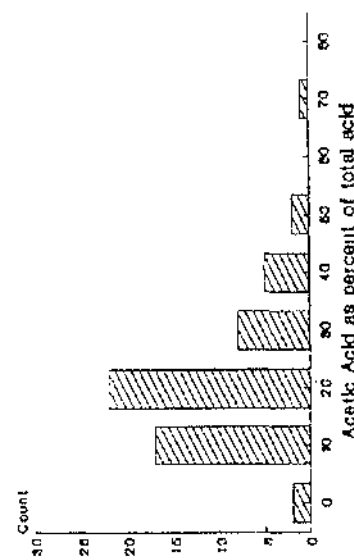
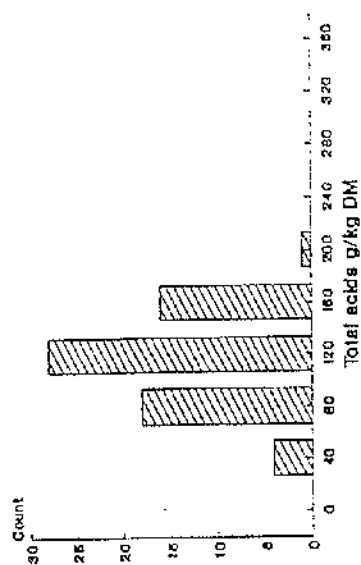
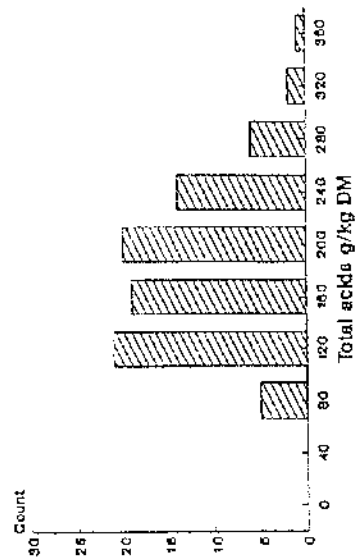


Figure 2.9

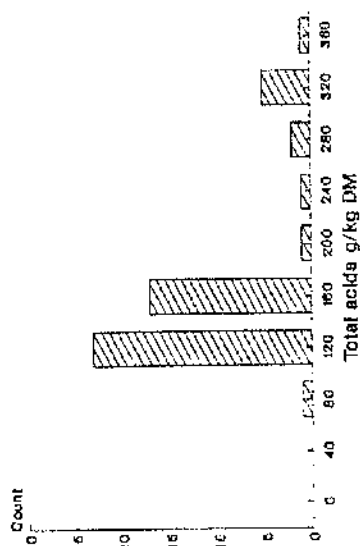
HISTOGRAM OF TOTAL ACIDS
GROUP 2



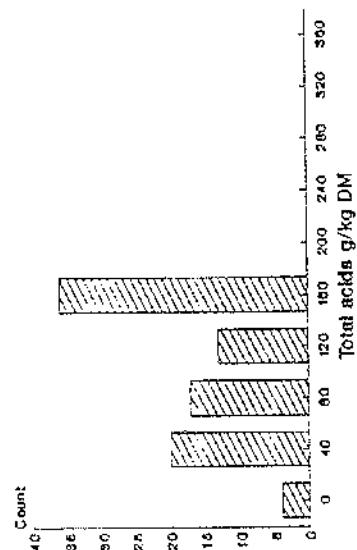
HISTOGRAM OF TOTAL ACIDS
GROUP 4



HISTOGRAM OF TOTAL ACIDS
GROUP 1



HISTOGRAM OF TOTAL ACIDS
GROUP 3



2.3.1.4 Additive Use

The presence and type of additives in each of the four classification groups were also investigated (Table 2.14). Using the Chi Squared test on these silages, Group 1 was found to have a significantly greater number of silages with no additive ($p < 0.001$). Similarly Group 4 had a significantly greater number of silages with the additive Sylade (ICI Chemicals Ltd) ($p < 0.001$) and Group 3 had a significantly greater number of silages with the additive Add-F (BP Chemicals Ltd) ($p < 0.001$).

2.3.2 Group Allocation of Silages

New silages can now be allocated to one of the four groups by plotting a point on the graph. To calculate the x co-ordinate, the natural logarithms of each of the eight chemical constituents are multiplied by the first vector loading for that constituent and the resultant figures are summed (Table 2.12). The y-axis co-ordinate is given by the sum of the second vector loadings multiplied by their natural logarithmic values. The position on the graph indicates in which of the four groups the silage lies. The third dimension, the z-axis, plays a very small part in group discrimination accounting for only 3% of the variation and is not included. A detailed example of this allocation is given in Appendix 2.

2.3.3 Group Correlations

Once the four groups had been quantified, the interrelationships between the chemical constituents were examined by the construction of correlation matrices.

Firstly a correlation matrix was calculated for the entire data set. The strongest correlations were found between propionic acid and butyric acid ($r = 0.774$) and ammonia nitrogen and butyric acid ($r = 0.734$). Other important relationships occurred between;

| | | |
|-------------------------------------|--------------|--------------|
| pH and ammonia nitrogen | $r = 0.636$ | |
| pH and butyric acid | $r = 0.586$ | |
| pH and lactic acid | $r = -0.527$ | |
| total nitrogen and acetic acid | $r = 0.510$ | |
| ammonia nitrogen and propionic acid | $r = 0.547$ | |
| acetic acid and propionic acid | $r = 0.486$ | (Table 2.15) |

Table 2.14

ADDITIVE DISTRIBUTION

| | Group | | | |
|----------------|-------|----|----|----|
| | 1 | 2 | 3 | 4 |
| No Additive | 58 | 28 | 15 | 15 |
| Add-F | 8 | 25 | 35 | 7 |
| Sulphuric acid | | 2 | | |
| Sylade | 27 | 44 | 5 | 70 |
| Silaform | | 5 | 8 | |
| Formaldehyde | | 9 | 14 | 1 |
| + formic acid | | | | |
| Formalin | | | 2 | |
| Ecosyl | | | | 7 |
| Biomax | | | | 1 |
| Innoculant | 10 | | 8 | |
| Enzyme | | 2 | 2 | |

Table 2.15

TOTAL DATA SET CORRELATION MATRIX

| | | | | | | | | |
|-------|--------|--------|-------|--------|-------|--------|--------|-------|
| DM | 1.000 | | | | | | | |
| pH | 0.134 | 1.000 | | | | | | |
| TN | 0.005 | 0.340 | 1.000 | | | | | |
| NH3-N | -0.040 | 0.636 | 0.381 | 1.000 | | | | |
| AA | -0.243 | 0.164 | 0.510 | 0.384 | 1.000 | | | |
| PA | -0.225 | 0.433 | 0.370 | 0.547 | 0.486 | 1.000 | | |
| BA | -0.047 | 0.586 | 0.377 | 0.734 | 0.358 | 0.774 | 1.000 | |
| LA | -0.171 | -0.527 | 0.147 | -0.138 | 0.211 | -0.062 | -0.137 | 1.000 |
| | DM | pH | TN | NH3-N | AA | PA | BA | LA |

In both Groups 1 and 2, the highest correlations occurred between pH and lactic acid. ($r = -0.652$ in Group 1; $r = -0.679$ in Group 2) This relationship was not as important in Groups 3 and 4 where $r = -0.473$ and -0.489 respectively. In Group 3 the correlation of pH and acetic acid was of equal importance ($r = -0.492$) whilst in Group 4 pH and ammonia nitrogen were very highly correlated. ($r = 0.703$) (Tables 2.15, 2.16, 2.17, 2.18, 2.19).

Several other noteworthy relationships found in the individual groups also occurred in the whole data set: in Group 1 total nitrogen was positively correlated to acetic acid and in Group 4 ammonia nitrogen was positively correlated to pH and butyric acid. The only sizable relationship which was common only to Groups 1 and 2 was dry matter and propionic acid being negatively correlated ($r = -0.494$ and $r = -0.432$), but this does not have such importance in the whole data set where $r = -0.225$ (Tables 2.15, 2.16, 2.17, 2.18, 2.19).

These relationships highlight some of the main group characteristics. In Groups 1 and 2, pH and lactic acid are important variables, whilst in Groups 3 and especially 4, they are of less importance. In the latter two groups components of less well fermented silages e.g. ammonia nitrogen and butyric acid had stronger correlations and were more important. The fact that variables are highly correlated does not give an indication of their importance for classification. The variables were selected because they contributed to the overall group classification, each one being as important as the others.

2.4 ADVISORY SILAGES

2.4.1 Materials

Some 3,535 silage analyses were obtained from the Scottish Agricultural Colleges Advisory Service. All of the data were obtained from routine analysis of farm silages or the analysis of silages where there had been a problem. They were all pit silages, with 61% being classified as high quality and 39% as medium quality according to the SAC quality rating (Appendix 3). The samples originated from all parts of Scotland, from Shetland and Orkney in the north to the Borders and Dumfries and Galloway in the south. The south-western corner of Scotland produced the highest concentration of

Table 2.16

CORRELATION MATRIX FOR GROUP 1

| | | | | | | | | |
|-------|--------|--------|--------|--------|--------|--------|-------|-------|
| DM | 1.000 | | | | | | | |
| pH | 0.022 | 1.000 | | | | | | |
| TN | 0.199 | 0.179 | 1.000 | | | | | |
| NH3-N | 0.383 | 0.373 | 0.453 | 1.000 | | | | |
| AA | 0.108 | 0.167 | 0.570 | 0.342 | 1.000 | | | |
| PA | -0.494 | 0.342 | -0.007 | -0.012 | 0.275 | 1.000 | | |
| BA | 0.059 | 0.110 | 0.389 | 0.299 | 0.477 | 0.396 | 1.000 | |
| LA | 0.034 | -0.652 | 0.302 | -0.008 | -0.009 | -0.206 | 0.144 | 1.000 |
| | DM | pH | TN | NH3-N | AA | PA | BA | LA |

Table 2.17

CORRELATION MATRIX FOR GROUP 2

| | | | | | | | | |
|-------|--------|--------|--------|-------|-------|--------|--------|-------|
| DM | 1.000 | | | | | | | |
| pH | 0.337 | 1.000 | | | | | | |
| TN | 0.059 | 0.045 | 1.000 | | | | | |
| NH3-N | -0.086 | 0.174 | 0.249 | 1.000 | | | | |
| AA | -0.208 | -0.306 | 0.375 | 0.308 | 1.000 | | | |
| PA | -0.432 | 0.151 | -0.163 | 0.041 | 0.068 | 1.000 | | |
| BA | 0.234 | 0.317 | -0.013 | 0.355 | 0.056 | -0.076 | 1.000 | |
| LA | -0.147 | -0.679 | 0.270 | 0.082 | 0.436 | -0.192 | -0.180 | 1.000 |
| | DM | pH | TN | NH3-N | AA | PA | BA | LA |

Table 2.18

CORRELATION MATRIX FOR GROUP 3

| | | | | | | | | |
|-------|--------|--------|--------|--------|--------|-------|--------|-------|
| DM | 1.000 | | | | | | | |
| pH | 0.522 | 1.000 | | | | | | |
| TN | -0.025 | 0.376 | 1.000 | | | | | |
| NH3-N | 0.493 | 0.269 | -0.026 | 1.000 | | | | |
| AA | -0.289 | -0.492 | -0.147 | 0.212 | 1.000 | | | |
| PA | -0.190 | -0.116 | 0.179 | -0.227 | -0.127 | 1.000 | | |
| BA | 0.305 | 0.214 | -0.023 | 0.202 | -0.099 | 0.242 | 1.000 | |
| LA | -0.186 | -0.473 | -0.034 | 0.076 | 0.433 | 0.006 | -0.158 | 1.000 |
| | DM | pH | TN | NH3-N | AA | PA | BA | LA |

Table 2.19

CORRELATION MATRIX FOR GROUP 4

| | | | | | | | | |
|-------|--------|--------|-------|--------|-------|--------|--------|-------|
| DM | 1.000 | | | | | | | |
| pH | -0.057 | 1.000 | | | | | | |
| TN | 0.242 | 0.055 | 1.000 | | | | | |
| NH3-N | 0.173 | 0.703 | 0.028 | 1.000 | | | | |
| AA | -0.210 | -0.107 | 0.346 | -0.131 | 1.000 | | | |
| PA | -0.111 | 0.221 | 0.252 | 0.105 | 0.365 | 1.000 | | |
| BA | 0.252 | 0.539 | 0.209 | 0.646 | 0.029 | 0.333 | 1.000 | |
| LA | 0.089 | -0.489 | 0.363 | -0.394 | 0.263 | -0.064 | -0.191 | 1.000 |
| | DM | pH | TN | NH3-N | AA | PA | BA | LA |

samples, with 13% originating from the Dumfries area. Only one sample originated from the Stornoway area.

The additive used, if any, and its type were both given in the data (Table 2.20). Out of the total number of silages, 76% had been made without the use of an additive. The most popular additive was the acid-based Add-F (BP Chemicals Ltd) which had been used on 3.5% of the silages. Other additives used varied considerably ranging from acids, formaldehyde and inoculants to sugar beet pulp, molasses and potatoes.

The cut number of the silage had been given for most of the silage data. Just over half (55%) of the silages analysed had been first cut silage, 25% were second cut and only 1%, third cut. Silage made with a mixture of cuts accounted for 7%, and the cut number of the remainder (12%) was not recorded.

The range of analyses for the advisory silages was more limited than that of the research silages. Oven dry matter, pH, ammonia nitrogen, crude protein (total nitrogen), DOMD value, ME and ash, were the only chemical constituents given (Table 2.21).

The values of these parameters varied considerably. Oven dry matter values ranged from 109 g/kg to 701 g/kg, pH from 3.4 to 9.3 with the greatest range of values occurring in ammonia nitrogen: from a low 8 g/kg TN to a high 684 g/kg TN (Table 2.21).

Both the very high pH and high ammonia nitrogen values belonged to the same silage which was made in the Inverness advisory area in 1988. It was a pit silage of unknown cut, made without an additive. The very high dry matter silage was made in the St Boswells advisory area in 1988. It was a first cut silage again made without an additive. In the year that these silages were made, Scotland experienced an exceptionally hot summer. This uncharacteristic weather could explain how a silage with such a high dry matter was made. The low ammonia nitrogen silage was also a first cut 1988 silage, originating from Shetland and made without an additive.

Table 2.20

ADVISORY SILAGE ADDITIVE USE

| | |
|--------------------------------|------|
| No Additive | 2705 |
| Innoculant | 250 |
| Formic acid based | 218 |
| Inorganic acid based | 98 |
| Formaldehyde/acid based | 18 |
| Formic acid/formaldehyde based | 66 |
| Absorbant | 41 |
| Enzymes | 36 |
| Formate/nitrate based | 12 |
| Sugars | 9 |
| Unknown | 16 |
| | 3534 |

Table 2.21**SPREAD OF ADVISORY DATA**

| | Min | Mean | Max |
|---------------|------------|-------------|------------|
| DM g/kg | 109.70 | 224.50 | 701.10 |
| pH | 3.40 | 4.02 | 9.30 |
| NH3-N g/kg TN | 8.00 | 106.49 | 684.00 |
| TN g/kg DM | 4.24 | 21.23 | 37.50 |

Unusual Advisory Silage Analyses

| DM | pH | NH3-N | CP | IVOD | ME | ASH | |
|--------|-----|-----------|--------|------|---------|--------|-------------|
| (g/kg) | | (g/kg TN) | (g/kg) | (%) | (MJ/kg) | (g/kg) | |
| 543.2 | 9.3 | 684 | 156.4 | 48.0 | 7.7 | 31.7 | Inverness |
| 701.1 | 5.1 | 28 | 111.3 | 62.1 | 9.9 | 69.1 | St Boswells |
| 188.1 | 3.7 | 8 | 142.4 | 65.5 | 10.5 | 71.2 | Lerwick |

Despite ME and ash content being given, these parameters were not used in any data analysis, because they had not been used in the research silages' analysis.

2.4.2 Analysis of Advisory Silages

The advisory silages were analysed using the same statistical package as above.

The method of analysis was kept as close to that used in the final classification of the research silages in order to allow straightforward comparisons to be made.

Although a better classification of research silages was obtained when initially eleven groups were formed and the number of groups was reduced by one repeatedly (Section 2.2.3.2) until the optimum group number was reached, it was impractical to follow that procedure in this case because of the vast amount of time, both actual and computing, needed to classify this large number of silages. It was only possible to partition the data into five groups and then to alter the grouping to the required four groups. As this was the best group number previously determined using research silages, it was the group number selected for investigation.

The parameters used in the silage classification were dry matter, pH, ammonia nitrogen and total nitrogen. These were the chemical constituents which were common to both the advisory silages and the final classification of the research silages.

2.4.3 Results

The classification resulted in four groups, using dry matter, pH, ammonia nitrogen and total nitrogen as the discriminatory variables (Figure 2.10). There was a group containing silages of good quality, one of bad quality, and two intermediate groups. The good and bad quality groups are quite distinct and characterised by a low

ammonia nitrogen content, (Group 1), and a high pH, (Group 4), respectively. The two intermediate groups can be distinguished by the first (Group 2), having a higher pH than the second (Group 3), and containing the low dry matter silages (Table 2.22).

Ammonia nitrogen was the most important parameter contributing towards group classification, pH the second most important, with total nitrogen and dry matter coming third and fourth respectively. This can be seen from the vector loadings, with the most important parameter having the highest value (Table 2.23).

Most of the variation between groups is due to the first vector loading. This accounted for 97% of all variation. The second vector loading accounted for the remaining 3%, with the third vector loading being insignificantly small.

When the advisory silages were plotted graphically in their four groups, there were, due to the large amount of data, often two or more points which were too close to be distinguished with the resolution available on the graph. The statistical package used identified such "coincident" points by a colon. It was unfortunately not possible to identify these points in any way (Figure 2.10).

Graphical examination of the advisory silages illustrated that the good quality and bad quality groups are found on different sides of the graph compared to previous plots for the research silages. This is due to the scales of the axis being arbitrary and therefore good and bad is determined by constituents not right and left (Figure 2.10).

2.5 COMPARISON OF RESEARCH SILAGE CLASSIFICATION AND ADVISORY SILAGE CLASSIFICATION

In order to facilitate comparison between the research silage classification and the advisory silage classification, a further analysis was carried out.

