CALCIUM HYDROXIDE TREATMENT OF SPENT GRAINS FROM THE SCOTCH WHISKY INDUSTRY.

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ABREVIATIONS

ADAS	Agricultural Development and Advisory Service
ADF	Acid Detergent Fibre
ARA	Arabinose
BOD	Biochemical Oxygen Demand
CP	Crude Protein
CSFA	Calcium Soaps of Fatty Acids
CV	Coefficient of Variation
DE	Digestible Energy
DM	Dry Matter
DMI	Dry Matter Intake
ED	Energy Digestibility
FUC	Fucose
g	gramme
GAL	Galactose
GC	Gas Chromatography
GE	Gross Energy
GLC	Glucose
H	Hours
ISOMD	In Sacco Organic Matter Digestibility
ISNDFD	In Sacco NDF Digestibility
IVOMD	In Vitro Organic Matter Digestibility
k_f	Efficiency of Utilisation of ME for Fattening or Gain
kg	Kilogramme
LWG	Liveweight Gain
MAFF	Ministry of Agriculture, Fisheries and Food
MAN	Mannose
MDG	Malt Distiller's Grains
ME	Metabolisable Energy
mg	milligramme
mmol	millimole
MJ	Megajoule
MSBS	Molassed Sugar Beet Shreds
N	Nitrogen
NCD	Neutral Detergent/Cellulase Digestibility
ND	Nitrogen Digestibility

NDF	Neutral Detergent Fibre
NDF	Neutral DEtergent Fibre Digestibility
NH_3-N	Ammonia Nitrogen
NID	Nutritionally Improved Draff
NOSCA	North of Scotland College of Agriculture
OM	Organic Matter
OMD	Organic Matter Digesibility
PAS	Pot Ale Syrup
RHA	Rhamnose
SAC	Scottish Agricultural College
SD	Standard Deviation
SE	Standard Error
SED	Standard Error of Difference
TMD	$Ca(OH)_2$-Treated Maize Draff
TP	True Protein
UMD	Untreated Maize Draff
UV	Ultra Violet
VFA	Volatile Fatty Acid
WSC	Water Soluble Carbohydrate
XYL	Xylose

SUMMARY

The literature in the following areas is reviewed:

a) The nutritional characteristics of spent grains from grain and malt whisky distillation and their use in ruminant diets.

b) The effects of fat in ruminant diets.

c) The degradation of fibre in the rumen.

d) The effects of alkali treatment on the chemical structure and digestibility of fibre.

A series of small experiments were carried out to investigate the effects of alkali treatment on malt distillers spent grains. Parameters used as indications of increases in nutritive value included *in vitro* organic matter digestibility (IVOMD) and *in sacco* digestibility of organic matter (ISOMD) and neutral detergent fibre (ISNDFD). Treatment with 80 g NaOH/kg DM at 60°C increased the IVOMD of spent grains from 0.37 to 0.50. It was shown that substitution of NaOH with $Ca(OH)_2$ produced greater increases in IVOMD.

$Ca(OH)_2$-treatment of spent grains reduced the NDF content and increased the, OM the digestibility *in sacco* and *in vitro* was increased from 0.63 to 0.84 and from 0.51 to 0.65 respectively. These effects were thought to be due to the upgrading of the fibre fraction of the spent grains and to the formation of insoluble soaps of fatty acids reducing the inhibitory effects of the unsaturated oil on rumen micro-organisms. Soap formation was measured as approximately 60 % of the total oil in the grains. The effects of different treatment conditions (treatment time, temperature and level of $Ca(OH)_2$ application) for $Ca(OH)_2$-treatment of spent grains were investigated. Optimal conditions were found to be 80 g $Ca(OH)_2$/kg DM 60°C overnight (18 hours). These treatment conditions were adopted as the standard conditions for $Ca(OH)_2$-treatment to prepare nutritionally improved draff (NID).