2.5.1 Classification of Research Silage Data Using Advisory Silage Parameters

The research silage data were classified into four groups, starting from eleven groups, using dry matter, pH, total nitrogen and ammonia nitrogen as the discriminating variables (Figure 2.11). These are the same parameters as those used as variables in the advisory silage data. As with the advisory silage classification there was a group of

Table 2.22

ADVISORY GROUP MEAN VALUES

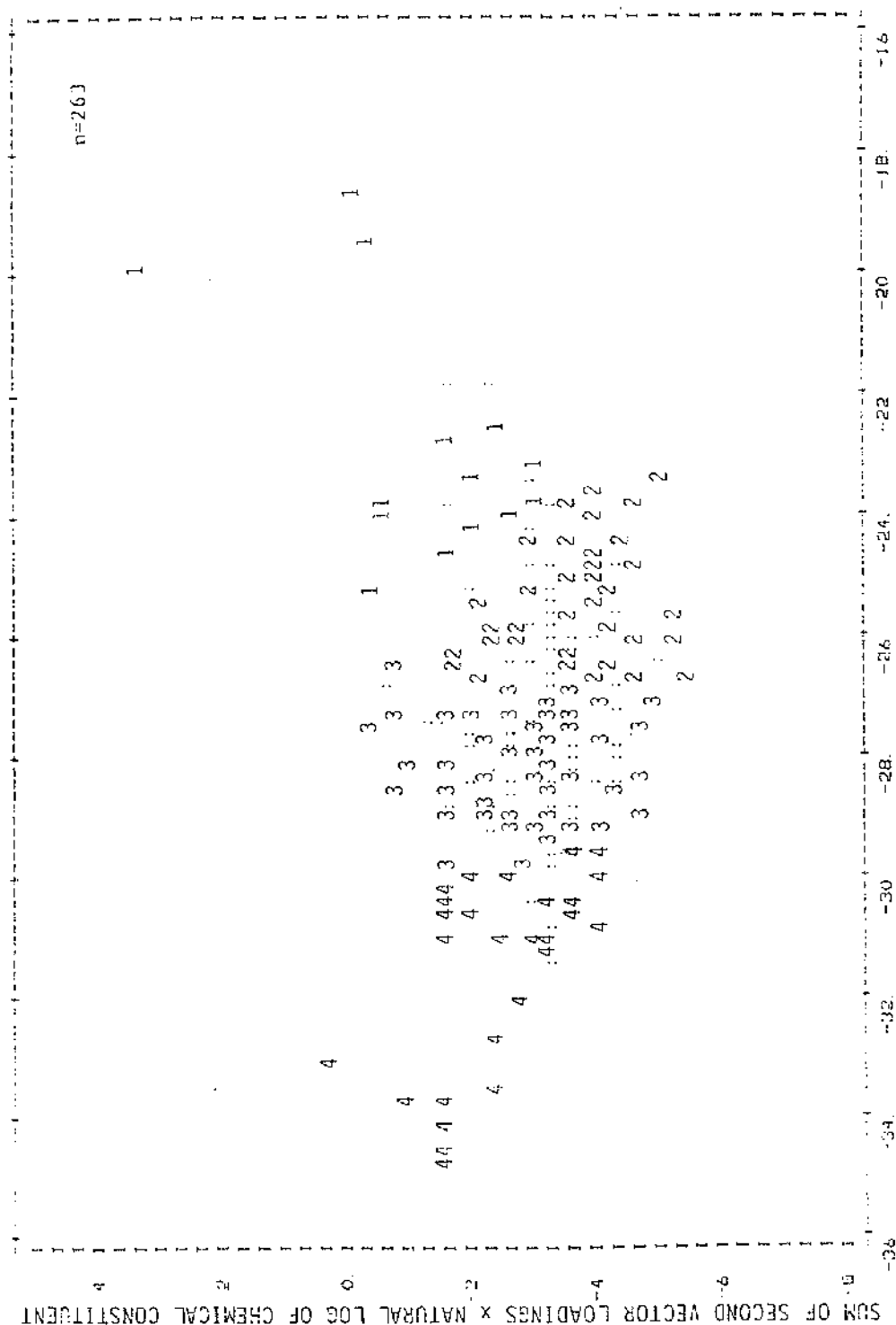
| | | Min | Mean | Max | Total |
|-------------------|---------------|-------|-------|-------|-------|
| Group 1 | DM g/kg | 143.0 | 218.8 | 458.5 | 1486 |
| | pH | 3.40 | 3.87 | 4.50 | |
| | NH3-N g/kg TN | 68.0 | 90.9 | 150.1 | |
| | TN g/kg DM | 11.1 | 21.7 | 34.2 | |
| Group 2 (bad) | DM g/kg | 127.2 | 203.2 | 659.8 | 278 |
| | pH | 4.20 | 4.78 | 9.30 | |
| | NH3-N g/kg TN | 43.0 | 239.4 | 648.7 | |
| | TN g/kg DM | 5.2 | 25.0 | 38.5 | |
| Group 3 (good) | DM g/kg | 154.0 | 232.3 | 702.0 | 892 |
| | pH | 3.50 | 3.85 | 6.80 | |
| | NH3-N g/kg TN | 9.0 | 57.2 | 82.0 | |
| | TN g/kg DM | 9.0 | 20.4 | 36.0 | |
| Group 4 | DM g/kg | 110.7 | 220.3 | 478.7 | 862 |
| | pH | 3.60 | 4.19 | 5.50 | |
| | NH3-N g/kg TN | 50.0 | 134.0 | 267.0 | |
| | TN g/kg DM | 11.4 | 23.0 | 35.6 | |

Table 2.23

ADVISORY SILAGE VECTOR LOADINGS

| | 1 | 2 | 3 |
|-------------|--------|--------|--------|
| DM | -0.643 | -1.564 | -5.907 |
| pH | 2.435 | 3.459 | 0.268 |
| NH3-N | 4.187 | -2.071 | -0.266 |
| TN | 0.860 | -1.477 | -2.482 |
| % Variation | 96.87 | 3.0 | 0.13 |

RESEARCH SILAGES CLASSIFIED BY ADVISORY PARAMETERS



SUM OF FIRST VECTOR LOADINGS x NATURAL LOG OF CHEMICAL CONSTITUENT

good quality silage (Group 1), a group of bad quality silage (Group 4) and two intermediate groups. The good quality group is characterised by a low pH and low ammonia nitrogen, and the bad quality group by high ammonia nitrogen values. The two intermediate groups were distinguished by their pH and ammonia nitrogen values, one group having a lower pH than the other (Table 2.24).

As with the advisory silage analysis, most of the between- group variation was due to the first vector loading, which accounted for 94% (Table 2.25). The second and third vector loadings accounted for 5% and 1% respectively.

It should be noted that the same order of importance of parameters occurred in this analysis as in the advisory analysis. Again ammonia nitrogen was the most important discriminator with pH, total nitrogen and dry matter coming second, third and fourth respectively.

2.5.2 Comparison of Research Silage Classification with Eight and Four Parameters

Where the research silage classifications and the advisory silage classification were studied, some similarities became apparent. Compared to the research silage classification based on eight parameters *viz* toluene dry matter, pH, total nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid and lactic acid (RSC8), the research silage classification based on four parameters *viz* oven dry matter, pH, total nitrogen and ammonia nitrogen (RSC4) is more dependent upon its first vector loading. In RSC8 only 79% of the variation is due to the first vector loading compared to 94% for RSC4.

On comparing the graphical solutions of RSC8 and RSC4 it can be easily seen that RSC4 had more outlying points, with more dispersed and less evenly distributed groups (Figures 2.4, 2.11). Group comparison between these two classification schemes highlighted a good quality group and a bad quality group in both schemes.

Similarities were found to exist between the research silage classification of the four parameters (RSC4) and the advisory silage classification (ASC). The range of chemical constituents for each group was found to correspond well between the two systems. In particular two groups, good and bad quality silage respectively, were common to both

Table 2.24

**GROUP VALUES FOR RESEARCH SILAGE DATA
CLASSIFICATION USING ADVISORY VARIATES**

| | | Min | Mean | Max |
|-------------------|---------------|-------|-------|-------|
| Group 1 (bad) | tDM g/kg | 166.0 | 221.8 | 349.0 |
| | pH | 4.40 | 4.78 | 5.70 |
| | TN g/kg DM | 21.4 | 32.6 | 47.6 |
| | NH3-N g/kg TN | 157.0 | 233.0 | 409.1 |
| Group 2 | tDM g/kg | 160.0 | 230.2 | 393.9 |
| | pH | 3.90 | 4.34 | 5.09 |
| | TN g/kg DM | 16.9 | 31.2 | 42.4 |
| | NH3-N g/kg TN | 57.0 | 126.8 | 201.9 |
| Group 3 (good) | tDM g/kg | 165.0 | 210.0 | 335.0 |
| | pH | 3.61 | 3.98 | 4.60 |
| | TN g/kg DM | 15.7 | 23.7 | 36.6 |
| | NH3-N g/kg TN | 9.3 | 35.5 | 53.0 |
| Group 4 | tDM g/kg | 154.0 | 229.1 | 515.9 |
| | pH | 3.60 | 3.97 | 4.61 |
| | TN g/kg DM | 15.0 | 24.4 | 37.1 |
| | NH3-N g/kg TN | 57.0 | 83.8 | 138.9 |

Table 2.25**VECTOR LOADINGS FOR RESEARCH SILAGE DATA USING
ADVISORY SILAGE PARAMETERS**

| | 1 | 2 | 3 |
|-------------|----------|----------|----------|
| DM | 0.458 | -1.984 | 1.656 |
| pH | -2.400 | 3.134 | -1.255 |
| TN | -1.303 | 1.147 | 3.988 |
| NH3-N | -3.253 | -2.007 | -0.339 |
| % Variation | 93.97 | 5.05 | 0.98 |

RSC4 and ASC, with the good quality groups in both classifications having low pH and low ammonia nitrogen values and the bad quality groups having high pH and high ammonia nitrogen values. Additionally the other two groups in both RSC4 and ASC fell between the above two groups as far as quality was concerned.

The vector loadings for groups in the two classification systems were generally the same size and hence of equal importance, but of opposite direction (Tables 2.23, 2.25). Graphically ASC and RSC4 were difficult to compare as two coincident points were illustrated as a colon on the graph and hence the group to which they belonged could not be identified (Figures 2.10, 2.11). Whilst RSC4 had some dispersion and outlying values, ASC had an even greater number of outlying values away from the tight nucleus of the group. This is particularly evident for ASC Group 4, which contains the bad quality silages: there is a tight group nucleus with several outlying values indicating silages of bad quality, more extreme in one parameter than the rest.

Underlying the comparison of the research silage classification (RSC) and the advisory silage classification (ASC) is the fundamental question of how good is classification based on eight parameters compared to that of four parameters. In an attempt to resolve this question the research silage classification based on eight parameters (RSC8) was compared to the research silage classification based on four parameters (RSC4). The group numbers of the silages of RSC4 were tabulated against those of RSC8 so that comparison of the two classification systems could be made. Any movement of silages or reforming of groups would thus be apparent (Table 2.26). On initial examination of the tabulation, it appeared as if the silages were in completely different groups in the two classification systems. On closer examination, however, some trends were revealed. Nearly all of the bad quality silages (Group 4), of RSC4 lay in the bad quality group, of RSC8. The remainder of the bad quality group came from the second worst quality silage group of RSC4. None of the good quality silages (Group 1), in RSC8, were in the good quality group of RSC4. The good quality group of RSC4 was predominantly made up of silages from the second best quality group of RSC8. The two intermediate quality groups do not appear to have formed any clear-cut pattern for between-group movement.

Comparison of RSC8 and RSC4 has answered the question of whether existing advisory silage analyses are adequate to classify silages accurately into the four groups. Based upon the above evidence, advisory silage analysis is not sufficiently detailed to

Table 2.26

**GROUP POSITION OF SILAGES THAT BELONG TO RSC4
COMPARED TO RSC8**

| RSC4 RSC8 | 1 | 2 | 3 | 4 |
|----------------------|----------|----------|----------|----------|
| 1 | 0 | 14 | 0 | 37 |
| 2 | 3 | 39 | 1 | 23 |
| 3 | 0 | 9 | 22 | 26 |
| 4 | 33 | 48 | 0 | 8 |

classify silages except into extremes of good and bad.

Advisory silage analysis seems, however, to be adequate to separate most of the poor quality silages from the rest. The four chemical constituents *viz* dry matter, pH, total nitrogen and ammonia nitrogen seem inadequate to classify silages into four groups. This would be a consequence of the large loss of information when acetic, propionic, butyric and lactic acids were omitted.

2.6 DISCUSSION

2.6.1 Comparison with Other Systems

Once the final classification system of the research silages had been clearly defined, it was of interest to see how it compared to the other systems described in Chapter 1 and whether groups of silages with the same characteristics were common to both this system and other schemes.

2.6.1.1 Simplest Classification Systems

The simplest classification schemes based solely on organoleptic criteria could not be compared to the final classification of research silages because of the highly subjective nature of classification.

2.6.1.2 Intermediate Classification Schemes

Comparison was possible, however, with the intermediate classification schemes because defined values controlled group classification limits. One severe limitation of many of these schemes was, however, that they defined good quality silage only. Archibald *et al* (1954), Breirem and Ulvesli (1954) and Ulvesli and Saue (1965) all categorised only good quality silage, whilst Hellberg (1963) defined both good and poor quality. The primary objective of most of these authors was not to classify silage but to help discuss silage quality. Only in one case, that of the recent classification by Wilkinson *et al* (1981), was the method or basis of group discrimination described.

Classification schemes which are more similar to the final classification of research silages with four groups were those of Langston *et al* (1958) with three groups,

Nilsson *et al* (1956) with five groups, Wieringa (1966) with three groups and Rydin (1961) with six groups. These schemes do not just separate what they define as good quality silage from the rest but divide the silages up into groups of varying quality based on the amounts of chemical constituents. The above four classification schemes were difficult to compare to the final research classification scheme in this work, the main reason being the smaller number of chemical constituents used to classify silages, and secondly the values are quoted on a fresh weight basis, with no dry matter given.

In Langston's (1958) classification, however, his good quality silage group was not as good as the calculated classification good quality silage, Group 1. The pH values were considerably higher in his scheme, 3.9 to 4.8, as compared with 3.6 to 4.5, with a mean of 4.0, for the final classification. Langston's poor quality silage group was considerably worse than the final research silage classification's poor group, Group 4, with a pH range of 5.2 to 5.7 as compared to 3.7 to 5.7 with a mean of 4.5. His intermediate quality group lay between the good and bad quality groups in the research silage classification (Table 1.1).

The classification of Nilsson *et al* (1956) roughly approximates to that calculated in the final classification. Whilst the two schemes do not correspond exactly, the trends and magnitude of the differences tend to be the same for both systems. The values for the percentage nitrogen as ammonia nitrogen found by Nilsson *et al* (1956) appear to match the final classification values reasonably well, whereas the butyric acid ranges tend to be higher in the system of Nilsson *et al* (1956) than in the final classification. His very good and good groups, correspond closely to the final good group, Group 1. The intermediate group in the final classification, Group 2, covers a slightly larger range of values than that of Nilsson *et al* (1956). For example, ammonia nitrogen in g/kg of total nitrogen is 52 to 220 for the final classification and 150 to 175 for the intermediate group in the scheme of Nilsson *et al* (1956). Butyric acid values are considerably higher in the classification of Nilsson *et al* (1956), the mean value being up to four times higher in comparison of the bad quality groups (Table 1.1).

Wieringa's (1966) classification scheme has many similarities with the final classification. Despite quantifying according to only three variables, his values are in accordance with those in the final solution. Both systems classify silages with a pH of less than 4.2 as good quality and with a pH greater than 4.5 as poor quality. Whilst Wieringa's good quality silage has less than 0.2% butyric acid and the final solution

has a mean value of 0.15%, the values for the poor quality silage groups are in disagreement. Wieringa (1966) proposed a value of greater than 0.5% but the final solution gives a mean value of 1.6%. The trend with ammonia is similar to that of butyric acid with the good quality groups being in accordance with each other whilst the poor quality silage group has lower values in Wieringa's scheme (Table 1.1).

Rydin's (1961) classification is comparable only in respect of ammonia values. These are of the same magnitude as in the final solution, but a full comparison cannot be made on the basis of only one parameter (Table 1.1).

The intermediate classification schemes, whilst generally agreeing with the groups found in the final solution, all suffer from one drawback, namely they do not encompass an adequate number of chemical constituents. In many cases the group values were not defined clearly, making interpretation by other workers impossible and perhaps not worthwhile as the values cannot be used as the basis for further study or comparison.

2.6.1.3 Recent Classification Schemes

More chemically detailed group classifications as per McDonald and Edwards (1976), Thomas and Thomas (1985), Wilkinson *et al* (1981) and Heikonen (1979) are more easily compared with the final solution.

McDonald and Edwards (1976) classify silages into one of five groups, *viz* lactate, butyrate, acetate, wilted and chemically restricted. These groups coincide with the types of silages characterised in the final solution, with Group 1 corresponding to the lactate silages, Group 2 the acetate silages, Group 3 the wilted and chemically restricted silages and Group 4 the butyrate silages (Table 1.2).

The ammonia nitrogen values in the two classification systems agree quite closely, except that McDonald's butyrate silage group is of poorer quality than the poor quality group in the final solution. Amounts of fermentation acids in the final classification agree quite closely with those in McDonald's groups. In particular the amounts of lactic acid in the two classification systems agree very closely.

The chemical constituents selected as being important for classification were those

which McDonald and Edwards (1976) found to be important in their classification: "Of the fermentation characteristics of these five types of silage, the concentrations of fermentation acids, residual WSC and nitrogen components are particularly relevant to the ruminant."

The classification system of silages proposed by Thomas and Thomas (1985) is a modification of that by Wilkinson *et al* (1981). The former authors state that attempts to classify silages are necessarily over-simplistic but their classification based on the analysis of Wilkinson *et al* (1981) serves to illustrate the wide range of silage types that can be encountered. This classification is the same as that by Wilkinson (1981), namely three groups and eight subgroups.

- Well fermented A high lactic, low acetic
- B medium lactic, high acetic
- Badly fermented C low lactic, high acetic
- D low lactic, high acetic, high butyric
- E high acetic, high butyric
- Restricted F restricted fermentation - wilted
- G restricted fermentation - acid
- H restricted fermentation - formaldehyde

These groups are more detailed than those of the research silage classification, with one final group encompassing several of those above.

- e.g. Group 1 approximates to A
- Group 2 B, C
- Group 3 F, G, H
- Group 4 D, E

Hence the research silage classification Group 1 is equivalent to the very best silages described by Wilkinson *et al* (1981). Group 2 in the research silage classification is equivalent to the mediocre silages, whilst Group 4 is equivalent to the badly fermented silages described by Wilkinson *et al* (1981). Group 3 in the research silage classification is similar to the three restricted fermentation groups described by Wilkinson *et al* (1981); wilted, acid treated and formaldehyde treated.

Wilkinson *et al* (1981) divided their silages into two groups (good and bad fermentation quality) and seven subgroups. From inspection the research silage

classification agrees quite well with this system, with one research silage group encompassing one or more of their subgroups (Table 1.2).

e.g. Group 1 approximates to B, C

Group 2 D

Group 3 A

Group 4 E, F

Group G from the classification by Wilkinson *et al* (1981) is of far poorer quality than that found in the final classification's Group 4. As it had only two silages with a mean pH of 6.8 it was felt to be too extreme to be comparable to Group 4.

Both the absolute values and the relative trends between the groups agree well for the two classification systems: pH, total acids, propionic and butyric acids and ammonia nitrogen are the most closely matched constituents, with acetic and lactic acids producing few discrepancies. Despite using different data sets, the agreement between the classification of Wilkinson *et al* (1983) which is currently the most well known and accepted system, and the research silage classification is good.

Another classification system is that by Heikonen *et al* (1979). Whilst not strictly falling into this group, it is more like an update of the early organoleptic systems. Classification is based on organoleptic judgement and the analysis of pressed juice. The simplicity of this system does not allow more than the separation of good quality silage from the rest. Despite this, the pH of this system agrees with that of the research silage classification. Ammonia nitrogen and sugars, whilst being taken into account when assessing the silage quality, cannot unfortunately be related to the absolute amounts in the research classification due to analyses being conducted on the pressed juice.

The recent classification schemes, whilst not always defining their group values accurately, seem to validate those found in the research silage solution. Only Wilkinson *et al* (1981) actually defined their group values. A major flaw in their classification system is, however, the small size of some of the groups and the fact that all of the silage analyses were from laboratory silos. It is not clear whether these are representative of the whole silage population, or are just a few unusual silages. In this respect the research silage classification is superior as it is taken from a much wider data set.

Ideally the recent classification schemes would be directly compared with the research silage classification. If the vector loadings were multiplied by the natural logarithms of the mean values of the classifications by McDonald and Edwards (1976) and Wilkinson *et al* (1981), these silages could be plotted on the final research silage graph and assigned a group number. Unfortunately this was not possible in either case because only six out of the eight chemical components were given i.e. total nitrogen and propionic acid were missing in the former study whilst dry matter and total nitrogen were absent in the latter study.

2.7 CONCLUSION

Classification of a wide range of silages into distinct groups was found to be possible. Silages could be classified into four distinct groups on the basis of toluene dry matter, pH, total nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid and lactic acid.

Silages with a more limited analysis of oven dry matter, pH, total nitrogen and ammonia nitrogen were not found to have a sufficiently detailed analysis for classification into the same groups. These parameters did enable the good and bad silages to be identified, but advisory silage analyses did not enable these silages to be allocated into the four classification groups.

CHAPTER 3

3.1 INTRODUCTION

In Chapter 2 a scheme for classifying silages into groups was devised. However, it is not known whether silages included in these groups have characteristics which are relevant to the animal both in terms of intake and/or response to the type of concentrate offered with the silage.

The aim of the experiment described here was to examine the interaction between the characteristics of each silage group and concentrate type with respect to silage intake. This was achieved by conducting four linked trials in which the intakes of silages of different types fed with different concentrates were compared. A silage representative of each of the four classification types in Chapter 2, namely a low pH, normal, high dry matter and a poorly fermented silage (Table 2.11) was fed with each of five different concentrate types: a standard concentrate, the standard plus bicarbonate, and a high starch, a high protein and a high fibre concentrate to Freisian bull calves.

3.2 MATERIALS AND METHODS

3.2.1 Diets

Concentrate was fed at 13 g/kg LW per day in two equal meals with silage fed ad libitum. At this rate, assuming that the animals would eat approximately 25 g/kg LW per day, the concentrate would comprise about half of the total diet.

3.2.1.1 Silages

From amongst the silages which were available in sufficient quantity for the trials, silages were chosen which were expected to be representative of each of the groups described in Chapter 2. In order to confirm this the amount of each of the chemical constituents used to classify the silage was multiplied by the vector loadings as described in section 2.3.2 and compared with the final group classification. The results are shown in Figure 3.1. This indicates that the normal, high DM and bad silages fall clearly within the range of values expected for Groups 2, 3 and 4. It was also found,

Group Classification Showing Experimental Silages

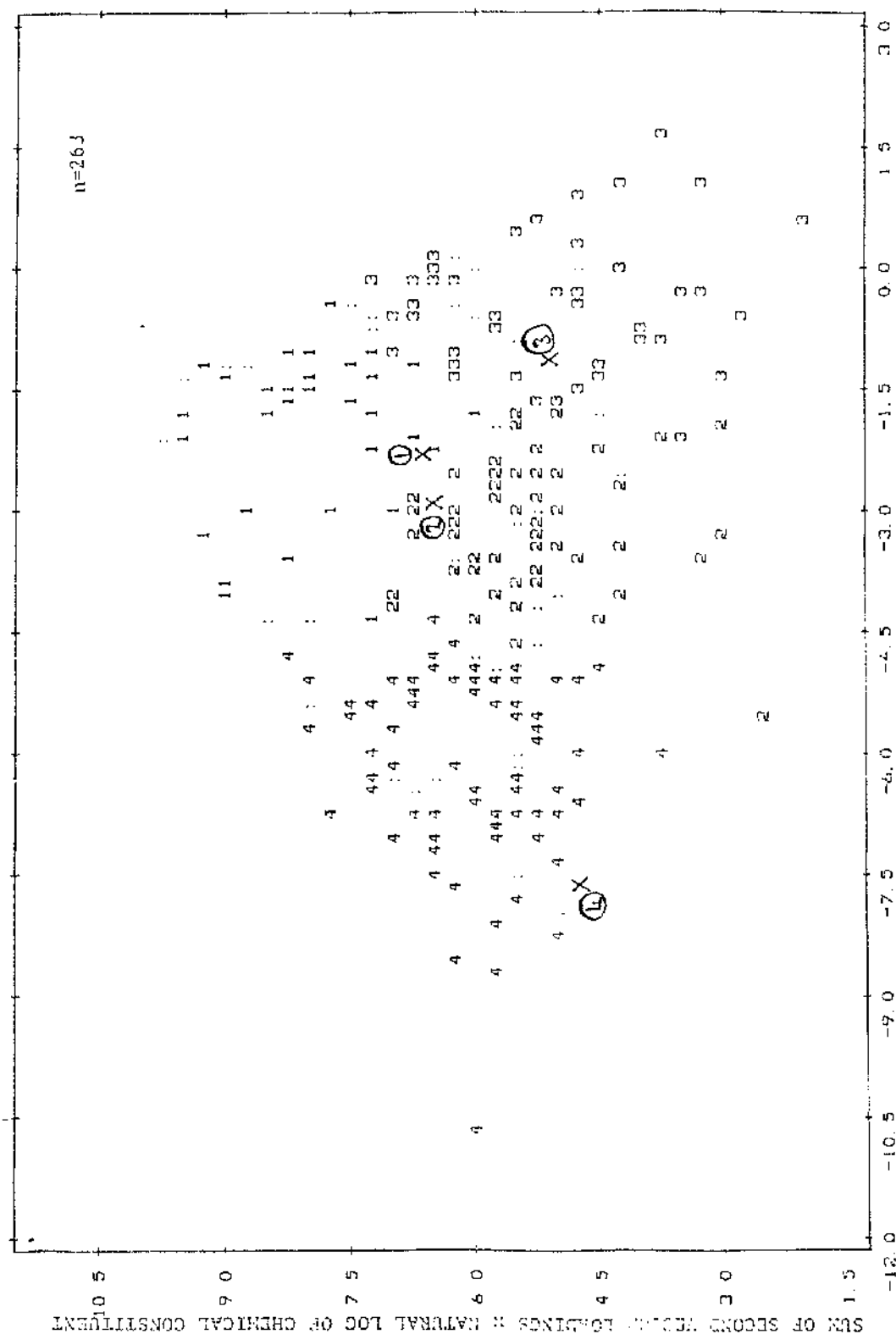


Figure 3.1

however, that the low pH silage lay on the border of Groups 1 and 2 in Figure 3.1. That this silage is only a marginal Group 1 silage is due to the pH of the silage being higher than that of the samples taken earlier. Thus the position of this silage within its group was not as central as intended. Nevertheless, whilst this silage is only a marginal Group 1 silage, for ease of presentation it will be referred to as a Group 1 silage.

The silage selected as being characteristic of a **Group 1** or "low pH" silage (Table 2.11) in terms of its chemical composition was a first cut perennial ryegrass (Lolium perenne) silage made in Strathaven, Lanarkshire, on 28 to 30 May 1990. The grass had been cut with a disc mower and minimally wilted (6-12 hours) prior to lifting with a forage harvester. During harvest Add-F (formic acid 85% ; BP Nutrition Ltd) was applied at a rate of 3 l/t. The grass was ensiled in a clamp silo, covered with polythene to exclude air and weighted down with tyres.

The silage representative of a **Group 2** silage was termed a "good quality" or "normal" silage (Table 2.11) and originated from Crichton Royal Farm, Dumfries. The silage was made from the primary growth of perennial ryegrass (L. perenne), cut on 23 and 24 May 1989. It had been harvested using a drum mower and chopped to a nominal chop length of 20mm using a precision chop forage harvester within 6-12 hours of harvesting. Sulphuric acid (45%) was applied at a rate of 3 l/t and the grass was ensiled in a clamp silo, sealed with polythene and weighted down with straw bales.

The silage selected as being characteristic of a **Group 3** or "high dry matter" silage (Table 2.11) was made at SAC Auchincruive from the second cut of predominantly perennial ryegrass (L. perenne), on 20 and 21 July 1989. It was wilted for approximately 24 hours prior to being harvested with a precision chop forage harvester. Add-Safe (ammonia complex of formic acid 55% / propionic acid 15% ; BP Nutrition Ltd) was applied during chopping at a rate of 3 l/t. The grass was ensiled in a clamp silo, sealed with polythene and weighted down with tyres.

The **Group 4** silage selected, identified as a "badly fermented" silage (Table 2.11), originated from a farm near Symington, Ayrshire. It was made from a second cut of perennial ryegrass (L. perenne) harvested on 26 July 1990 by a disc mower and wilted before it was lifted with a precision chop forage harvester, ensiled without an additive in a clamp silo, covered with polythene and weighted down with straw bales. The

silage fed in the trial came from the top 1.5 metres of the clamp as this was the only section of the clamp that was of the required poor quality. It is possible that the grass in this layer had been cut and left lying in the field for a number of days of poor weather before it was ensiled.

3.2.1.2 Concentrates

The compositions of the experimental concentrates used are shown in Table 3.1. The standard concentrate was formulated to have a CP level of 160 g/kg DM and intermediate levels of starch and fibre. The bicarbonate concentrate was the standard concentrate with the addition of 57.5 g/kg of sodium bicarbonate (Alkakarb, ICI Nutrition). This concentration provided approximately 125g of bicarbonate per animal per day. All of the five concentrates were estimated to be isoeNERgetic at 13.0 MJ/kg DM and were formulated using wheat, barley, wheatfeed, molassed sugar beet pulp, hipro soya (480 g CP/kg), and fishmeal (660 g CP/kg) in the following proportions (Table 3.2).

A mineral/ vitamin mix was added at 30 g/kg to all concentrates. The cereals were ground before inclusion in the mix which was fed in a loose meal form.

3.2.2 Animals and Allocation

For each of the 4 trials thirty 12 week old Friesian bull calves, weaned at 6 weeks of age, were stratified in order of their pre-trial liveweights and divided into five blocks of six calves. From within each of these blocks the six animals were allocated at random to one of the six experimental groups: the control group which was fed silage only or one of the 5 groups fed silage plus concentrate. Thus five animals were allocated to each treatment, each treatment occupying a separate pen.

The animals were fed individually using Calan Broadbent gates. They were allowed 10 days to learn to operate the doors and to accustom themselves to a new diet of dried grass pellets. This was used as the covariate diet. A barley/soya mix (681 g barley/kg, 292 g soya/kg with 27 g minerals and vitamins/kg) was also fed to ensure adequate nutrition. During the next 14 days the animals were fed dried grass pellets ad libitum and the barley/soya mix at 5.5 g/kg LW per day, fed in two meals. The changeover to silage and the experimental concentrate took 7 days. For the subsequent 14 days

Table 3.1

**TARGET COMPOSITION OF EXPERIMENTAL CONCENTRATE
(g/kg DM)**

| | Standard and Bicarb | High Starch | High Fibre | High Protein |
|----------------|--------------------------------|------------------------|-----------------------|-------------------------|
| protein | 160 | 160 | 160 | 330 |
| NDF | 245 | 180 | 300 | 130 |
| starch | 265 | 380 | 150 | 140 |

Table 3.2**FORMULATION OF EXPERIMENTAL CONCENTRATES (g/kg)**

| | Standard | High Starch | High Fibre | High Protein | Bicarb |
|--------------|-----------------|--------------------|-------------------|---------------------|---------------|
| wheat | 319 | 425 | - | 157 | 302 |
| barley | - | 220 | 131 | - | - |
| wheatfeed | 330 | 49 | 350 | 183 | 312 |
| molassed SBP | 275 | 183 | 440 | 61 | 260 |
| hipro soya | 36 | 120 | 30 | 599 | 34 |
| fishmeal | 40 | 3 | 49 | - | 38 |
| bicarbonate | - | - | - | - | 57.5 |

silage was fed ad libitum, with the aim of having 100 g/kg fresh weight of the silage left uncaten after 24 hours. The concentrate was fed at 13 g/kg LW per day in two equal meals. The amounts of silage and concentrate offered to and refused by the calves were recorded daily. The animals were weighed at the same time each week, and concentrate allocation was then adjusted accordingly. Water was freely available at all times.

3.2.3 Sampling and Chemical Analysis

Samples of dried grass pellets, both offered and refused, were collected daily and bulked into weekly samples for analysis. The barley/soya mix was also sampled daily and bulked weekly for analysis. These analyses are given in Appendix 4. Both the grass pellets and barley/soya mix were analysed to ensure that the covariate diet had not changed in chemical composition across the four trials.

During the middle 5 days of the first silage intake recording week, both faecal and silage samples were taken to determine the apparent digestibility of the feed from the IADF concentration. Two hours after morning feeding a faecal grab sample was obtained from each animal. These samples were bulked for each animal over the 5 days and analysed (Appendix 5). Silage refusals were also sampled from each animal daily and again bulked over the 5 days for analysis. Samples of silage offered per pen were collected, bulked over the 5 days and analysed. Likewise the concentrate fed was sampled daily, bulked and analysed for DM and for IADF (Penning and Johnson, 1983). All of the concentrate allocation was consumed, hence there were no refusals.

Using IADF values of both feed and faeces (which are given in Appendix 6), the apparent digestibility of the complete ration for each animal was calculated using the following equation:

$$\begin{array}{lcl} \text{Apparent digestibility} & & \text{faecal IADF - feed IADF} \\ \text{of the dry matter} & = & \text{-----} \times 100 \\ (\%) & & \text{faecal IADF} \end{array}$$

where each IADF sample is expressed in g/kg DM.

During the second recording week (silage intake period), daily samples of the silage

offered to and refused by the calves were collected from each pen for analysis. A bulked sample of the concentrate offered per pen was collected and analysed. Again all of the concentrate was consumed. The chemical methods of analysis and calculation of ME are given in Appendix 7.

3.2.4 Methods of Statistical Analysis Used

All data on feed intake were analysed using the Genstat V Statistical Package (Release 2.1) (Copyright 1990, Lawes Agricultural Trust, Rothamsted Experimental Station). The four individual feeding trials were analysed separately. For trial 4 this resulted in a total of 29 degrees of freedom, 20 residual. For trials 1 and 2 28 degrees of freedom, 19 residual (1 missing value per trial) and for trial 3 27 degrees of freedom, 18 residual (2 missing values). An attempt was also made to analyse all the intake data together (115 degrees of freedom, 75 residual with 4 missing values). An example of the analysis of variance is given in Appendix 8. Explanation of the missing values is given in Section 3.4.

The silage intake data were analysed by Analysis of Variance using the consumption of the dried grass pellets as a covariate. Intake of the dried grass pellets was used as a covariate as the knowledge of an animal's voluntary intake of a standard diet (dried grass pellets) enabled comparison of silage intake between experiments, to be calculated.

In the absence of covariance no attempt was made to amalgamate the data on digestibility across the four trials. The data from each trial were examined by Analysis of Variance using Minitab Release 6.2 Standard Version, Copyright Minitab Inc. 1987.

3.3 RESULTS

3.3.1 Composition of Feeds

3.3.1.1 Composition of Silages

The low pH (Group 1) silage fed had the highest lactic acid content of the four silages (Table 3.3). There was no butyric acid present but the ammonia nitrogen level was higher than had been anticipated. The normal (Group 2) silage fed was well fermented

Table 3.3

EXPERIMENTAL SILAGE ANALYSIS

| Silage Group | 1 low pH | 2 normal | 3 high DM | 4 bad |
|------------------------------|-------------|-------------|--------------|----------|
| pH | 3.8 | 3.8 | 4.0 | 5.5 |
| tol DM (g/kg) | 203 | 197 | 281 | 219 |
| TN (g/kg DM) | 26.6 | 28.8 | 33.9 | 39.2 |
| NH ₃ -N (g/kg TN) | 122 | 111 | 95 | 520 |
| (g/kg DM) | | | | |
| ash | 85 | 47 | 71 | 46 |
| WSC | 25.3 | 45.5 | 167.9 | 7.6 |
| Lactic acid | 125 | 115 | 78 | 0 |
| Acetic acid | 34.5 | 24.0 | 23.4 | 46.9 |
| Propionic acid | 5.1 | 6.4 | 3.0 | 12.8 |
| Butyric acid | 0.0 | 0.1 | 3.0 | 51.0 |
| ethanol | 4.0 | 5.3 | 2.0 | 3.4 |
| propanol | 5.5 | 1.0 | - | 2.9 |
| NDF | 578 | 501 | 443 | 569 |
| ADF | 379 | 329 | 279 | 395 |
| OM | 915 | 953 | 929 | 954 |
| Bc (meq/kg DM) | 1045 | 1024 | 751 | 670 |
| in vitro DOMD | 62 | 69 | 64 | 54 |
| ME (MJ) | 9.8 | 10.9 | 10.4 | 9.2 |
| DMD in vivo | 67 | 81 | 76 | 54 |

with a low pH and with somewhat lower lactic and acetic acids and ammonia nitrogen than the low pH silage and a small amount of butyric acid. However, as was reported earlier, the low pH silage was not as representative of the Group 1 silage as would have been desired and hence the distinction between the low pH and normal silages used was not as great as intended. The high DM (Group 3) silage was a silage with a high DM content, low ammonia nitrogen and lactic acid contents and with a high level of residual water soluble carbohydrates. Finally the bad (Group 4) silage fed was a badly fermented, poor quality silage with a high pH, very high ammonia content, high levels of acetic acid and butyric acid and a low water soluble carbohydrate content but with no lactic acid present. Complete analyses of the silages offered to each group of animals in each experiment are given in Appendix 9. Unless stated otherwise, all analyses are quoted on an ethanol corrected toluene DM basis.

3.3.1.2 Composition of Concentrates

There were only slight differences in the chemical compositions of the concentrates between each experiment and mean values are given in Table 3.4. The actual chemical compositions of the experimental concentrates differed slightly from the formulations (Table 3.2): in particular the crude protein of the standard concentrate was higher than intended and the energy values were lower (Table 3.4). It had been intended that the concentrates would comprise 500 g/kg of the total diet. However, due to greater than anticipated silage intakes, they made up approximately 400 g/kg of the total DM intake.

3.3.2 Apparent Digestibility

Analysis of the data were restricted to within trial (silages) comparisons. The apparent digestibilities of the DM of all diets are given in Table 3.5. The differences between the apparent digestibilities for different concentrates fed with the same silage were significant (Table 3.5).

For the normal (Group 2) silage the highest digestibilities occurred in the animals fed either silage alone or the protein concentrate and the lowest digestibility was found in the animals fed silage plus the fibre concentrate.

For the remaining three silages (the low pH silage, high DM and poorly preserved

Table 3.4

CONCENTRATE ANALYSIS
(g/kg DM UNLESS OTHERWISE STATED)

| | standard | bicarb | starch | fibre | protein |
|---------------|-----------------|---------------|---------------|--------------|----------------|
| DM (g/kg) | 858 | 864 | 858 | 844 | 847 |
| Ash | 92 | 117 | 73 | 103 | 89 |
| Ether extract | 32.7 | 26.5 | 23.9 | 26.7 | 27.0 |
| NDF | 219 | 245 | 195 | 299 | 206 |
| ADF | 106 | 103 | 79 | 121 | 85 |
| CP | 194 | 167 | 174 | 181 | 345 |
| WSC | 70.7 | 83.4 | 72.2 | 123.2 | 90.4 |
| NCD | 601 | 777 | 848 | 787 | 824 |
| Starch | 249 | 219 | 367 | 156 | 134 |
| ME (MJ/kg DM) | 11.8 | 11.5 | 12.5 | 11.4 | 12.0 |
| IADF | 26.1 | 25.4 | 16.5 | 31.3 | 16.7 |

Table 3.5

**APPARENT DIGESTIBILITIES OF TOTAL DM
CALCULATED FROM IADF (%)**

| | Silage Type | | | |
|--------------------|-------------|--------|---------|---------|
| | Low pH | Normal | High DM | Bad |
| Concentrate | | | | |
| Standard | 71.57 | 74.20 | 83.10 | 71.39 |
| High starch | 75.79 | 76.97 | 85.27 | 74.14 |
| Fibre | 76.13 | 73.93 | 79.99 | 66.33 |
| Protein | 75.92 | 80.24 | 85.35 | 76.46 |
| Bicarbonate | 71.87 | 75.50 | 81.15 | 69.22 |
| Silage only | 66.84 | 80.77 | 76.34 | 54.55 |
| SED | 0.80 | 0.76 | 0.75 | 1.48 |
| | p<0.001 | p<0.05 | p<0.001 | p<0.001 |

silage) the lowest digestibility was found in the animals fed silage alone. There was no consistent trend of one concentrate type giving the highest or lowest digestibility for all four silages.

3.3.3 Voluntary Intake

The voluntary intake of the dried grass pellets which was used as the covariate diet is given in Appendix 10.

Initially the results had been expressed in three forms; firstly on an absolute basis (g DM/day), secondly on the basis of bodyweight (g DM/kg LW per day) and finally on the basis of metabolic liveweight (g DM/kg LW^{0.75} per day). In an attempt to determine the best method of data expression several correlation coefficients were calculated. The logarithm of total DM intake was plotted against the logarithm of liveweight and the slope of the line $y=a+bx$ was 0.968. This correlation suggests that quoting the data on the basis of gDM/kg LW is the best method of expression. Secondly total DM intake was plotted against LW, LW^{0.75} and LW^{0.968} and the respective correlation coefficients were 0.566, 0.567 and 0.566. Thus data expressed as LW, LW^{0.75} and LW^{0.968} are equally good methods of expression. Finally silage DM intake in kg/day, g/kg LW and g/kg LW^{0.75} was plotted against liveweight and the respective correlation coefficients were 0.318, 0.096 and -0.005. This implies that metabolic LW^{0.75} is the best method of data expression. In view of this investigation proving inconclusive, the data given by metabolic LW has been selected as the basis for all further discussions; this accords with the conclusions of Rook and Gill (1990). The intake data in the form of g DM/day and g DM/kg LW are given in Appendix 11.

The silage intake data was analysed with the use of a covariate. This was found to reduce the residual variability. In the following discussion reference to a particular silage type should be interpreted as the trial in which that silage was fed. The results are presented in Tables 3.6 and Table 3.7.

For each silage type the animals fed silage alone ate significantly more silage than the animals fed silage plus a concentrate (Table 3.6). Although the addition of concentrate reduced silage intake ($p<0.001$) it significantly increased total DM intake (Table 3.7). There was no consistent effect of concentrate type on either silage or total intake nor was there any evidence of interactions between concentrate type and silage.

Table 3.6

SILAGE INTAKE
(g DM/kg metabolic LW)

| | Silage Alone | Standard | High Starch | High Fibre | High Protein | Bicarb | SED | Mean |
|------------------------|-----------------|----------|----------------|---------------|-----------------|--------|------|------|
| Low pH Silage | 68.4 | 53.4 | 50.4 | 51.2 | 52.1 | 56.1 | 2.66 | 55.3 |
| Normal silage | 74.0 | 60.8 | 58.6 | 60.8 | 58.6 | 63.4 | 3.04 | 62.7 |
| High dry matter silage | 96.7 | 69.2 | 70.4 | 71.7 | 63.4 | 73.8 | 7.90 | 74.2 |
| Badly fermented silage | 72.9 | 57.0 | 54.0 | 59.1 | 55.4 | 58.1 | 3.40 | 59.4 |

SED AND SIGNIFICANCE OF EFFECTS

| | SED | Significance |
|------------------------------------|------|--------------|
| Silage (between trials) | 2.87 | p<0.001 |
| Silage alone v rest (concentrates) | 6.42 | p<0.001 |
| Within concentrate treatments | 6.31 | NS |

Table 3.7

TOTAL DRY MATTER INTAKE
(g DM/kg metabolic LW)

| | Silage Alone | Standard | High Starch | High Fibre | High Protein | Bicarb | SED | Mean |
|------------------------|-----------------|----------|----------------|---------------|-----------------|--------|------|-------|
| Low pH silage | 68.4 | 90.3 | 92.9 | 88.2 | 87.9 | 95.2 | 2.52 | 87.1 |
| Normal silage | 74.0 | 99.5 | 97.9 | 100.7 | 98.6 | 103.1 | 3.08 | 95.6 |
| High dry matter silage | 96.7 | 110.5 | 111.2 | 115.1 | 104.4 | 112.8 | 7.92 | 108.5 |
| Badly fermented silage | 72.9 | 99.1 | 94.7 | 100.8 | 97.3 | 100.5 | 3.38 | 94.2 |

SED and Significance of Effects

| | SED | Significance |
|-----------------------------------|------|--------------|
| Silage (between trials) | 2.70 | p<0.001 |
| Silage alone v rest (concentrate) | 6.33 | p<0.001 |
| Within concentrate treatments | 6.23 | NS |

The high DM silage was consumed in greater quantities than any of the other 3 silages regardless of whether or not the silage was supplemented with a concentrate. The intake of the remaining 3 silages did not differ greatly. (Tables 3.6, 3.7).