The effects of $Ca(OH)_2$ treatment of spent grains on their digestion in the rumen were investigated further using an *in vitro* method which measured the volumes of gas produced over six hours from rumen liquor incubated with quantities of the feed. The rate of gas production was increased with $Ca(OH)_2$ treatment from 31 to 45 mls/ 6 hours. This increase was proportionately substantially less than the increase in 0 time ISOMD. The discrepancy in measurements of initial digestion rate between the two techniques may be due to increased leakage of small particles from the nylon bags with $Ca(OH)_2$-treatment. Rate of gas production from $Ca(OH)_2$-treated grains were directly related to the length of time for which the grains had been held at 60^oC. It was suggested that this was due to the slow liberation of monosaccharide units from the ends of cell wall polysaccharides by the action of alkali.

The effect of calcium soap formation on the digestibility of spent grains was investigated further *in vitro*. Oil was extracted from the spent grains and treated with NaOH and $Ca(OH)_2$. The extracted oil-free residue and the alkali-treated draff oil were then incubated *in vitro*. Reaction with $Ca(OH)_2$ reduced the inhibitory effect of draff oil on IVOMD whilst it was increased by NaOH.

The *in vivo* digestibility of NID fed to sheep as the sole diet was measured. OM, NDF, gross energy (GE) and crude protein (CP) digestibilities were found to be 0.642, 0.732, 0.666 and 0.560 respectively, significantly higher than measured in untreated grains by other workers.

The effects of $Ca(OH)_2$-treatment of spent grains on animal production were measured in three groups of 10 finishing lambs. In this experiment NID was compared to mineralised draff or a proprietary concentrate as supplements when fed with hay. A concurrent experiment measuring the *in vivo* digestibility when fed with hay was carried out and showed that digestibility was increased with $Ca(OH)_2$-treatment. OM, GE and NDF digestibilities were increased from 0.53, 0.58 and 0.55 respectively for mineralised draff to 0.67, 0.65 and 0.79 for NID. The *in sacco* degradation of OM, N, and NDF of each feed was also measured.

In sacco measurements showed that NID degradation in the rumen was akin to the proprietary concentrate, whereas the mineralised draff degradation was more akin to the hay.

Lambs offered the proprietary concentrate or NID ate significantly more supplement and hay than those offered mineralised draff. The former groups also had higher liveweight gains and significantly more lambs were ready for slaughter on day 60 of the experiment. Samples of the adipose tissue from the carcasses were taken and analysis of the fatty acid profile showed an increase in unsaturated fatty acids in NID fed lambs.

It was observed that NID had enhanced water-holding capacity compared to untreated dried draff and a small experiment was conducted to investigate the use of NID as a silage effluent absorbent. It was found not to be suitable for this purpose since its enhanced absorptive properties were lost after 48 hours under the fermentative conditions of grass silage, and because it buffered strongly at around pH 5.5 resulting in the production of butyric silages.

A larger experiment was conducted using untreated maize draff as an effluent absorbent in grass silage. Compared to the control silages produced without absorbents, grass ensiled with 80 kg dried maize draff per tonne of grass FW produced significantly less effluent (134 vs. 29 kg/t grass) resulting in significantly lower organic matter effluent losses from the silo (6.49 vs. 1.78 % grass OM).

An experiment was conducted in which these silages were fed to growing bulls. Four groups of 5 bulls received either the control silage with proprietary concentrate, $Ca(OH)_2$-treated maize draff or untreated maize draff, or the absorbent silage with proprietary concentrate. This experiment showed that bulls offered silage containing maize draff as an absorbent ate more and grew faster than those fed the other diets. An *in sacco* study of the rumen degradation of these feeds showed that the response of the grains to $Ca(OH)_2$-treatment was different for maize than for barley draff and that the increase in ISOMD over short periods of time was lost after 48 hours incubation.

The absorptive properties of NID were combined with the ability of $Ca(OH)_2$ to form calcium soaps by producing oil-supplemented NID. It was shown *in vitro* that this product would act as a carrier for the oil and reduce the inhibitory effect of oil on microbial function. An experiment in which oil-supplemented products were fed to fistulated sheep showed that this was a potential method for including high levels of oil in ruminant diets without disrupting rumen function. In a further experiment in which these products were fed to lactating goats, showed that, although the oil supplemented NID could increase milk-fat levels under conditions of low-milk-fat syndrome, very little of the unsaturated oil escaped ruminal biohydrogenation.