3.4 DISCUSSION

In general the health of all the animals was good. A few of the animals, however, did catch pneumonia. In trial 1 one of the calves fed silage alone was removed from the trial on the second day of recording silage intake. In trial 2 one of the animals fed the high starch concentrates was removed from the trial on the seventh day of recording silage intake. In trial 3 two animals were removed from the trial in the middle of recording silage intake. One animal was being fed the standard concentrate, the other the high fibre concentrate. It was not possible to replace these animals due to the replacements not having been fed the correct concentrate type. Hence there were missing values in the data analysis.

3.4.1 Digestibility

The comparisons between the apparent digestibilities of the DM of different silages were not statistically analysed as the effects may not have been solely due to the silages, but to other factors such as the environment and animals (Table 3.5). The apparent digestibilities of the silages fed without concentrates calculated from the IADF measurements are in the same relative order as the values determined in vitro; the normal silage being the most digestible and the poorly preserved silage being the least digestible (Table 3.3, 3.5). Additionally the digestibilities of the silages calculated from the IADF measurements were higher than the in vitro D in three of the four silages (Tables 3.3, 3.5). For the normal and high DM silages the differences in digestibility were quite large, whilst for the poorly preserved silage the digestibilities were the same.

On reflection, perhaps the use of IADF to determine digestibility was not the ideal method to have used. Whilst Penning and Johnson (1983) found the use of IADF to be a suitable internal marker able to predict OM digestibility more precisely than the in vitro digestibility technique for fresh and dried grass, they had not used this technique for silage. Furthermore they concluded that more work was required to test this

technique over a wider range of herbage species and digestibilities.

With the exception of the low pH silage, apparent digestibilities of the diets with a high starch supplement were higher than those with a fibrous supplement. This agrees with the observations by Thomas *et al* (1986). A fibrous concentrate fed with silage was, however, found by Sutton *et al* (1987) to result in a higher apparent digestibility than a starchy concentrate fed with silage. This effect also occurred with the low pH silage in the present work. El Shazly *et al* (1961) proposed that the depression in the digestion of cellulose by starch in a ration is due to competition for essential nutrients, primarily nitrogen, with the result that starch digesting micro-organisms proliferate preferentially. This suggests that the addition of nitrogen may improve digestibility. Some caution is needed, however, when interpreting the relationship between nitrogen content and improved digestibility. When the protein supplement was fed with both the normal and bad silages, the apparent digestibility was observed to be higher than when the high starch concentrate was fed. With the high DM and the poorly preserved silages the protein concentrate gave the highest digestibilities.

In conclusion feeding a concentrate increased the digestibility of the low pH, high DM and bad silages. When the normal silage was supplemented there was a trend for decreasing digestibility in the diet. There was no consistent trend of one concentrate type giving the highest or lowest digestibilities for all four silages.

3.4.2 Analysis of Silage Intakes

The intended progression of the analyses was to analyse each experiment separately and then amalgamate them to compare the results between the silages.

Although it is invalid to state that the differences arising from the comparison of the four trials were solely due to the effect of the silages, statistical procedures cannot distinguish between the confounding effects of silage, animals, environment and time in these experiments. The use of intake during a standard feeding period prior to each experiment, as a covariate in the unamalgamated analysis, greatly increases the confidence that differences between experiments were due to the effects of silage.

The silage representative of Group 1 in the previous classification, the **low pH silage**, was consumed at 55.3 g DM/kg LW^{0.75} per day with a concentrate and at 68.4 g

DM/kg LW^{0.75} per day for silage fed alone. It had been anticipated that intake of this silage would be lower than that of the other silages selected as intake of silages of low pH is generally lower than that of less acidic silages (Harris *et al* 1966). However, in the current experiment, the silage did not have as low a pH as anticipated. When the experimental low pH silage was originally sampled, the pH of the silage samples were 3.6 and 3.7. During feeding the samples of the silage had pH values of 3.7-3.9 and the acid contents were not particularly high (Table 3.3) when compared with mean values for silage in data from MAFF (1990). The silage as fed was less acidic and had a lower free-acid content than had been expected with an average buffering capacity. It was in fact quite similar to the composition of the normal silage and was found to have a comparable silage intake.

If this silage had been truly representative of low pH silages, there are two theories which would help to explain the expected low intake of such silages.

If the silage has a high buffering capacity, which helps to maintain a low rumen pH, this leads to a low voluntary intake (Bhattacharya and Warner 1968). Feeding a low pH as compared to a high pH silage was found to lower the rumen pH in heifers (Ndwiga *et al* 1990). This may be a partial reason why the intake of acidic silages has been found by several workers to be low (Thomas *et al* 1961, Harris *et al* 1966).

Alternatively acid/base imbalance may be responsible for the low intake of this silage type; increasing the free-acid content of silage by the addition of lactic acid resulted in a decrease in voluntary intake (McLeod *et al* 1970). Thus it appears that high acid contents contribute to low levels of silage consumption and consequently to low levels of production by animals fed entirely on silage.

Based on the results of Orth and Kaufman (1966) and Thomas and Chamberlain (1982) it had been anticipated that the bicarbonate concentrate would interact with the low pH silage, partially neutralising it and resulting in a higher silage intake. Additionally McLeod *et al* (1970) and Farhan and Thomas (1978) achieved elevated silage intakes of 20.7% in five month old cattle and 9% in dairy cows respectively, when they included bicarbonate in the diet. In terms of intake the effect of feeding sodium bicarbonate with the silage used in the present experiment, however, was non-significant as was also found by Lancaster and Wilson (1975).

It was unclear as to why a response in intake to the inclusion bicarbonate in the diet had not occurred in the present work, whereas other workers had found an effect. An examination of the silage pH values of these other studies was therefore carried out. The pH of the silage used by Farhan and Thomas (1978) was 3.9 whilst the pH of the silage fed by McLeod *et al* (1970) was 4.0; both of these silages had higher intakes after partial neutralisation. Contrary to this, when (Lancaster and Wilson 1975) added bicarbonate to silages of similar or lower pH (3.8 and 3.9) a decrease in silage intake resulted. This suggests that the efficacy of bicarbonate is not related to the pH of the silage and could perhaps be due to the amount of bicarbonate added.

McLeod *et al* (1970) included bicarbonate in the diet at 16 g/kg fresh weight whilst Farhan and Thomas (1978) fed two levels of bicarbonate, 8 g/kg and 16 g/kg fresh weight. Both of these experiments resulted in an increase in silage DM. Lancaster and Wilson (1975) included bicarbonate in their diet at 25 g/kg fresh weight and did not achieve a silage DM increase with its inclusion. This suggests that at low rates of inclusion bicarbonate increases silage DM intake but at higher levels, its inclusion has no effect and possibly even reduces intake. These findings are further confused by animal species, digestibility and silage composition.

In conclusion, the low pH silage was not truly representative of a silage of low pH. Its intake was similar to that of two of the other silages and no response to bicarbonate was found.

The **normal silage**, the silage representative of Group 2, had an intake of 74.0 g DM/kg LW^{0.75} per day for silage fed alone and 62.7 g DM/kg LW^{0.75} per day for silage fed with a concentrate. As already discussed, the composition of this silage differs only slightly from that of the low pH silage and therefore it is not unexpected that the silage intakes are similar. The normal silage was quite leafy with very few long fibrous stalks. Both the ADF and NDF values reflect this, they fall into the lower half of values quoted by MAFF (1990). The *in vitro* D value, *in vivo* DMD and the M/D value are higher for the normal silage than those of the low pH silage indicating a more digestible silage made from grass cut at a younger stage of growth.

The relationship between silage intake and digestibility has been studied by many workers. Campling and Murdoch (1966) found that as digestibility increases, silage

intake increases with the relationship between digestibility and intake being very variable. One of the main points arising from a study by Thomas (1980) was the very large variation around the mean increase in silage DM intake of 0.15 kg per unit increase in D value depending on the fermentation characteristics: two silages of different digestibility can thus have similar intakes and two silages of similar digestibility can have very different intakes.

Consumption of the **high DM** silage was high at 96.7 g DM/kg LW^{0.75} per day for silage intake alone and 74.2 g DM/kg LW^{0.75} per day for silage intake fed with a concentrate. It had a higher intake than the other experimental silages.

Silage DM intake has been found to be positively correlated with DM content accounting for 15.8 % (Wilkins *et al* 1971) and 44.4 % (Rook and Gill 1990) of the variation in intake. Several workers have found that the water content of the silage appears to affect intake levels. For example when the DM content of *P. purpureum* was increased from 120 g/kg to 146 g/kg, voluntary intake was increased by 0.15 (Grant *et al* 1974). The most comprehensive study of the effect of water in fresh grass was conducted by Verite and Journet (1970) with lactating cows; voluntary intake increased by 0.337 kg DM for each 10 g/kg fall in water content above a DM content of 181 g/kg.

Contrary to this, however, it may not be the DM content *per se* which affects intake but rather the changes in fermentation pattern associated with DM content (Zimmer and Wilkins 1984). For example wilting prior to ensiling reduces the fermentation acid content of the silage (Jackson and Forbes 1970, Forbes and Jackson 1971). Thus high dry matter silages such as the high DM silage used in the current experiment will tend to have low levels of lactic, butyric and acetic acids and a low buffering capacity. Furthermore wilting has been found to restrict proteolysis, leading to a higher protein nitrogen content (McDonald *et al* 1991). The high DM silage also contained a high level of residual sugar (168 g/kg DM) supporting the view of McDonald *et al* (1968) that the more a silage is pre-dried, the higher will be the level of sugars it contains. Both residual sugar and protein nitrogen contents were found to be important in determining intake of silage by lambs (Henderson *et al* 1984).

Despite the positive association between silage DM content and silage DM intake, Rook and Gill (1990) concluded that there was little to be gained in intake terms by

wilting to DM contents above 250 g/kg.

In conclusion some studies have found that the DM content *per se* of the silage plays an important role in determining silage intake with higher DM silages having higher intakes. The extent of the increase in silage DM intake is influenced by the original DM of a silage and the difference in silage DM between that silage and the drier one. Also, whether a silage of lower DM will have a lower intake than a higher DM silage will depend on whether the lower DM is due to moisture contained in the grass or the effect of adding water. Contrary to this, however, other studies suggest that it is the change in fermentation pattern associated with the DM content that determined intake.

The **badly fermented** silage had undergone a secondary fermentation as is clearly shown by the chemical analyses, in particular the very high ammonia, acetic acid and butyric acid contents and complete absence of lactic acid. These attributes are characteristic of a butyrate silage according to the classification of McDonald and Edwards (1976) and are termed high butyrate clostridial silages by McDonald (1981).

There are two possible reasons why this silage was of such poor quality. Firstly the silage may have been cut at a young stage of growth but left lying in the field for a period of time in poor weather prior to ensiling: the fibre values of the bad silage are lower than would be expected for that digestibility. Alternatively it is possible that there was poor compaction at ensilage as the badly fermented silage is very similar in composition to that of McDonald *et al* (1991) where high levels of oxygen were artificially passed through the silage (Table 3.8). In either of these situations, if the water soluble carbohydrate levels were low in the standing crop, there would be inadequate sugar in the grass in the silo to produce sufficient lactic acid and to inhibit clostridial growth.

The intake of the bad silage was greater than might have been expected from examination of the chemical composition (Tables 3.3, 3.6) as a silage containing such high levels of ammonia and acetic acid generally has a low voluntary intake potential (Wilkins *et al* 1971). Thus it was surprising that intake was not substantially lower than the 'normal' silage. The silage DM intakes for this experimental silage were 72.9 g DM/kg LW^{0.75} per day when fed alone and 59.4 g DM/kg LW^{0.75} per day when supplemented, these levels being comparable with the low pH and normal silages (Table 3.6).

Table 3.8

**THE EFFECT OF DIFFERENT OXYGEN LEVELS ON SOME GRASS
SILAGE CHARACTERISTICS**

| Amount of oxygen passed through the silage | LOW | HIGH |
|---|------|------|
| pH | 3.91 | 5.72 |
| NH₃-N (g/kg TN) | 112 | 308 |
| (g/kg DM) | | |
| WSC | 10 | 13 |
| AA | 26 | 54 |
| PA | 0 | 12 |
| BA | 0 | 32 |
| LA | 144 | 0 |

(McDonald *et al* 1991)

An observation by Buchanan-Smith (1989) may help to explain the high intake of this silage. He suggested that the odour or taste of acetic acid causes an adverse response by sheep to silage but that this effect appears to be neutralised by elevated levels of other constituents such as ammonia and butyric acid. In this experimental silage the levels of butyric acid and acetic acid were high and that of ammonia very high. It is possible that the odour or taste of the high levels of acetic acid are thus masked and this is why the resultant silage intake was higher than anticipated.

The conclusions of Buchanan-Smith (1989) appear to be contradicted by Wilkins *et al* (1971) who observed that acetic acid, total acids and ammonia were negatively correlated with silage intake. They did not attempt to correlate intake with butyric acid or any combination of these factors.

High intakes by sheep of silages with high pH and high butyric acid contents were found by Harris *et al* (1966). Intakes of both wilted and unwilted silages which had been aerated were 0.12 greater than the corresponding unaerated silages (Table 3.9). These aerated silages had the higher pH values and contained more butyric acid and, in most cases, less lactic acid. Thus they would have been classed as poor silages and yet their intakes were higher than those of the well fermented silages. Harris *et al* (1966) concluded that, whilst butyric acid levels may be so high as to restrict intake due to unpalatability, a low pH and a high level of lactic acid are possibly even more significant factors limiting silage intake.

In conclusion, the similar silage intakes of the low pH and normal silages reflect their similar chemical compositions. The high intake of the high DM silage is in agreement with the established relationship between DM and intake. Whilst the intake of the bad silage was higher than had been expected from examination of its chemical composition, similar results have been obtained in experiments by other workers.

3.4.3 Concentrate Effects on Silage Intake

When ruminants fed silage *ad libitum* are given concentrates, the supplement generally depresses the intake of silage (Thomas 1987). This decrease in the intake of silage DM per kg increase in concentrate DM is termed the substitution rate.

Table 3.9

COMPOSITION AND INTAKE OF AERATED SILAGES

| | DM (g/kg) | pH | Butyric Acid (g/kg DM) | Lactic Acid (g/kg DM) | Silage Intake (gDM/kg metLW) |
|-----------------------------|--------------|-----|------------------------------|-----------------------------|------------------------------------|
| Unwilted | 136 | 4.2 | 0 | 79 | 47.9 |
| | 130 | 4.5 | 12 | 55 | 49.1 |
| | 160 | 3.9 | 0 | 98 | 43.6 |
| Unwilted aerated | 142 | 4.9 | 22 | 44 | 51.9 |
| | 155 | 4.8 | 35 | 67 | 59.4 |
| | 162 | 4.0 | 16 | 100 | 56.6 |
| Wilted | 213 | 4.2 | 0 | 101 | 56.3 |
| | 197 | 4.5 | 7 | 87 | 56.3 |
| | 229 | 4.0 | 0 | 83 | 63.6 |
| Wilted aerated | 213 | 4.2 | 28 | 54 | 60.8 |
| | 210 | 5.0 | 38 | 48 | 64.8 |
| | 226 | 4.0 | 6 | 78 | 61.5 |

from Harris *et al* (1966)

In the current experiment there was clear evidence that supplementation reduced the intake of silage. However, there were no significant effects of concentrate type on silage intake nor did there appear to be any interaction between concentrate type and the different silages.

There were, however, some trends apparent in the substitution rates calculated from the silage intake data (covariate corrected) (Table 3.10). Mean substitution rates were highest for the high DM silage and lowest for the normal silage. Furthermore there were some differences in substitution rates between concentrate types.

Before the effects of the different concentrate types on substitution rate are examined it is intended briefly to examine the mean substitution rates for each trial (silage).

The magnitude of the depression in intake for the high DM silage is similar to that recorded by Bines (1985) who found a substitution rate of 0.68 for what he termed a good grass silage. The relative order of the substitution rates (Table 3.10) tends to agree with the observation by Wilkins (1974); that the substitution rate in silages is related positively to the intake of the silage as a sole feed. The high DM silage, which had the highest intake, had the highest substitution rate. Also the low pH, normal and bad silages which had approximately the same silage intakes had very similar substitution rates (Table 3.6, Table 3.10).

Despite the substitution rate being proportional to digestibility in hay (Leaver 1983) this relationship does not necessarily hold for silages. Individual trials have shown, however, that increasing the digestibility of silages induces high substitution rates. This effect is only likely to be apparent when differences in fermentation characteristics between silages are small. Nevertheless the data of Phipps *et al* (1987) explained that even in silages there exists an underlying relationship between digestibility and substitution rate.

There are three specific concentrate effects which will be examined in some detail: the effect of feeding a fibre as compared to a starch concentrate, the effect of feeding a high protein concentrate and the effect of feeding bicarbonate.

High fibre concentrates are increasingly being used in preference to starch as they are believed to result in lower substitution rates. There is, however, some debate as to

Table 3.10

**SUBSTITUTION RATES FOR THE DIFFERENT CONCENTRATES FED
WITH THE FOUR SILAGE TYPES**

| | Conc Type | | | | | |
|-------------|-----------|-----|-----|-----|-----|------|
| | S | HS | F | P | B | Mean |
| Silage Type | | | | | | |
| Low pH | .41 | .42 | .46 | .46 | .31 | .41 |
| Normal | .34 | .39 | .33 | .39 | .27 | .34 |
| High DM | .67 | .64 | .59 | .81 | .58 | .66 |
| Bad | .38 | .46 | .33 | .42 | .35 | .39 |
| Mean | .45 | .48 | .43 | .52 | .38 | |

whether this is entirely true. This is illustrated by the work of Castle *et al* (1981) who found little difference in intake with fibrous and starchy concentrates whilst Thomas *et al* (1986), Sutton *et al* (1987) and Castle and Watson (1975) found higher silage intakes with a fibrous supplement and Mayne and Gordon (1984) reported higher intakes where a starchy supplement was fed. Thomas *et al* (1986) and Castle *et al* (1981), however, found no differences in substitution rate when either starch or fibre concentrates were fed with silage.

In the current experimental work a lower substitution rate was found when the high fibre concentrate was fed as compared to the high starch concentrate. This occurred for all of the normal, high DM and bad silages (Table 3.10). For the low pH silage, however, a lower substitution rate was found when the high starch concentrate as compared to the high fibre concentrate was fed.

A study by Recvc *et al* (1986) illustrated that for either low or high concentrate inputs, silage intake was higher and the substitution rate lower when a high protein concentrate (403 g/kg DM) as compared to a low protein concentrate (214 g/kg DM) was fed. Additionally Castle and Watson (1976) found a higher silage intake and a lower substitution rate when a 540 g CP/kg DM concentrate as compared to a 100 g CP/kg DM barley concentrate was fed. Thomas and Gill (1988) found that protein supplementation, in the form of fishmeal, of silages with high nitrogen content and high intake characteristics caused a marked depression in silage intake.

The interaction between the high protein concentrate and the high DM silage resulted in an exceptionally high substitution rate. In contrast the interactions between the protein concentrate and the low pH, normal and bad silages were comparable to those with other concentrate types.

The final effect to be studied was that of the bicarbonate concentrate. In comparison with the other concentrate types the bicarbonate produced the lowest substitution rate for the low pH, normal and high DM silages and the second lowest for the bad silage.

It must be recognised, however, that the difference between the mean substitution rate for the different concentrate types is relatively small: standard concentrate 0.45, high starch concentrate 0.48, protein concentrate 0.52, fibre concentrate 0.43 bicarbonate concentrate 0.38. Furthermore there is no clear pattern of one concentrate type

consistently giving a higher or lower substitution rate across all silage types. The high starch concentrate does give the highest substitution rate for the bad silage and joint highest for the normal silage whilst the high fibre concentrate gives the lowest substitution rate for the bad silage. For all of the experimental silages except the low pH silage the high starch concentrate exhibits a higher substitution rate than that of the fibre concentrate. This tends to support the commonly held view that fibrous concentrates have a lower substitution rate than starchy ones.

In conclusion, the results show that supplementation with concentrate reduced silage intake and this reduction was greatest with the high DM silage. Although the effect of type of concentrate on substitution rate was relatively small there was a trend for the fibre and bicarbonate supplements to produce the lowest substitution rates.

3.5 CONCLUSION

The aim of this set of trials was to examine whether silages, characteristic of the Cluster Groups derived in Chapter 2 are reflected by differences in voluntary intake and response to concentrate type.

Silages characteristic of Group 2(normal) , 3 (high DM content) and 4 (bad) were obtained and fed to calves. A silage of low pH (Group 1), however, was not obtained as the silage that had been intended to be representative of this group was on the border of silages characteristic of Group 2 (normal).

The calves consumed significantly more silage of high DM content (Group 3) than of the other silages. It was surprising that a silage of poor fermentation characteristics (Group 4) had intakes similar to those observed with normally fermented silages.

There was a trend for high intake silages typified by Group 3 to have the highest substitution rates. Differences between the other silages were small. There was a trend for concentrates which were high in fibre or contained sodium bicarbonate to result in a lower substitution rate. These effects were, however, small in relation to the influence of silage characteristics.

CHAPTER 4

4.1 GENERAL DISCUSSION

Intake of silage by cattle has long been studied but an understanding of the factors determining silage intake is as yet far from complete. The review of the literature on this topic which was carried out at the start of this work confirmed that the type of fermentation that proceeds when grass is ensiled is determined by a number of factors including the chemical composition of the grass, the presence of air in the silo and the use of additives. Depending on the fermentation patterns which occur, a wide range of silages of varying chemical composition are produced.

Several previous attempts have been made to classify this range of silages by grouping them on the basis of their chemical composition. Both simple and more complex classification schemes (McDonald and Edwards, 1976 and Wilkinson *et al* 1981) have been devised, with the latter classifying silages according to fermentation pathways. These classification schemes, however, have not been found to bear any relation to the nutritive value of the silage.

The voluntary intake of food by cattle is controlled by both physical and physiological factors. Where the diet is of a bulky, fibrous nature physical factors (e.g. gastric distension) will determine voluntary intake. Where physical bulk does not limit intake, however, metabolic control (i.e. glucostatic, chemostatic and osmotic mechanisms) regulates intake.

As well as the above general factors determining food intake there are additional elements affecting voluntary food intake which are specific to forage. Forage intake is primarily governed by the ratio of cell contents to cell walls. Osbourn *et al* (1974) showed that voluntary intake was more highly negatively correlated with the proportion of cell walls in the forage than that of digestible organic matter i.e. cell contents which was positively correlated. When forage is at an early stage of growth it has a high proportion of cell contents and is very digestible. Thus within a forage species intake is affected by digestibility. Factors influencing the cell contents to cell wall ratio and ultimately forage intake include the stage of growth of the plant, species and cultivars, soil fertility and climate during growth.

The voluntary intake of silage has generally been found to be less than that of the corresponding fresh forage or hay but the extent of such differences is highly variable (Demarquilly 1973). The physical effects of digestibility, which appear to exert a major control on hay intake, are modified by the presence of fermentation end products in the silage. The latter vary widely between silages depending on the original composition of the sward and the conditions during ensiling. Thus the intake of a specific silage is the combined response to a number of variables, of which the nature of the fermentation end products is clearly important.

In many cases the feeding of forage as a sole feed provides insufficient energy to achieve the required level of animal production; thus it is common practice to feed concentrates with the silage. Supplementation in this way not only increases the total ME energy intake but where an increased proportion of ME is derived from concentrates it may also improve the efficiency of utilisation of ME for growth and fattening (Gill *et al* 1989).

Feeding a concentrate with forage depresses voluntary intake. The decrease in intake of silage DM per kg increase in concentrate DM intake (the substitution rate) is dependent on the forage, the supplement and the animal (Thomas 1987). Both the type and the amount of concentrate fed also affect the substitution rate. Starch based supplements tend to decrease rumen pH and fibre digestion and, as a consequence, forage intake. Fibre concentrates are increasingly being fed instead of starch concentrates in the belief that they result in a lower substitution rate. The results of previous workers show that, whilst this effect has been observed in a number of experimental studies, results to the contrary have also been found.

Bicarbonate is frequently included in the diet either in the concentrate or mixed with the silage as it is generally believed to increase silage intake (Orth and Kaufman 1966) due to the alleviation of rumen acidity associated with the end products of fermentation. Several studies have shown a marked increase in silage DM intake on the inclusion of bicarbonate in the diet whilst other researchers have found no effect. Several reasons have been advanced for the differences in the response to bicarbonate, including the species and age of the animals, the type of silage and the level of supplementation (Thomas and Thomas 1982).

The development of a reliable means of predicting silage intake from composition would be an important step in improving the efficiency of animal production as it would enable the optimal utilisation of feed of different compositions. With this in mind a number of attempts have been made to develop a mathematical relationship between intake and silage composition using both single and multi-variable equations (Rook *et al* 1990); success has been variable, the latter not necessarily being more accurate than the former.

It became apparent from the review of the literature that, despite the considerable effort devoted to investigating this matter, there was only an imperfect understanding of the complex relationship between intake, concentrate type and the wide variety of factors affecting silage composition. Thus the main aim of this experimental work was to seek an improved understanding of the factors governing the intake of silage as a sole feed and of the effect on silage intake of different types of concentrate fed with silages of different qualities.

In an attempt to reduce the effects of variations in silage composition the feasibility of combining silages into groups of similar chemical composition was investigated. The statistical method which was selected in this work for the separation of silages into distinct groups with common characteristics was cluster analysis. Whilst this was not a new technique for silage classification, having been used by Wilkinson *et al* (1981), it had not previously been applied to such a large number of silages with such widely ranging characteristics.

It was found that, on the basis of their chemical analyses, silages fell into four groups, each group being distinguished by a certain combination of levels of concentration of chemical constituents. From among the wide range of constituents examined, eight chemical constituents, namely toluene dry matter, pH, total nitrogen, ammonia nitrogen, acetic acid, propionic acid, butyric acid and lactic acid, were found to be necessary to discriminate between groups. An attempt was made to reduce the number of these chemical constituents required for classification without success; the resultant groups were less clearly defined.

It is one of the limitations of cluster analysis of silages that all chemical constituents have to be present. Water soluble carbohydrates are not often measured in silage analysis and in order to keep the present data set as large as possible water soluble

carbohydrates were not used as a constituent in the cluster analysis. It should be noted, however, that both Wilkinson *et al* (1981) and McDonald and Edwards (1976) considered that the amount of water soluble carbohydrates present was an important indicator of silage quality and stated that at least 3% by weight in the fresh silage was required to demonstrate a good fermentation. This led to the inclusion of water soluble carbohydrates in the classification schemes developed by these workers for a limited range of silages. As further data become available on the water soluble carbohydrate content of silages it will be interesting to expand the cluster analysis of silages to establish the extent to which this factor is indeed relevant to the classification.

A classification scheme is of little practical use unless it is readily possible to allocate new silages to one of its established groups. It was shown in Chapter 2 that new silages can easily be assigned to one of the four groups, provided that they have been chemically analysed for the eight parameters used in the classification. Group classification is not, however, possible if even one of these constituents has not been determined.

Normally silages examined by the SAC Advisory Service were only analysed for dry matter, pH, total nitrogen, MAD fibre, ME, digestibility and ash. It had been hoped that these chemical constituents would be sufficient to allow silages to be classified into the same groups in which the research silages had been classified with the eight parameters listed above. Unfortunately this was not found to be possible and in order that new silages can be allocated to a group they require to be fully analysed for the above eight chemical constituents. It is unfortunate that analysing each silage for each of the chemical constituents currently entails a large amount of laboratory work. The development of near infra-red reflectance spectroscopy (NIR) should enable silages to be fully analysed in a shorter time and thus more easily allocated to one of the four groups.

In conclusion cluster analysis has proved to be a successful method of grouping silages of widely varying chemical composition into only four groups on the basis of eight parameters. In the light of this a new SAC advisory classification system of predicting silage intake for ration formulation has been developed based upon the method of cluster analysis and classifying silages into four groups (Offer *et al* 1993). Instead of classifying silages with respect to eight of the silage's chemical constituents, however, Offer *et al* (1993) discriminate between groups on the basis of parameters which can be

measured more readily. Following auto titration of the juice squeezed from the silage, classification is carried out on the basis of:

1. volatile fatty acids (g/kg - including lactic + formic acids)
2. ammonia acid nitrogen (g/kg total soluble nitrogen)
3. sugar
4. neutralising value (meq alkali required to raise the pH of 1kg fresh weight of silage to 6.5)

Thus Offer *et al* (1993) classified silages into four groups on the basis of only four parameters. It is easier to analyse silages for these four parameters than to measure the chemical components of silage in the traditional way as is required for the classification scheme developed in the current work, all four parameters being measured in one automated operation.

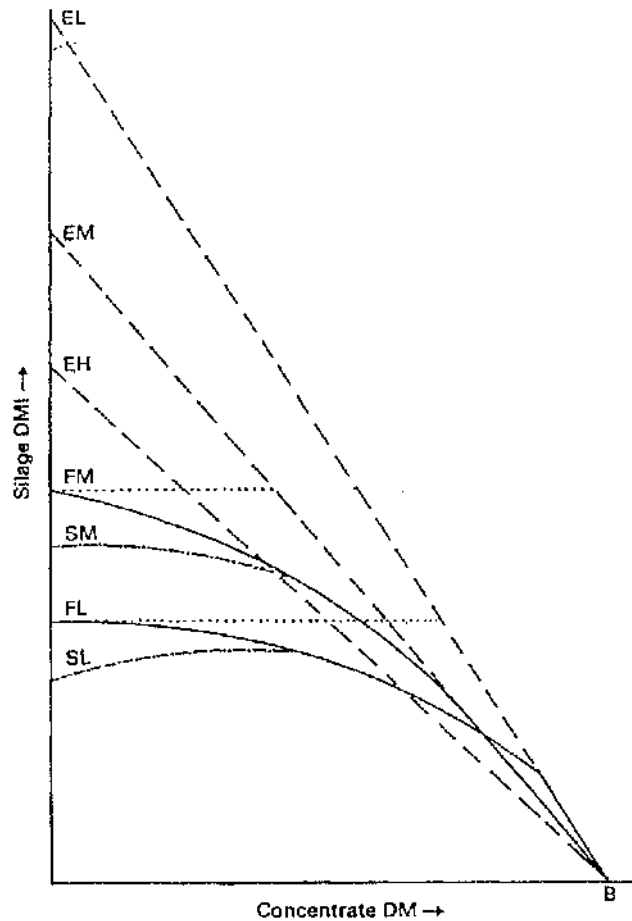
4.2 THE VOLUNTARY INTAKE OF SILAGE AND THE EFFECTS OF SUPPLEMENTATION

In the current work the intake by calves aged around four months of a silage characteristic of each of the four classification groups developed in Chapter 2 was investigated. It was found that the high DM silage was consumed in significantly greater quantities than either of the normal, low pH or bad silages which were consumed in equal amounts. On the basis of the predictions of the model of Thomas (1987) (Figure 4.1) it was expected that silages of low digestibility would have lower intakes than those of higher digestibility. In the experimental work, however, the bad silage which had the lowest digestibility was consumed in quantities equal to that of the normal and low pH silages (medium digestibility). A similar observation was made by Harris *et al* (1966) but the reason for this remains unclear.

The observation that the mean consumption of the normal, low pH and bad silages was similar was unexpected. It has to be recognised that there was some variation in intake among the animals fed the same silages. There were also wide variations in silage type as they were made from different grass at different times in different silos, with different additives and thus with different digestibilities. It would be interesting to repeat this work with a larger number of animals and with four silage types originating from the same grass sward, different treatments being used to yield silages of different chemical composition. A normal (Group 2) silage could be made from grass ensiled

Figure 4.1

Representation of the Relationship Between Silage Intake and Concentrate Supplementation



Schematic representation of the relationship between silage intake and concentrate supplementation (Thomas 1987, after Osbourn 1989). Low (L), medium (M), and high (H) digestibility. Lines EL, EM and EH to B, represent the intakes of silage of low, medium and high digestibility respectively, assuming that the animal could eat to maintain constant energy intake. The solid curves FM and FL to B represent the effect of concentrate in depressing cellulolytic activity in the rumen and hence increasing rumen 'fill', while the dotted lines (FM and FL to B) predict the intake of M and L if the concentrate had no effect on fill. Finally, the broken lines SM and SL represent the control of intake by factors related to fermentation characteristics of the silage.

under good conditions made without an additive. If an enzyme was added to this grass at ensiling, the resultant silage should be a low pH or Group 1 silage (McDonald *et al* 1991). The high DM (Group 3) silage would be achieved by wilting the grass and ensiling in good conditions, whilst the poorly fermented (Group 4) silage could be obtained by either adding water to the grass at ensiling, or shading the grass to reduce the water soluble carbohydrate content prior to cutting. Therefore any silage intake effects that result might be expected to result wholly from the silage chemical composition, not from the additive, digestibility or chemical composition of the original sward.

The production in this way from the same grass sward of four well preserved silages showing a gradation in the extent of fermentation was attempted by Chamberlain *et al* (1992). They found, however, that of the resultant silages three were of very similar good quality whilst the fourth had a restricted fermentation. Furthermore there were no significant differences in intake when these silages alone were fed to dairy cows.

It has long been recognised that feeding a concentrate decreases silage intake and the expected relationship between intake and concentrate supplementation is shown schematically in Figure 4.1 for silages of different digestibilities. As expected feeding a concentrate with silage in the current work was found to reduce silage intake for all silage types. The predictions of the Thomas (1987) model that a silage of high digestibility could have a low substitution rate are borne out by the normal silage (69 D, SR 0.34). Moreover the low pH and bad silages with lower digestibilities of 62 D and 54 D respectively have higher substitution rates (0.41 and 0.39) than that of the normal silage. This pattern is, however, contradicted by the high DM silage which has an intermediate digestibility (64 D) when compared with the other three silages but had much the highest substitution rate (0.66); this silage had a significantly higher intake than the others.

Although the intake of the high DM silage was significantly different from the intakes of the other three silages, there were only small and non-significant differences in the effect of the various concentrate types on the intake of all four silages. Despite the analysis of variance table in the current experimental work finding no significant differences in silage intake for the different concentrate types there was a trend, however, for the fibre and bicarbonate supplements to produce the lowest substitution rates. Once again, it should be recognised that differences between concentrates may

be masked by the variation in silage intake among the animals. A similar observation to this has, however, been made by Chamberlain *et al* (1992) who fed three different concentrate types (high fibre, high starch and high protein) with four silages made from the same grass sward and found no significant concentrate/ silage interactions.

In conclusion, neither the experiment by Chamberlain *et al* (1992) nor the current experimental work found any significant effect on silage intake of type of concentrate fed. This situation occurred with silages of similar chemical composition made from the same grass sward and from silages of different composition originating from different swards. As far as the practical feeding of cattle is concerned, this is an important conclusion since it allows the cheapest and most readily available concentrates to be used whilst maintaining a given energy value.

4.3 COMPARISON OF INTAKES WITH PREDICTION EQUATIONS

Once the experimental silage intakes had been established, they were compared with the intakes predicted using a number of equations derived by previous workers. It was hoped that such comparisons would help to give an understanding of which factors were the most important in controlling voluntary intake in the four experimental silages. The four groups derived from cluster analysis indicated that fermentation characteristics are important in differentiating between silages.

The first and simplest methods used to predict intake of silage are based on equations which seek to relate the intake of silage as a sole feed to a single variable. Two simple systems based on NDF and crude protein will be examined. The use of NDF for intake prediction was studied by Osbourn *et al* (1974) who found NDF to be highly correlated ($r = 0.83$) with intake. Furthermore Mertens (1987) has suggested that NDF would be the best chemical predictor of forage intake since it is strongly related to rate of fibre digestion, rate of chewing and digesta volume. Crude protein was selected as the basis for intake prediction by Wilkins *et al* (1971) who found voluntary intake of silage to be positively correlated with nitrogen content ($r = 0.325$) and by England and Gill (1985) who found a strong positive correlation between silage nitrogen and silage intake.

In the light of this, prediction equations were devised by LaForest *et al* (1986) (separate equations using NDF and crude protein) and by Wilkins *et al* (1971) (crude

protein) and these are given in Table 4.1.

The actual and predicted silage intakes for the three prediction equations are given in Table 4.2. Using the mean square prediction error (MSPE) the actual and predicted intakes were compared; the smaller the MSPE, the more accurate the prediction equation. The MSPE can be regarded as the sum of three components - mean bias, line bias and random (Bibby and Toutenburg 1977). Mean bias generally reflects differences between predicted and actual data while a large line bias is indicative of underlying inadequacies of the model. The remaining variation unaccounted for by mean or line bias is random. A very accurate prediction equation will have very small mean and line bias and a proportionately large random value. The mean prediction error (MPE) is the square root of the MSPE and is reported as a percentage of the mean observed intake.

Each of the four experimental silages was analysed for NDF and crude protein and the predicted voluntary intake obtained using the equations of LaForest *et al* (1986) and Wilkins *et al* (1971). The MSPE values were then calculated for each of the three equations.

Table 4.2 illustrates that the prediction of silage intake using NDF is more accurate than using crude protein as is shown by the lower MPE value. In particular the equation by LaForest *et al* (1986) gives a more accurate prediction of the high DM silage as this silage has the lowest NDF content (Table 3.3). Additionally this equation correctly predicts the relative intakes of the four silages.

The equations by Wilkins *et al* (1971) and LaForest *et al* (1986) predicting silage intake on the basis of crude protein are equally inaccurate at predicting silage intake. Both wrongly predict that the highest intake should be observed in the bad silage which has the highest total nitrogen content (Table 3.3). In addition these equations fail to predict the high intake in the high DM silage.

The MPE values for the crude protein prediction equations of LaForest *et al* (1986) and Wilkins *et al* (1971) are similar but the proportions of the MSPE due to mean and line bias differ; the equation of Wilkins *et al* (1971) has a large mean bias reflecting underprediction of the silage intake for all four silages whilst that of LaForest *et al* (1986) has sizeable line bias indicating the overall inadequacy of the model.

Table 4.1

SINGLE VARIABLE PREDICTION EQUATIONS

NDF

LaForest et al (1986)

$$\text{Voluntary Intake (g/kg metabolic LW)} = 132.7 - 0.105 x$$

where x = NDF (g/kg DM)

Crude Protein

LaForest et al (1986)

$$\text{Voluntary Intake (g/kg metabolic LW)} = 35.8 + 0.239x$$

Wilkins et al (1971)

$$\text{Voluntary Intake (g/kg metabolic LW)} = 17.7 + 0.237 x$$

where x = crude protein (g/kgDM)

Table 4.2

PREDICTION OF SILAGE AS A SOLE FEED
(g/kg metabolic LW/day)

| | Actual | Laforest et al (1986) NDF | Laforest et al (1986) CP | Wilkins et al (1971) CP |
|--------------------------------|--------|------------------------------|-----------------------------|----------------------------|
| Low pH | 68.4 | 79.87 | 76.17 | 46.60 |
| Normal | 74.0 | 85.49 | 81.78 | 53.34 |
| High DM | 96.7 | 89.76 | 87.24 | 66.37 |
| Bad | 72.9 | 84.38 | 109.78 | 67.94 |
| Mean | 78.0 | 84.84 | 89.11 | 58.84 |
| MSPE | | 392.22 | 685.39 | 629.54 |
| Prop ⁿ of MSPE Bias | | .220 | .268 | .444 |
| Line | | .014 | .241 | .046 |
| Random | | .766 | .491 | .510 |
| MPE | | 19.80 | 26.18 | 25.09 |

The intake of the experimental silages was then compared with the intake predicted by more comprehensive equations having a greater number of variables (Table 4.3). The equations by Rook *et al* (1990) and Lewis (1981) are based on fermentation characteristics whilst that of ARC (1980) is based on the energy value of the silage. These equations predict silage intake as part of a mixed diet as compared to the single variable equations of LaForest *et al* (1986) and Wilkins *et al* (1971) which only predict the intake of silage as a sole feed. The mean silage intakes predicted with respect to the particular silage and concentrate fed to each animal are given in Table 4.4. The MSPE and the proportional contribution of the mean bias, line bias and random are also given in Table 4.4. Examination of the MPE values illustrates that the five equations of Rook *et al* (1990) are essentially equally accurate in predicting silage intake whilst ARC (1980) and in particular Lewis (1981) are less accurate.

When the predictions in respect to individual silages are examined, the ARC (1980) equation is found to be inadequate being of approximately equal accuracy to the LaForest *et al* (1986) and Wilkins *et al* (1971) crude protein prediction equations. Examination of the predicted intakes of each silage type (Table 4.5) show that the ARC (1980) equation is very good for the bad silage. This equation is primarily based on the energy value of the silage; the ME value for the bad silage was low and this results in the low predicted silage intake. The predicted intake of the high DM silage is considerably lower than the actual intake because there is no positive coefficient for dry matter in the equation and the high actual intake of this silage is not totally reflected in the ME value.

The Lewis (1981) equation (Table 4.3) was by far the least accurate at predicting silage intake (Tables 4.4, 4.5). All of the predicted intakes were considerably lower than the actual intakes. Note, however, that the predicted intake of the bad silage is only an estimated value due to its ammonia nitrogen content (at 520 g/kg DM) being outwith the permissible range of values, the maximum ammonia nitrogen content to which the equation is meant to apply being 250 g/kg DM.

The observation that the Lewis (1981) equation predicted the silage intake to be so much lower than the actual intake was unexpected especially as AFRC (1991) had found this equation to be very reliable. Lewis (personal communication, 1995) has indicated, however, that the liveweight/substitution rate interaction (the I x C factor in

Table 4.3

MULTI-VARIABLE PREDICTION EQUATIONS

Rook *et al* (1990)

$$VI = -25.5 + 0.0155 \text{ CI} - 0.5816 \text{ DM} + 12.98 \text{ pH} - 0.42 \text{ BA} + 0.445 \text{ TN} + 0.0257 \text{ DOMD} \quad \text{Equation 1}$$

$$VI = 38.68 - 0.4591 \text{ CI} + 0.0754 \text{ DM} - 0.2318 \text{ BA} + 0.3688 \text{ NH}_3\text{-N} + 0.0184 \text{ DOMD} \quad \text{Equation 2}$$

$$VI = 9.92 - 0.578 \text{ CI} + 0.1196 \text{ DM} - 0.067 \text{ DOMD} \quad \text{Equation 3}$$

$$VI = 24.96 - 0.5397 \text{ CI} + 0.1080 \text{ DM} - 0.0264 \text{ NH}_3\text{-N} + 0.458 \text{ DOMD} \quad \text{Equation 4}$$

$$VI = 17.96 - 0.395 \text{ CI} + 0.1092 \text{ DM} - 0.123 \text{ BA} + 0.0525 \text{ DOMD} \quad \text{Equation 5}$$

where VI = voluntary intake (gDM/kg metabolic LW per day)
 LW = liveweight at start (kg)
 CI = concentrate intake (gDM/kg metabolic LW per day)
 DM = toluene DM (g/kg)
 BA = butyric acid (g/kgDM)
 TN = total nitrogen (g/kgDM)
 NH₃-N = ammonia nitrogen (g/kg TN)
 DOMD = digestibility (g/kg DM)

Lewis (1981)

$$I = 0.010 \text{ DM} + 0.0161 \text{ DOMD} - 0.0154 \text{ W} - 0.02 \text{ NH}_3\text{-N} + 13.6$$

$$\text{SDMI (g/kg LW)} = 0.92I - 0.027I \times C - 0.0247 (C \times C) + 1.0$$

where C = concentrate intake (g/kg LW)

ARC (1980)

$$\text{DMI (g/kg metabolic LW)} = 106.5 \times \text{ME}/18.4 + 37 \times \text{CProp}^n + 24.1$$

where CPropⁿ = concentrate proportion

Table 4.4

PREDICTION OF MEAN SILAGE INTAKE
(g/kg metabolic LW/day)

| <u>All Silage Types</u> | Mean | MSPE | Prop of MSPE | | | |
|--------------------------------|-------|--------|--------------|------|--------|-------|
| | | | Bias | Line | Random | MPE |
| Actual Intake | 62.9 | | | | | |
| Predicted Intake Rooketal 90 ① | 57.22 | 138.93 | .179 | .001 | .820 | 18.94 |
| ② | 59.64 | 135.36 | .049 | .001 | .950 | 18.70 |
| ③ | 57.17 | 153.04 | .166 | .007 | .827 | 19.90 |
| ④ | 57.55 | 152.16 | .142 | .007 | .851 | 19.80 |
| ⑤ | 61.86 | 141.53 | .001 | .007 | .992 | 19.10 |
| ARC (1980) | 65.94 | 229.65 | .060 | .118 | .822 | 24.40 |
| Lewis (1981) | 47.23 | 551.23 | .515 | .126 | .359 | 37.70 |

Table 4.5

PREDICTION OF INDIVIDUAL SILAGE INTAKE
(g/kg metabolic LW/day)

| Low pH Silage | Prop of MSPE (Bias) | | | | | |
|--|---------------------|--------|------|------|--------|------|
| | Mean | MSPE | Mean | Line | Random | MPE |
| Actual Intake | 55.3 | | | | | |
| Predicted Intake Rook <i>etal</i> 90 ① | 53.29 | 33.26 | .152 | .038 | .810 | 10.4 |
| ② | 59.94 | 43.86 | .405 | .0 | .595 | 11.9 |
| ③ | 57.26 | 31.75 | .076 | .102 | .822 | 10.1 |
| ④ | 58.28 | 35.10 | .187 | .064 | .749 | 10.6 |
| ⑤ | 63.39 | 86.16 | .683 | .006 | .311 | 16.7 |
| ARC (1980) | 69.19 | 218.24 | .832 | .005 | .163 | 26.5 |
| Lewis (1981) | 52.37 | 90.16 | .118 | .532 | .324 | 17.1 |

| Normal Silage | Mean | MSPE | Mean | Line | Random | MPE |
|--|-------|-------|------|------|--------|------|
| Actual Intake | 62.7 | | | | | |
| Predicted Intake Rook <i>etal</i> 90 ① | 54.46 | 70.2 | .560 | .035 | .405 | 13.8 |
| ② | 60.72 | 26.9 | .001 | .0 | .999 | 8.5 |
| ③ | 56.98 | 48.4 | .291 | .130 | .579 | 11.4 |
| ④ | 57.84 | 38.9 | .216 | .076 | .708 | 10.3 |
| ⑤ | 63.28 | 35.0 | .170 | .010 | .820 | 9.7 |
| ARC (1980) | 69.08 | 108.5 | .627 | .068 | .305 | 17.1 |
| Lewis (1981) | 42.29 | 209.5 | .622 | .258 | .122 | 23.8 |

| High DM Silage | Mean | MSPE | Mean | Line | Random | MPE |
|--|-------|--------|------|------|--------|------|
| Actual Intake | 74.2 | | | | | |
| Predicted Intake Rook <i>etal</i> 90 ① | 63.96 | 395.62 | .223 | .022 | .755 | 27.1 |
| ② | 67.23 | 355.21 | .107 | .053 | .840 | 25.6 |
| ③ | 63.42 | 402.41 | .245 | .031 | .724 | 27.3 |
| ④ | 64.38 | 390.38 | .207 | .040 | .753 | 26.9 |
| ⑤ | 69.13 | 340.53 | .054 | .109 | .837 | 25.1 |
| ARC (1980) | 63.90 | 509.66 | .171 | .033 | .796 | 30.7 |
| Lewis (1981) | 48.40 | 916.97 | .679 | .0 | .321 | 41.2 |

| Bad Silage | Mean | MSPE | Mean | Line | Random | MPE |
|--|-------|--------|------|------|--------|------|
| Actual Intake | 59.4 | | | | | |
| Predicted Intake Rook <i>etal</i> 90 ① | 57.21 | 62.90 | .065 | .154 | .781 | 13.3 |
| ② | 50.95 | 119.16 | .572 | .014 | .414 | 18.4 |
| ③ | 51.22 | 134.44 | .473 | .140 | .387 | 19.6 |
| ④ | 44.79 | 270.47 | .769 | .037 | .194 | 27.8 |
| ⑤ | 51.91 | 107.45 | .496 | .016 | .488 | 17.5 |
| ARC (1980) | 61.61 | 87.51 | .061 | .196 | .743 | 15.8 |
| Lewis (1981) * | 38.98 | 530.1 | .771 | .127 | .102 | 38.9 |

* Estimated value using max^m value of ammonia nitrogen as equation permits

his equation) is not expected to be very accurate at low liveweights as the concentrate substitution rate factors in this equation are proportional to the liveweight of the animal. Lewis (1981) , however, had not given any animal liveweights for his experiments. It was also confirmed that Lewis used 59 data sets with a mean animal liveweight of 318 kg (range 190 - 430) (Lewis, personal communication, 1995). The mean animal liveweight in the current experimental work was 155 kg (range 113 - 198 kg). Thus it is apparent that the range of applicability of the Lewis (1981) equation does not extend to the small animal liveweights.

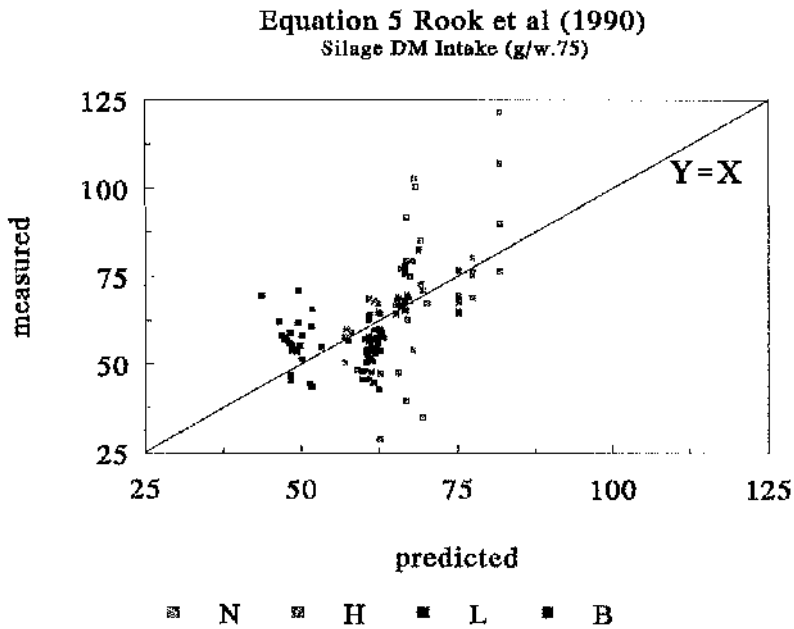
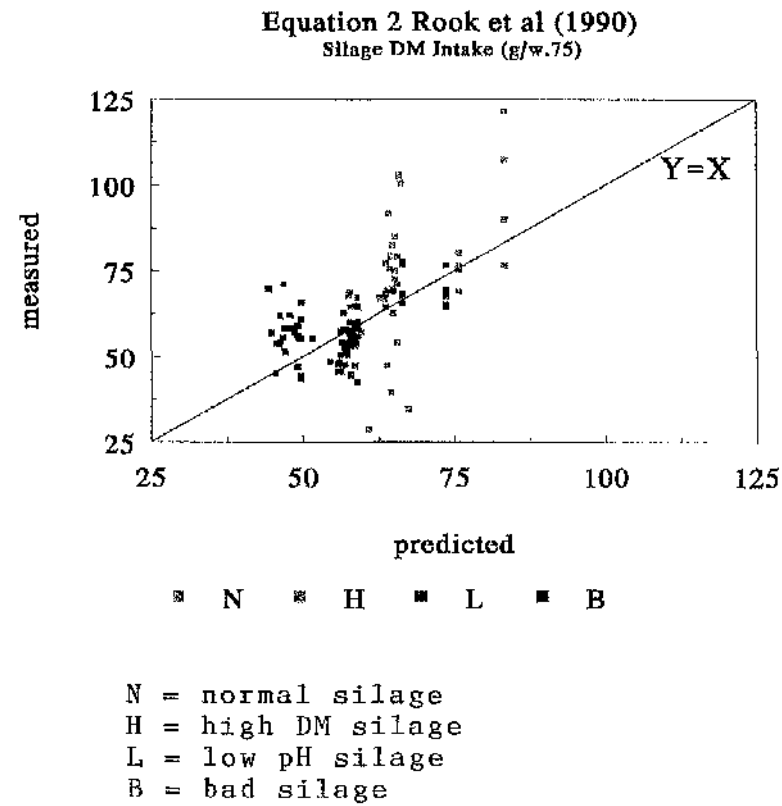
All of the Rook *et al* (1990) prediction equations were quite similar in their accuracy of mean intake prediction (Table 4.4). There were, however, some differences in accuracy between the equations when applied to the individual silage types (Table 4.5). For example equation 1 was the most accurate at predicting the intake of the bad silage as indicated by the low MPE value. The reason for this appears to be due to the large positive coefficient for pH (12.98), this variable not appearing in any of the other equations.

None of the equations by Rook *et al* (1990) was able accurately to predict intake of the high DM silage as is indicated by the high MPE values (Table 4.5). These equations are based on fermentation characteristics (Table 4.3) and their inability to predict the intake of this silage suggests that fermentation characteristics are not the sole factor in predicting intake of the experimental silages and that other factors play an important role.

The MPE of the Rook *et al* (1990) equations in Table 4.5 (mean 17.1, range 8.5 - 27.8) is similar to the MPE values (mean 14.9, range 8.1 to 25.5) they reported in the same study whilst testing the accuracy of the equations. In order to examine further the applicability of these equations, the actual intake was plotted against the predicted intake for two of their equations. Equations 2 and 5 were selected, equation 2 having the smallest MSPE and equation 5 having the lowest mean and line biases. Figure 4.2 contains the results for each animal and the large variation between animals fed one silage type and across silage types is again apparent. For both the normal and low pH silages (green squares and blue squares) the predicted intake is close to the actual intake. For the bad silage (black squares) the actual intake is greater than the predicted intake. The greatest deviation of predicted from actual intake is with the high DM silage (red squares).

Figure 4.2

Relationship Between Actual and Predicted Intakes



Several discrepancies were encountered whilst examining the applicability of the Rook *et al* (1990) prediction equations. Firstly there were no liveweights given for the animals used to measure the actual intakes. Despite the silage intakes being quoted on a metabolic liveweight basis, an indication of liveweight would have assisted in establishing the likely range of applicability of the equations. Secondly, and more importantly, Rook *et al* (1990) did not give either comprehensive silage analyses or a range of values of the silage chemical components. The inaccuracy in predicting the intake of the bad silage may be due to the small number of silages of similar chemical composition in the Rook *et al* (1990) data set. Unfortunately due to the omission of silage data this possibility cannot be substantiated.

The accuracy of the single variable and multi-variable equations was subsequently compared. Examination of the MPE values in Tables 4.2 and 4.4 clearly show that for the current data set for calves around 150 kg LW the equation by Lewis (1981) is the least accurate. The equations predicting intake with respect to crude protein by Laforest *et al* (1986) and Wilkins *et al* (1971) and the ARC (1980) equation are somewhat more successful and of similar accuracy. The multi-variable equations by Rook *et al* (1990) are of similar accuracy to the NDF equation by Laforest *et al* (1986) as is shown by the similar MPE values: these are the most accurate prediction equations examined. It is surprising that predicting silage intake with respect to NDF is as accurate as predicting intake with respect to several fermentation characteristics. This finding supports the view that fermentation characteristics alone are not sufficient to predict silage intake (Offer, personal communication, 1995). The observation that a simple relationship based on NDF can give an equally good prediction of intake suggests that some combination of these factors might be more successful in predicting intake. Unfortunately the limited data and variability found in the present work preclude any attempt to develop a meaningful new equation of this type based from these data.

4.4 INDICATIONS FOR FUTURE RESEARCH

During the course of this work the need for further research in a number of aspects of silage intake became apparent.

- * The low pH silage used in the present experimental work was not truly

representative of a Group 1 silage and bicarbonate was not found to have any effect on the intake of this silage. Further intake trials with a more typical example of this group might give a better indication of the intake potential of this type of silage and establish whether the inclusion of bicarbonate has any positive effect in increasing intake.

* The bad silage in the current experimental work exhibited a higher than expected intake. Hitherto very few trials had fed a bad silage and thus it is not clear whether the current recorded intake is typical or atypical. Further trials feeding a bad silage would indicate the intake potential of this type of silage; confirmation of this finding would be important given the widespread occurrence of bad silages.

* The inclusion in the present work of a high sugar concentrate was not possible because of lack of animal space. Molasses is often fed to cattle with silage and thus it would be useful to know if this leads to any increase in intake.

* A problem encountered during the current research was the absence of any quick and easy method of predicting digestibility using feed and faecal samples. This should now be possible using NIR and the development of a data base for this would be greatly beneficial to digestibility studies.

* Access to an alternative, equally large data set in which water soluble carbohydrates had been measured would enable this parameter to be examined for use in classifying silages into groups by cluster analysis. It would be interesting to see whether water soluble carbohydrates are important in group discrimination as silage intake has been found by Henderson *et al* (1984) to be correlated with the water soluble carbohydrate content.

* It would also be interesting to examine the effects on intake of silage characteristics other than fermentation. The results of the present work suggest that factors other than fermentation characteristics play a significant role in governing voluntary intake.

Silage is widely used as a cheap, nutritious fodder which is easy to store. Despite the efforts of many workers over many years, there still exists only a broad understanding of the factors governing silage intake. The current experimental work sought to classify all silages into groups of similar composition and to relate each group to

voluntary intake. The classification of silages was successful but attempts to establish a relationship between a representative of each silage group and intake were less successful. This may be due to the variables that were used for classification not being the key ones in determining silage intake. These variables also had the disadvantage that they were difficult to measure. Thus there is a need to establish whether there are other key variables which are easy to quantify and to relate these to intake. In particular it would seem worth exploring further whether some combination of fermentation parameters and NDF could be developed which would better describe intake.

Equations predicting the intake of the experimental silages were not developed in this work as only four silages were used with five concentrate treatments and five animals per treatment. This was too small and narrow a data base for developing prediction equations.

The large variations in silage intake between animals for each treatment points to the need for large scale feeding trials so that any significant relationships will be more clearly shown. There is also a need for greater restriction of variables such as additive use as the number of variables in the current work made clear interpretation of the results difficult. Further work of this nature should permit the development of a better understanding of the key factors affecting silage intake in ruminants.

APPENDIX 1

EXAMPLE 1

A simplified example illustrates how the criterion value can be calculated. For example nine points in two dimensions have been arbitrarily allocated to the following groups.

Group 1 (1,2), (4,5), (6,2)

Group 2 (1,1), (3,6), (5,3)

Group 3 (2,1), (3,5), (5,2)

For each group the value of the sum of the squares of the distances of each point in that group from the centre of gravity to the group is calculated. The sum of these values for each group is the criterion value of that particular grouping.

For Group 1 sums of squares of distances of each point from centre of gravity $= (1^2 + 4^2 + 6^2 - 11^2/3^2) + (2^2 + 5^2 + 2^2 - 9^2/3^2)$
 $= 12.666 \quad + 6$
 $= 18.666$

For Group 2 sums of squares $= (1^2 + 3^2 + 5^2 - 9^2/3^2) + (1^2 + 6^2 + 3^2 - 10^2/3^2)$
 $= 8 \quad + 12.666$
 $= 20.666$

For Group 3 sums of squares $= (2^2 + 3^2 + 5^2 - 10^2/3^2) + (1^2 + 5^2 + 2^2 - 8^2/3^2)$
 $= 4.666 \quad + 8.666$
 $= 13.333$ Total sums of squares of distances $= 52.666 = \text{Criterion value}$

If the points are assigned to different groups as follows:

Group 1 (5,3), (5,2), (6,2)

Group 2 (3,5), (3,6), (4,5)

Group 3 (1,2), (1,1), (2,1)

then the sums of squares, hence the criterion value is different.

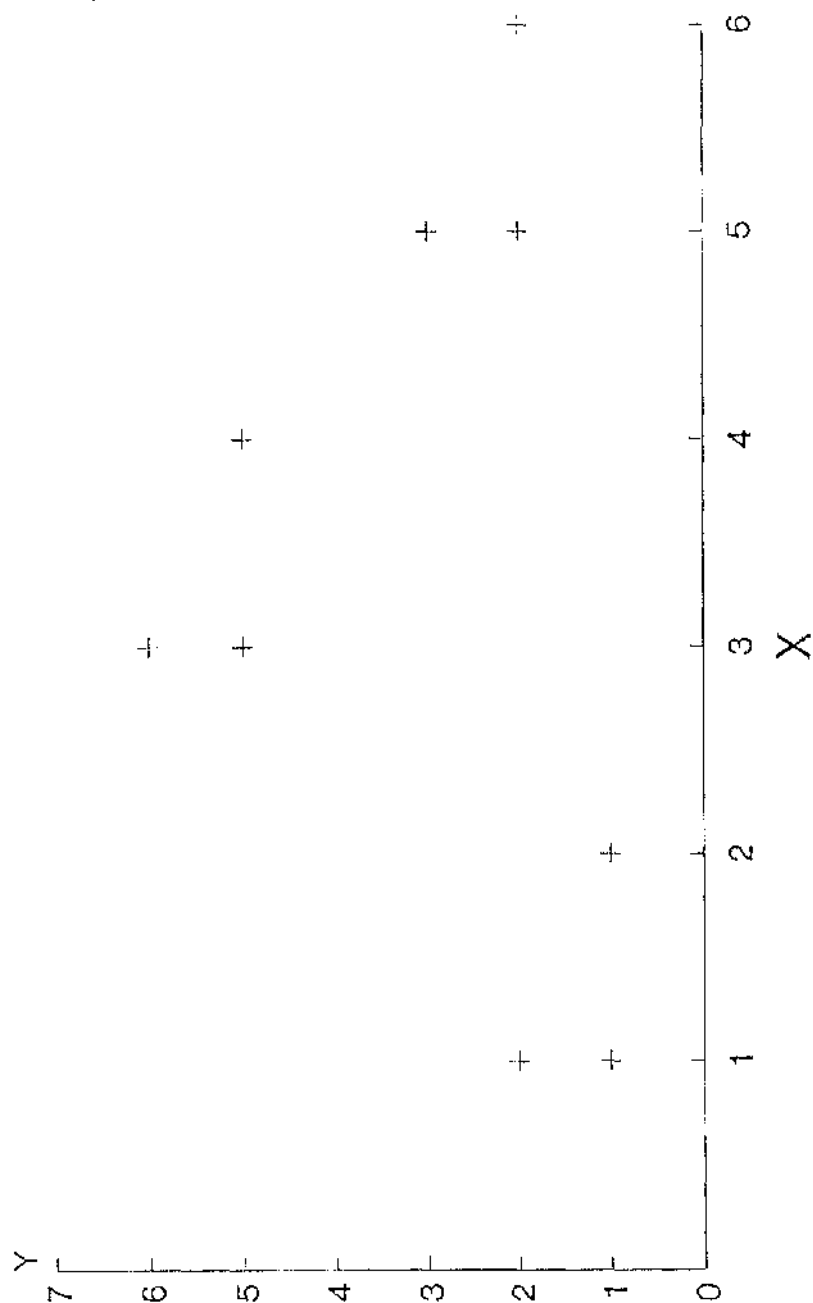
For Group 1 sums of squares $= (5^2 + 5^2 + 6^2 - 16^2/3^2) + (3^2 + 2^2 + 2^2 - 7^2/3^2)$
 $= 0.666 \quad + 0.666$
 $= 1.333$

| | |
|---|---|
| Group 2 | $= (3^2 + 3^2 + 4^2 - 10^2/3^2) + (5^2 + 6^2 + 5^2 - 16^2/3^2)$ $= 0.666 \quad + 0.666$ $= 1.333$ |
| Group 3 | $= (1^2 + 1^2 + 2^2 - 4^2/3^2) + (2^2 + 1^2 + 1^2 - 4^2/3^2)$ $= 0.666 \quad + 0.666$ $= 1.333$ |
| Total sums of squares = 4 = Criterion value | |

Thus by comparing the two criterion values, the second one is lowest, indicating that it is a better grouping of the points. This can be seen by examination of Appendix 1 Graph 1. The points are clearly separated into three groups, as per the second arrangement of the data points.

APPENDIX 1

GRAPH 1



EXAMPLE 2

This example demonstrates that the greater the number of groups the greater the number of centres of gravity, hence the smaller the criterion value.

Eight points (1,1), (1,2), (2,1), (2,2), (4,2), (4,3), (5,2) and (5,3) have been selected for division into four groups, three groups and two groups (Appendix 1 Graph 2).

For the four groups the criterion value is:

| | Criterion Value |
|--|-----------------|
| Group 1 (1,1), (1,2) | 0.5 |
| Group 2 (2,1), (2,2) | 0.5 |
| Group 3 (4,2), (4,3) | 0.0 |
| Group 4 (5,2), (5,3) | 0.5 |
| Criterion value for four groups = <u>2</u> | |

For three groups:

| | Criterion Value |
|---|-----------------|
| Group 1 (1,1), (1,2), (2,1), | 1.334 |
| Group 2 (2,2), (4,2) | 2 |
| Group 3 (4,3), (5,3), (5,2) | 1.334 |
| Criterion value for three groups = <u>4.668</u> | |

For two groups:

| | Criterion Value |
|--|-----------------|
| Group 1 (1,1), (1,2), (2,1), (2,2) | 2 |
| Group 2 (4,2), (4,3), (5,2), (5,3) | 2 |
| Criterion value for two groups= <u>4</u> | |

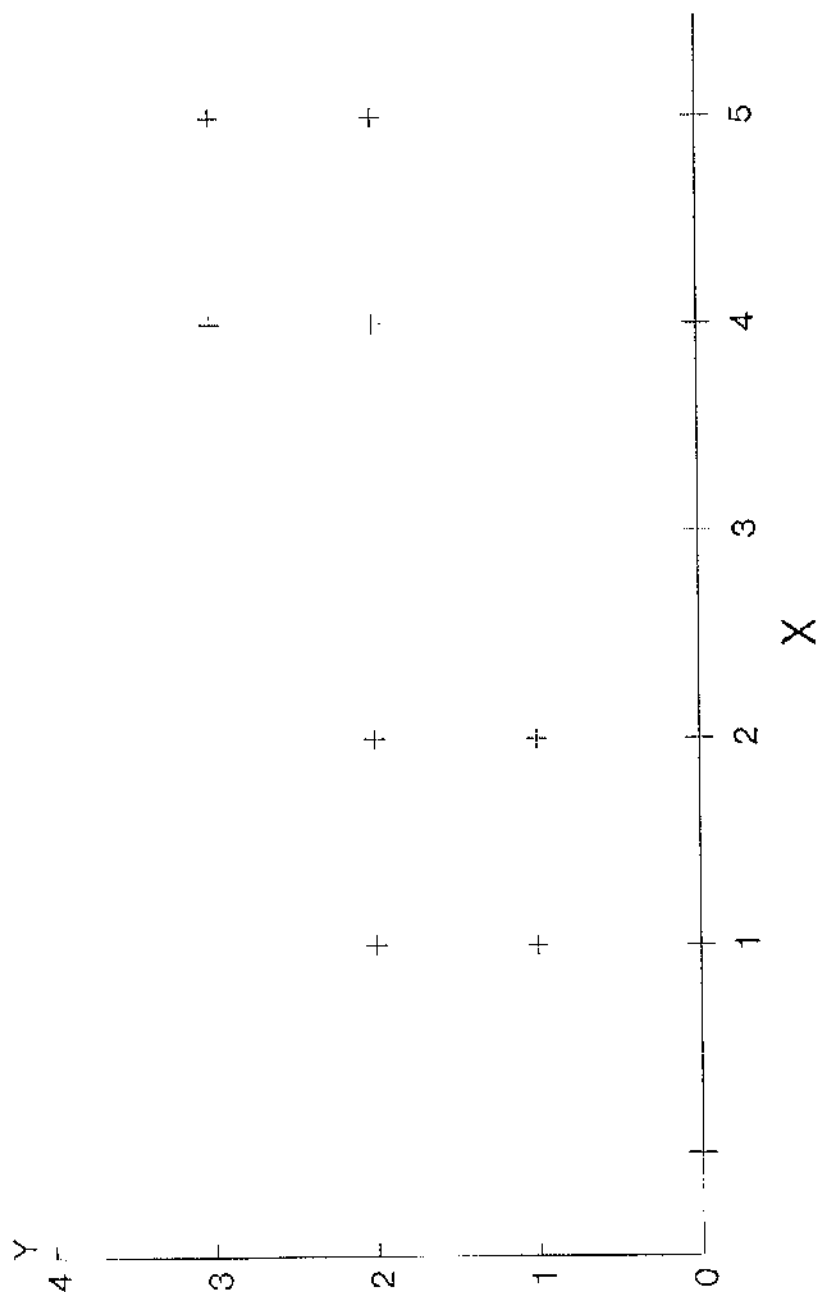
When selecting the optimum number of groups, the percentage difference in the criterion values are compared. The lowest percentage difference gives the best number of groups. In the above example the percentage difference on reduction from four to three groups is 57.2% and from three to two groups 14.3%.

| | Criterion Value | % Difference Between Groups |
|----------|-----------------|-----------------------------|
| 4 Groups | 2 | |
| 3 Groups | 4.668 | 57.2 |
| 2 Groups | 4 | 14.3 |

Thus by comparison of the criterion value percentage differences two groups is the best solution. This can be seen visually on inspection of Appendix 1 Graph 2.

APPENDIX 1

GRAPH 2



APPENDIX 2

For example a silage with the following analysis could be assigned to one of the groups.

| | Chemical Analysis | Natural Log | Vector Loading 1 | Vector Loading 2 |
|------------------|----------------------|----------------|---------------------|---------------------|
| Dry Matter | 213 | 5.361 | 3.436 | -0.034 |
| pH | 3.9 | 3.9 | -0.555 | 2.338 |
| Total Nitrogen | 25 | 3.219 | 0.879 | -5.004 |
| Ammonia Nitrogen | 96 | 4.564 | -1.535 | 4.090 |
| Acetic Acid | 51 | 3.932 | -2.304 | 5.365 |
| Propionic Acid | 2.4 | 0.875 | -0.688 | 0.406 |
| Butyric Acid | 1.5 | 0.405 | -0.510 | -0.576 |
| Lactic Acid | 96 | 4.564 | -0.512 | 1.464 |
| | | | -1.789 | 8.049 |

The first vector loading gives the x-axis coordinate, -1.789, and the second vector loading gives the y-axis coordinate, 8.049. Thus a point (-1.789, 8.049) can be plotted on Figure 2.5. This silage therefore belongs to Group 1, the good quality group.

APPENDIX 3

The SAC quality rating is based on a desirable range of chemical constituents for silage. Namely:

| | |
|----------------------------|--------------|
| Dry Matter (g/kg) | 180.0- 280.0 |
| pH | 3.8- 4.2 |
| Ammonia Nitrogen (g/kg TN) | 30.0- 100.0 |
| Crude Protein (g/kg) | 150.0- 180.0 |
| D Value | 63.0- 68.0 |
| ME (MJ/kg) | 10.0- 11.0 |
| Ash (g/kg) | 50.0- 90.0 |

From these a silage index is derived with values over 80 described as being very good quality, between 70 and 80 good quality, between 60 and 70 medium quality, and under 60 poor quality.

APPENDIX 4

COMPOSITION OF PRE-TRIAL DIETS

| | DM (g/kg) | TN (g/kg DM) | OM (g/kg DM) | IVOD in vitro (%) | M/D D | NDF (g/kg DM) | ADF (g/kg DM) | Starch (g/kg DM) | |
|----------------|-----------------------|-----------------|-----------------|----------------------|----------|------------------|------------------|---------------------|-----|
| Low pH silage | Grassnuts: offered | 908 | 27.4 | 882 | 66.2 | 58.4 | 9.8 | 509 | 285 |
| | refused | 891 | 27.7 | 872 | 67.1 | 58.5 | 9.9 | 476 | 329 |
| | Barley/soya | 856 | 30.9 | 959 | 87.6 | 83.9 | 13.5 | 131 | 61 |
| Normal silage | Grassnuts: offered | 907 | 24.0 | 9.5 | 65.2 | 59.0 | 9.9 | 553 | 304 |
| | refused | 871 | 24.0 | 901 | 65.3 | 58.8 | 9.9 | 540 | 314 |
| | Barley/soya | 853 | 32.3 | 930 | 86.5 | 80.4 | 12.9 | 142 | 52 |
| High DM silage | Grassnuts: offered | 887 | 24.5 | 902 | 63.4 | 57.3 | 9.6 | 550 | 331 |
| | refused | 818 | 25.9 | 895 | 63.4 | 56.7 | 9.5 | 608 | 361 |
| | Barley/soya | 828 | 42.2 | 926 | 86.8 | 80.3 | 12.9 | 210 | 68 |
| Bad silage | Grassnuts: offered | 874 | 29.3 | 880 | 65.6 | 57.7 | 9.7 | 539 | 295 |
| | refused | 856 | 17.6 | 884 | 66.2 | 58.5 | 9.9 | 531 | 310 |
| | Barley/soya | 841 | 38.6 | 937 | 87.3 | 81.8 | 13.1 | 184 | 67 |

APPENDIX 5

ANALYSIS OF FAECAL SAMPLES PER TREATMENT

| | Standard | High Starch | High Fibre | High Protein | Bicarb | Silage |
|-------------------------|----------|-------------|------------|--------------|--------|--------|
| Low pH silage | | | | | | |
| DM (g/kg) | 14.9 | 16.1 | 16.3 | 16.2 | 14.2 | 14.6 |
| ash (g/kg DM) | 145 | 136 | 140 | 153 | 143 | 134 |
| IADF (g/kg DM) | 11.16 | 10.30 | 11.79 | 10.39 | 11.41 | 14.16 |
| Normal silage | | | | | | |
| DM (g/kg) | 16.1 | 14.7 | 16.9 | 15.3 | 15.9 | 15.4 |
| ash (g/kg DM) | 136 | 129 | 117 | 142 | 141 | 128 |
| IADF (g/kg DM) | 14.59 | 13.30 | 13.87 | 13.71 | 13.51 | 15.79 |
| High dry matter silage | | | | | | |
| DM (g/kg) | 15.7 | 16.9 | 15.7 | 14.4 | 15.7 | 15.1 |
| ash (g/kg DM) | 128 | 124 | 122 | 136 | 127 | 114 |
| IADF (g/kg DM) | 13.89 | 12.64 | 16.03 | 12.74 | 14.26 | 12.66 |
| Poorly fermented silage | | | | | | |
| DM (g/kg) | 18.2 | 17.0 | 18.5 | 16.1 | 17.7 | 18.7 |
| ash (g/kg DM) | 174 | 169 | 177 | 171 | 174 | 174 |
| IADF (g/kg DM) | 14.00 | 13.31 | 14.21 | 15.57 | 13.28 | 12.92 |

APPENDIX 6

IADF VALUES

| | S | HS | F | P | B | Silage |
|-------------------------|-------|-------|-------|-------|-------|--------|
| Low pH | | | | | | |
| silage | 29.4 | 29.4 | 29.4 | 29.4 | 29.4 | 29.4 |
| concentrate | 25.0 | 14.7 | 28.7 | 12.9 | 22.2 | |
| faeces | 111.6 | 103.0 | 117.9 | 105.2 | 114.1 | 147.0 |
| Normal silage | | | | | | |
| silage | 44.4 | 44.4 | 44.4 | 44.4 | 44.4 | 44.4 |
| concentrate | 21.3 | 14.6 | 26.7 | 13.0 | 21.3 | |
| faeces | 145.9 | 133.0 | 138.7 | 137.1 | 135.1 | 157.0 |
| High DM | | | | | | |
| silage | 46.0 | 46.0 | 46.0 | 46.0 | 46.0 | 46.0 |
| concentrate | 30.2 | 19.1 | 35.8 | 19.6 | 31.0 | |
| faeces | 138.9 | 126.4 | 100.3 | 127.4 | 142.3 | 126.6 |
| Poorly fermented | | | | | | |
| silage | 61.5 | 61.5 | 61.5 | 61.5 | 61.5 | 61.5 |
| concentrate | 27.7 | 17.4 | 34.1 | 21.1 | 26.9 | |
| faeces | 137.7 | 133.1 | 142.1 | 149.7 | 132.8 | 128.5 |

APPENDIX 7

METHODS OF ANALYSIS

Chemical analyses were carried out on fresh material or on a material dried at 60 C in a forced-draught oven for 48 hours and then hammermilled in order to pass through a 1 mm screen.

Dry Matter (DM)

Samples were dried in a force-draught oven at 60 C for 48 hours. This is referred to as oven dry matter (ODM).

The DM values of the silage offered to the animals were determined using the toluene distillation method (TDM) of Dewar and McDonald (1961) and corrections were made for ethanol and volatile fatty acids (East of Scotland College of Agriculture Annual Report 1978, p75).

pH

The pH of the silages were determined on aqueous macerates of the silage using a Pye combined electrode and pH meter previously calibrated on a pH 7.0 and on a pH 4.0 solution.

Buffering Capacity (Bc)

The buffering capacity was determined on silage according to the method of Playne and McDonald (1966).

10 g of well mixed silage was macerated with 250 ml (chilled) distilled water for two minutes and transferred to a beaker.

The initial pH was measured by means of a combined glass electrode and pH meter. The above macerate was taken to pH 3 with 0.05 M sulphuric acid and then brought back to pH 4.0 and titrated from 4.0 to pH 6.0 using 0.1 M sodium hydroxide. The volume of standard alkali required between 4.0 and 6.0 was

recorded. (The macerate was acidified first to remove bicarbonate which would act as a buffer if present).

The buffering capacity is expressed as m.equiv. of alkali required to change the pH from 4.0 to 6.0 per kg DM.

Total Nitrogen (TN)

The total nitrogen was determined in the fresh herbage or silage using the Kjeldahl method. Approximately 10 g of fresh material was accurately weighed into a polythene bag and placed into a 500 ml Kjeldahl flask. 5 g of potassium sulphate \ selenium catalyst and 40 ml concentrated sulphuric acid were added and the flask heated on the digestion stand until the contents were clear and then for a further 2 hours. The flask was then cooled, the contents diluted with water and a few grains of anti-bumping granules added.

A known quantity of 0.05 M sulphuric acid was measured into a receiving flask and 130 ml sodium hydroxide poured carefully into the Kjeldahl flask. The distillation apparatus was then connected up and the Kjeldahl flask heated for 30 minutes fairly gently and then for 15 minutes strongly.

The excess of 0.05 M sulphuric acid in the receiving flask was titrated with standard 0.1 M sodium hydroxide and the amount of nitrogen in the silage calculated.

Ammonia Nitrogen (NH₃-N)

20 g of freshly minced silage is added to a Waring blender along with 200 ml of distilled water. The mixture is blended at low speed for five minutes, filtered and the extract used for the determination of ammonia.

0.1 ml of extract is diluted with 10 ml of a phenol/sodium hydroxide/sodium nitroprusside solution and 0.5 ml of a

trisodium orthophosphate/disodium hydrogen orthophosphate/sodium hydroxide/ sodium hypochlorite solution is added. 20, 40 and 60 ppm standard solutions are diluted at the same time. The blue colour is allowed to develop at room temperature for 60 minutes and read on a spectrophotometer at 584 nm.

The ammonia is calculated as g/kg of total nitrogen.

Lactic Acid (LA)

10 g chopped silage were pressed down firmly in a glass screw-topped bottle. Sufficient 0.3 M sulphuric acid was added to cover the silage sample (usually 20 ml) and a few small thymol crystals. The bottle was kept under refrigeration for a week.

The contents were then squeezed through cloth and centrifuged at 3000 RPM for 20 minutes. Aliquots of this extract were then used to determine lactic acid by the method of Barker and Sommerson (1941).

Five ml of the extract were made up to 50 ml in a volumetric flask with distilled water. Then 10 ml of this solution were treated with one ml of 20% copper sulphate solution, one gram calcium hydroxide and 17 ml of distilled water in a test tube. The contents were well mixed, then filtered through hardened Whatman paper (Grade 50, 11mm diameter). Five ml of this filtrate was made up to 50 ml in a volumetric flask with distilled water.

One ml of this diluted filtrate was added to 9 ml ice cold concentrated sulphuric acid in a boiling tube slowly with swirling. The tube was placed in a boiling water bath for 10 minutes, taken out and cooled for five minutes under running water and then placed in an ice bath. Then 0.05 ml 4% copper sulphate solution and 0.1 ml 4-hydroxy biphenyl reagent were added. The tube was well mixed using a rotamixer.

After cooling in an ice bath (deep freeze) for one hour the tube was plunged into boiling water for 90 seconds, cooled then placed back in an ice bath for 5 minutes. Finally the tube was brought back to room temperature and read at 560 nm in a spectrophotometer and compared with a blank and different concentrations of lactic acid standards.

The lactic acid content was calculated from a graph of lactic acid standards whose concentration was known.

Volatile Fatty Acids (VFA) and Ethanol

The ethanol and volatile fatty acid contents (acetic, propionic and butyric acids) were determined by gas chromatography on a sample of the acid extract of silage prepared for the determination of lactic acid.

A Hewlett-Packard, model 5790 gas chromatograph equipped with dual 2.3 m glass columns packed with Chromosorb 101, mesh size 60 to 80 was used with nitrogen as the mobile phase. The injector and detector temperature were 150 and 200 C. The column oven temperature was programmed at 5 C per minute from 130 to 200 C and the analysis took 22 minutes.

Water Soluble Carbohydrates (WSC)

1. Silage

WSC in silage were determined by the method of McDonald and Henderson (1964) except for the final colorimetric method where somogyi reagent (Somogyi 1945) and arsenomolybdate reagent (Nelson 1944) were added.

25 g of chopped silage were mixed with 200 ml distilled water (chilled) using an electric blender. The macerate was filtered through a cloth into a Buchner flask.

100 ml of the filtrate was transferred to a 250 ml conical Erlenmeyer flask using a measuring cylinder. The conical flask contained 5 ml of 0.05 M sulphuric acid plus powder filter aid. This was brought to the boil.

The conical flask was then cooled under running water and the contents filtered through Whatman folded filter paper (Grade 113v) into a volumetric flask. The residue was washed and the flask made up to the mark with distilled water.

15 ml of the filtrate were pipetted into a large test tube and 5 ml 1 M sulphuric acid added. The tube was stirred well using a rotamixer. The test tube was then placed in a water bath for 10 minutes, removed and cooled under running water for 5 minutes. One drop of methyl red indicator was added to the tube. The contents were then neutralised with 40% sodium hydroxide (approximately 6 drops), until the pink solution became yellow. Then 1 M sulphuric acid was added dropwise until a yellow-brownish colour was achieved. The contents of the tube were transferred to a 200 ml volumetric flask, and made up to the mark with distilled water.

Two ml of extract were pipetted into a test tube and 2 ml of somogyi reagent added (Somogyi 1945). Water blanks and fructose standards of different concentrations were also used. The tubes were placed in a water bath for 20 minutes, removed and cooled under running water for 3 minutes.

Two ml of arsenomolybdate reagent (Nelson 1944) were added to the tubes. The tubes were well shaken using a rotamixer. The contents were then transferred into 50 ml flasks and made up to the mark with distilled water. After 20 minutes the absorbance of the solutions was determined at 540 nm using a spectrophotometer.

The amount of WSC expressed as fructose was calculated from a standard graph of the fructose standards whose concentration is

known.

2. Concentrates

The sugars are extracted with aqueous ethanol and determined after inversion by the Luff-Schrool method (Statutory Instruments 1982).

2.500 g of the dried, ground sample was placed in a 1 l bottle, 200 ml 40% ethanol was added and this was then mixed on a rotary shaker for 1 hour. 5 ml of Carrez solution (zinc acetate dihydrate and glacial acetic acid) was added and the bottle stirred again for one minute. A further 5 ml of Carrez solution was added to the bottle and stirred for one minute. The residue was transferred quantitatively into a 250 ml graduated flask using 40% ethanol. This was mixed and approximately 125 ml was removed and evaporated to approximately half volume in order to eliminate most of the ethanol. The cool residue was transferred quantitatively into a 100 ml graduated flask using distilled water and made up to the mark with distilled water.

50 ml of the extract was transferred to a 250 ml Quickfit flask and three drops of methyl orange solution added. Using a pasteur pipette 4 N hydrochloric acid was added with continuous stirring until the liquid turns pink. 15 ml of 0.1 N hydrochloric acid was added and the liquid was refluxed for 30 minutes. This was cooled rapidly to 2 C and 15 ml of 0.1 N sodium hydroxide added. The liquid was transferred to a 100 ml graduated flask and made up to the mark with distilled water and mixed.

25 ml of Luff-Schrool reagent (copper sulphate solution/citric acid solution/sodium carbonate solution) was transferred into a 150 ml Erlenmeyer flask and 25.0 ml of the sugar extract, some antibumping granules and 1 ml of amylalcohol. The flask was fitted with a reflux condenser and brought to the boil in

2 minutes and allowed to simmer for 10 minutes. The flask was cooled immediately and left for 5 minutes before titrating.

10 ml of potassium iodide solution and 25 ml of 6 N sulphuric acid were added in increments. This was titrated with sodium thiosulphate solution until a dull yellow colour appeared. 1 ml of starch indicator was added and the titration was completed. The end point was milky-white, flesh coloured or off-white depending on the colour of the extract.

The same titration (without boiling) was carried out on a mixture of 25 ml Luff-Schrool reagent and 25 ml of distilled water after adding 10 ml potassium iodide solution and 25 ml 6N sulphuric acid. The amount of glucose is calculated from the sample titration minus the blank titration and determined from a standard graph.

Ash and Organic Matter (OM)

A 2 g dry sample in a previously weighed silica basin was put in a muffle furnace at 550 C for 3 hours. After cooling it was put in a desiccator until completely cool and weighed.

The difference between the weight of the basin and the weight of the basin plus the ash gives the total ash content. The difference between the dry matter and the ash content is the organic matter.

Acid Detergent Fibre (ADF)

ADF was determined on dried samples of silage and concentrate by the method of Van Soest (1963a, 1963b).

To 1 g of sample, 100 ml of cold acid detergent solution (1 N sulphuric acid/cetyl trimethyl ammonium bromide) was added. This was heated until boiling and then refluxed for 60 minutes. The solution was filtered and washed three times with

hot water and twice with acetone before weighing. The sample was then put in a muffle oven and ashed for 3 hours at 500 C before weighing.

Neutral Detergent Fibre (NDF)

NDF was determined on samples of dried silage and concentrate by the method of Van Soest and Wine (1967).

The procedure was similar to that for the determination of ADF. The neutral detergent solution was made from sodium lauryl sulphate, EDTA, sodium borate decahydrate, disodium hydrogen phosphate (anhydrous) and 2-ethoxy ethanol.

Indigestible Acid Detergent Fibre (IADF)

IADF was determined on dried samples of silage and concentrate by the method of Penning and Johnson (1983).

One gram samples are put through a standard ADF determination, omitting the acetone washes. The samples are washed through Whatman 54 filter paper with 200 ml of distilled water. The residue was transferred to plastic pots with approximately 50 ml enzyme solution (Trichoderma viride dissolved in a citric acid/anhydrous disodium hydrogen orthophosphate solution of pH 4.6) and covered before agitation.

The pots were incubated at 40 C for 10 days with daily agitation. The samples were washed into preweighed No.1 Gooch crucibles with 200 ml of distilled water and a little acetone. These were then dried overnight at 100 C and weighed and finally ashed overnight at 450 C and weighed. The IADF is quoted ash free.

Starch

The determination of starch in the concentrates was carried

out by the Unilever procedure (DAFS 1981).

To a 0.2 g sample of the finely ground material approximately 20 ml of hot (80 C) 85% ethanol was added. This removed the free glucose and other and other sugars and also deactivated the natural enzymes. The solution was mixed on a Whirlimixer, left to stand for 5 minutes, mixed again and centrifuged at 2500 RPM for 10 minutes. The supernatant was discarded then hot ethanol was added and the solution was mixed, decanted, mixed, centrifuged and the supernatant discarded once again. The residue was then haeted with 20 ml of water to gel the starch. It was mixed , the tube was loosely stoppered and placed in a boiling water bath for 2 hours. This extract was cooled below 40 C and 5 ml of acetate buffer was added and mixed. 2 ml of supernatant from the freshly prepared amyloglucosidase enzyme suspension was added with 2 drops of toluene which degrades the starch to glucose. This solution was mixed, lightly stoppered and incubated at 50-55 C overnight. After dilution glucose is measured colorimetrically using the specific enzyme glucose oxidase. The tube was removed from the incubator and quantitatively transferred to a 100 ml volumetric flask. This was cooled to room temperature, diluted to volume and mixed. The digest was filtered through 541 filter paper. 5 ml of filtrate was diluted to 50 ml. 0.2 ml of a standard glucose solution, 0.2 ml of the reagent blank and 0.2 ml water were all pipetted into seperate 10 ml Exelo tubes. 5.0 ml buffer/enzyme/chromogen reagent was added to all tubes which were then stoppered and mixed. The tubes were stood at room temperature, avoiding exposure to sunlight, for 25-50 minutes. Absorbance measurements of each solution were made against the water blank at 610 nm. The starch content of the sample could then be calculated.

Digestibility (D)

The method used was that by Alexander (1969) which is a development of the rumen liquor and pepsin procedure of Tilly

et al (1960).

One litre of rumen liquor was extracted from each of three rumen fistulated sheep. After filtration through muslin and saturation with carbon dioxide the rumen liquor was suspended (1+4) in McDougall's buffer (McDougall 1948). Molar ammonium sulphate solution was then added, 1 ml per 50 ml rumen liquor buffer mixture. Inoculations were made by adding 50 ml of the mixture to each tube. The tubes were swept with carbon dioxide, closed with stoppers fitted with Bunsen valves and placed in a water bath at 38.5 C. The digests were adjusted electrometrically to a pH of 6.9 at 24 hours and the rumen liquor stage was terminated at 48 hours by injection of hydrochloric acid (20% v/v). Aqueous pepsin solution (0.12 g in 5 ml) was added to each tube after electrometric adjustment of the pH to 1.2. After a further 48 hours digestion the residues were recovered in the presence of an inert filter aid (hyflo supercel) by filtration through a fibre glass paper using special filtration equipment. The residues were then dried at 100 C, weighed, ignited at 480 C and weighed again. A parallel determination of total organic matter enabled the digestibility coefficient of the organic matter to be calculated after allowing for residual organic matter in control tubes.

Calculation of Metabolisable Energy (ME)

The ME in MJ/kg DM for silages was calculated using regression equations based on the in vitro digestibility measurement.

$$(a) \text{ (OMD\% x 1.207) - 10.21 = } \underline{\text{in vitro}} \text{ OMD\%}$$

$$(b) \text{ } \underline{\text{in vivo}} \text{ OMD\% x } \frac{\text{(organic matter g/kg)}}{1000} = \text{D value}$$

$$(c) \text{ D value x 0.16 = ME}$$

The ME of the concentrates was again calculated from the in vitro digestibility

$$\text{OMD\% x } \frac{\text{(organic matter g/kg)}}{1000} \text{ x 0.16 = ME}$$

APPENDIX 8

ANALYSIS OF VARIANCE INTAKE (g/kg metabolic LW) WITH INTAKE OF DRIED GRASS PELLETS AS A COVARIATE

| | df | ss | ms | vr | cov ef | Fpr |
|----------------------------|--------|----------|---------|-------|--------|--------|
| Silage.Block.Stratum | | | | | | |
| Silage | 3 | 5933.74 | 1977.91 | 17.87 | 0.90 | <0.001 |
| Covariate | 1 | 122.73 | 122.73 | 1.11 | - | 0.309 |
| residual | 15 | 1660.20 | 110.68 | 1.17 | 1.01 | |
| Silage.Block.Treat.Stratum | | | | | | |
| Silage.yorn | 4 | 5925.31 | 1481.33 | 15.70 | 0.98 | <0.001 |
| Silage.treat.silageyorn | 16 | 570.74 | 35.67 | 0.38 | 0.99 | 0.984 |
| Covariate | 1 | 755.77 | 755.77 | 8.01 | 0.99 | 0.984 |
| Residual | 75(4) | 7074.87 | 94.33 | - | 1.09 | - |
| Total | 115(4) | 21511.79 | | | | |

silage yorn = silage fed alone v rest of concentrate treatments

APPENDIX 9

SILAGE ANALYSES FOR EACH TREATMENT FOR EACH TRIAL (G/KG DM)

| silage | concentrate | pH | tdm (g/kg) | ash | tn | nh ₃ -N | wsc | la | ra | pa | ba | ethanol | propanol | ndf | adf | om | ivod (%) | d | m/d |
|---------|-------------|-----|---------------|-----|------|--------------------|-------|-----|------|------|------|---------|----------|-----|-----|-----|-------------|------|------|
| Group 1 | standard | 3.8 | 309 | 101 | 33.4 | 114 | 30.4 | 112 | 32.5 | 5.5 | 0.0 | 3.2 | 4.6 | 596 | 381 | 899 | 72.2 | 0.65 | 11.8 |
| | starch | 3.9 | 206 | 85 | 25.1 | 137 | 20.4 | 138 | 32.5 | 4.0 | 0.0 | 4.4 | 5.6 | 581 | 379 | 919 | 70.9 | 0.63 | 11.6 |
| | fibre | 3.8 | 201 | 71 | 23.7 | 116 | 34.9 | 116 | 32.8 | 5.9 | 0.0 | 4.3 | 4.5 | 562 | 390 | 929 | 71.5 | 0.63 | 11.6 |
| | protein | 3.8 | 202 | 91 | 27.6 | 114 | 20.1 | 133 | 36.7 | 5.5 | 0.0 | 3.9 | 5.2 | 582 | 374 | 909 | 61.1 | 0.63 | 9.9 |
| | bicarbonate | 3.9 | 203 | 74 | 25.0 | 145 | 20.1 | 135 | 39.8 | 4.0 | 0.0 | 4.3 | 7.4 | 575 | 376 | 920 | 73.5 | 0.66 | 11.9 |
| Group 2 | silage only | 3.7 | 206 | 87 | 24.3 | 105 | 25.6 | 117 | 32.9 | 4.3 | 0.0 | 3.9 | 4.6 | 582 | 374 | 912 | 73.2 | 0.66 | 12.0 |
| | standard | 3.8 | 201 | 14 | 29.9 | 119 | 52.3 | 126 | 30.6 | 6.0 | 0.8 | 5.8 | 1.4 | 504 | 336 | 986 | 80.5 | 0.55 | 12.6 |
| | starch | 3.9 | 213 | 31 | 29.6 | 112 | 31.4 | 134 | 27.0 | 5.9 | 0.0 | 5.9 | 1.2 | 493 | 336 | 989 | 77.9 | 0.71 | 11.4 |
| | fibre | 3.9 | 195 | 29 | 29.1 | 96 | 54.0 | 125 | 21.2 | 7.4 | 0.0 | 5.4 | 1.0 | 511 | 323 | 971 | 75.2 | 0.70 | 11.1 |
| | protein | 4.0 | 185 | 68 | 25.8 | 117 | 36.0 | 100 | 17.6 | 8.4 | 0.0 | 2.8 | 0.5 | 490 | 321 | 932 | 78.0 | 0.71 | 11.3 |
| Group 3 | bicarbonate | 3.8 | 201 | 30 | 29.4 | 99 | 59.0 | 101 | 22.9 | 5.6 | 0.0 | 5.2 | 0.9 | 492 | 328 | 970 | 76.4 | 0.71 | 11.3 |
| | silage only | 3.8 | 195 | 11 | 28.5 | 120 | 39.3 | 103 | 24.4 | 5.1 | 0.0 | 6.8 | 1.0 | 517 | 332 | 989 | 75.7 | 0.69 | 11.0 |
| | standard | 4.2 | 276 | 59 | 34.9 | 110 | 118.3 | 90 | 25.0 | 1.0 | 2.0 | 2.0 | | 449 | 283 | 941 | 73.2 | 0.68 | 10.8 |
| | starch | 4.0 | 281 | 75 | 32.8 | 109 | 215.0 | 83 | 22.0 | 3.0 | 3.0 | 2.0 | | 446 | 283 | 925 | 70.9 | 0.65 | 10.4 |
| | fibre | 3.9 | 289 | 47 | 34.4 | 93 | 197.0 | 98 | 24.0 | 3.0 | 2.0 | 2.0 | | 443 | 275 | 953 | 74.1 | 0.63 | 11.0 |
| Group 4 | protein | 4.0 | 271 | 96 | 33.8 | 58 | 160.0 | 63 | 23.0 | 4.0 | 6.0 | 2.0 | | 451 | 277 | 904 | 71.4 | 0.65 | 10.3 |
| | silage only | 3.9 | 275 | 50 | 34.1 | 99 | 149.6 | 67 | 23.0 | 3.0 | 1.0 | 2.0 | | 424 | 266 | 910 | 70.8 | 0.65 | 10.4 |
| | standard | 5.6 | 212 | 45 | 40.5 | 502 | 5.7 | 0.0 | 45.9 | 14.1 | 53.7 | 3.5 | 3.7 | 571 | 411 | 955 | 67.6 | 0.55 | 9.5 |
| | starch | 5.5 | 216 | 24 | 38.8 | 529 | 8.4 | 0.0 | 48.9 | 12.1 | 46.2 | 3.4 | 2.7 | 563 | 402 | 976 | 66.9 | 0.52 | 10.1 |
| | fibre | 5.5 | 243 | 64 | 44.1 | 556 | 4.9 | 0.0 | 40.8 | 12.1 | 43.6 | 2.8 | 2.6 | 549 | 340 | 936 | 66.9 | 0.58 | 9.4 |
| | protein | 5.5 | 215 | 78 | 35.4 | 501 | 8.6 | 0.0 | 53.0 | 13.2 | 52.0 | 2.9 | 2.3 | 579 | 400 | 922 | 69.6 | 0.57 | 9.1 |
| | bicarbonate | 5.5 | 213 | 29 | 34.6 | 488 | 6.9 | 0.0 | 45.2 | 12.3 | 57.9 | 4.0 | 2.9 | 581 | 412 | 971 | 63.9 | 0.53 | 10.0 |
| | silage only | 5.5 | 216 | 37 | 45.1 | 543 | 11.6 | 0.0 | 47.6 | 13.0 | 52.6 | 3.7 | 3.0 | 571 | 408 | 963 | 66.4 | 0.53 | 10.1 |

APPENDIX 10

DRIED GRASS PELLET INTAKE AND LIVEWEIGHT AT START (g/kg metabolic LW) (kg)

| | Silage Alone | | Standard | | High Starch | | High Fibre | | High Protein | | Bicarb | |
|-------------------|--------------|-------|----------|-------|-------------|-------|------------|-------|--------------|-------|--------|-------|
| | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd |
| Low pH silage LW | 95.5 | 16.11 | 87.2 | 6.79 | 95.4 | 4.94 | 94.7 | 14.66 | 98.9 | 9.09 | 96.1 | 8.48 |
| | 116 | 10.8 | 120 | 13.8 | 119 | 12.9 | 122 | 14.9 | 118 | 10.8 | 116 | 8.6 |
| Normal silage LW | 114.3 | 14.21 | 98.1 | 9.28 | 100.3 | 12.89 | 117.1 | 12.33 | 97.7 | 10.26 | 87.7 | 23.43 |
| | 112 | 7.7 | 112 | 9.5 | 112 | 8.9 | 111 | 7.0 | 110 | 8.0 | 113 | 7.1 |
| High DM silage LW | 87.1 | 23.77 | 94.5 | 15.49 | 103.6 | 5.37 | 97.0 | 15.56 | 98.9 | 9.9 | 98.3 | 18.52 |
| | 126 | 11.9 | 126 | 12.7 | 122 | 9.5 | 123 | 10.8 | 124 | 12.8 | 123 | 8.4 |
| Bad silage LW | 92.3 | 11.30 | 91.5 | 11.51 | 90.1 | 7.08 | 91.0 | 5.62 | 91.3 | 8.40 | 85.5 | 10.91 |
| | 125 | 10.9 | 127 | 11.4 | 129 | 15.1 | 131 | 13.2 | 127 | 10.9 | 125 | 9.8 |

APPENDIX 11

SILAGE INTAKE (kg DM/day)

| | Silage Alone | Standard | High Starch | High Fibre | High Protein | Bicarb | Mean | SED |
|------------------------|-----------------|----------|----------------|---------------|-----------------|--------|------|------|
| Low pH silage | 2.97 | 2.37 | 2.18 | 2.21 | 2.32 | 2.37 | 2.40 | 0.11 |
| Normal silage | 3.12 | 2.76 | 2.66 | 2.72 | 2.72 | 2.85 | 2.81 | 0.09 |
| High dry matter silage | 4.47 | 3.17 | 3.34 | 3.38 | 2.99 | 3.39 | 3.46 | 0.41 |
| Badly fermented silage | 3.44 | 2.71 | 2.71 | 2.89 | 2.75 | 2.78 | 2.88 | 0.16 |

SED and Significance of Effects

| | SED | Significance |
|------------------------------------|------|--------------|
| Silage (between trials) | 0.13 | p<0.001 |
| Silage alone v rest (concentrates) | 0.31 | p<0.001 |
| Within concentrate treatments | 0.30 | NS |

**TOTAL DRY MATTER INTAKE
(kg DM/day)**

| | Silage Alone | Standard | High Starch | High Fibre | High Protein | Bicarb | Mean | SED |
|------------------------|-----------------|----------|----------------|---------------|-----------------|--------|------|------|
| Low pH silage | 2.97 | 5.64 | 5.81 | 5.54 | 5.59 | 5.38 | 5.16 | 0.11 |
| Normal silage | 3.12 | 6.17 | 6.10 | 6.12 | 6.26 | 6.34 | 5.69 | 0.11 |
| High dry matter silage | 4.47 | 6.83 | 7.00 | 6.98 | 6.68 | 7.00 | 6.49 | 0.42 |
| Badly fermented silage | 3.44 | 6.56 | 6.58 | 6.74 | 6.69 | 6.63 | 6.11 | 0.18 |

SED and Significance of Effects

| | SED | Significance |
|-----------------------------------|------|--------------|
| Silage (between trials) | 0.20 | p<0.001 |
| Silage alone v rest (concentrate) | 0.37 | p<0.001 |
| Within concentrates treatments | 0.36 | NS |

SILAGE INTAKE
(g DM/kg LW)

| | Silage Alone | Standard | High Starch | High Fibre | High Protein | Bicarb | Mean | SED |
|------------------------|--------------|----------|-------------|------------|--------------|--------|------|------|
| Low pH silage | 19.4 | 15.1 | 14.4 | 14.6 | 14.7 | 16.1 | 16.1 | 0.08 |
| Normal silage | 21.3 | 17.1 | 16.4 | 17.2 | 16.3 | 17.9 | 17.7 | 0.10 |
| High dry matter silage | 27.0 | 19.4 | 19.4 | 19.9 | 17.5 | 20.6 | 20.6 | 0.21 |
| Badly fermented silage | 20.2 | 15.8 | 14.6 | 16.2 | 15.1 | 16.0 | 16.3 | 0.10 |

SED and Significance of Effects

| | SED | Significance |
|------------------------------------|------|--------------|
| Silage (between trials) | 0.09 | p<0.001 |
| Silage alone v rest (concentrates) | 0.18 | p<0.001 |
| Within concentrate treatments | 0.18 | NS |

**TOTAL DRY MATTER INTAKE
(g DM/kg LW)**

| | Silage Alone | Standard | High Starch | High Fibre | High Protein | Bicarb | Mean | SED |
|------------------------|-----------------|----------|----------------|---------------|-----------------|--------|------|------|
| Low pH silage | 19.4 | 41.5 | 42.8 | 41.5 | 42.9 | 40.5 | 38.1 | 0.08 |
| Normal silage | 21.3 | 44.7 | 44.4 | 45.0 | 44.7 | 46.3 | 40.4 | 0.11 |
| High dry matter silage | 27.0 | 47.0 | 48.6 | 49.7 | 46.9 | 49.0 | 44.7 | 0.21 |
| Badly fermented silage | 22.2 | 46.2 | 44.6 | 46.4 | 45.7 | 46.5 | 41.6 | 0.10 |

SED and Significance of Effects

| | SED | Significance |
|-----------------------------------|------|--------------|
| Silage (between trials) | 0.08 | p<0.001 |
| Silage alone v rest (concentrate) | 0.18 | p<0.001 |
| Within concentrates treatments | 0.17 | NS |

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