More detailed studies of the effects of $Ca(OH)_2$ treatment on the carbohydrate fraction of spent grains were carried out. These included measurements of the monosaccharide composition and the lignin content of the whole grains and the soluble and insoluble fractions of the grains. The polysaccharides present in the soluble fraction of NID were fractionated and two arabinoxylans were identified, one with a particularly high arabinose to xylose ratio (2.71) suggesting a highly substituted or branched xylan backbone.

In the penultimate chapter the long term storage characteristics of $Ca(OH)_2$-treated spent grains were investigated. Two experiments showed that at high levels of $Ca(OH)_2$ (75 - 100 g $Ca(OH)_2$/kg DM) spent grains could be stored over a period of 2 months. However, at 50 g $Ca(OH)_2$/kg DM mould growth was enhanced. It was concluded that $Ca(OH)_2$-treated grains were unsuitable for ensilage (ie. storage at a low pH produced by fermentation) but that alkaline storage may be feasible.

Finally the findings of the project are discussed with particular reference to the commercial viability of the process at distilleries.

CHAPTER ONE

LITERATURE REVIEW

1.1 The Production of Spent Grains

There is no documentation of when the art of beer or wine production was first discovered. It is thought that it was developed between 5000 and 8000 years ago and that there were several independent discoveries. The earliest evidence is provided by the ancient Egyptians whose illustrations detail the brewing procedure. The discovery of distillation seems to have occurred at a later date, again in more than one civilisation. The earliest references to alcohol distillation are for the production of Arak from sugar cane. The earliest reference to distilling whisky is in the Scottish Exchequer Rolls of 1494, (cited by Black *et al.*, 1984) however, long before this the Celts were producing potable spirits which they called 'Uisge Beatha' or 'Uisge Baugh' which translates as 'the water of life'.

In the Middle Ages brewing was considered to be a mystery, the details of which were closely guarded by the master brewers and their guilds. At this time, although the details of the brewing and distilling procedures were well documented, there was no explanation as to how or why it happened. The same was true for the production of malt. Whilst the conditions necessary to produce a softer, sweetened grain suitable for fermentation were known, there was no underlying science behind the skill. It was recognised that the production of malt for brewing or for whisky making required cool conditions restricting malting to temperate regions during the autumn, winter and spring time.

At the turn of the century the brewing and distilling processes were industrialised in common with other industries and the scale of production and the technology surrounding beer and spirit production increased dramatically. The malt whisky industry on the other hand

was, and still is, resistant to change and has maintained the traditional ways of producing whisky. Consequently, malt whisky is still produced on a relatively small scale and commands a premium in the shops over the blended whiskys and other spirits.

The underlying mechanism for alcohol production depends on the fermentative action of yeast on a substrate of simple sugars to produce ethanol and carbon dioxide. The fermentable substrate can be provided directly from sucrose-rich sources such as sugar cane, or more commonly, indirectly, from starchy feedstuffs such as cereal grains or potatoes. If starch is to provide the carbohydrate for fermentation, it must first be converted to its component monosaccharide glucose. This is achieved by physical processing, usually some form of coarse milling, followed by cooking to disrupt the starch grains and to facilitate the enzymic hydrolysis of the glycosidic bonds. Subsequent exposure to amylase enzymes which degrade the starch polysaccharides to component monosaccharides which are a source of fermentation substrate for the yeast.

It is the main by-product of the whisky making process, the spent grains (or draff), which are sold as a ruminant feed, and the nutritional characteristics of which are the subject of this thesis. In order to give an appreciation of how these characteristics arise the malting and mashing procedures are described in outline.

Malting

Malt is cereal grain which has been allowed to germinate and then dried. Malting can be carried out on any cereal grain but is most successful with barley. Malting wheat is not so successful as barley because of problems of microbial growth on the surface of the grains during germination. Maize is rarely malted because its fat becomes rancid. In some African countries the indigenous grains are used, in particular sorghum, to produce native beers. In the UK the term 'malt' generally refers to malted barley and it is this that is used as the source of fermentation substrate in malt whisky production.

The malting process can be broken down into three main steps; steeping, germination and kilning or drying.

Steeping

During steeping the barley grains are immersed in water at 15°C. Air is perfused through the steep water to maintain aerobic conditions as respiration by the barley embryos increases. The water is drained off after approximately 12 hours and the grains are allowed to 'air rest'. After a few hours, the grains are reimmersed and the alternation of steeping and resting is continued until the grains have reached 42% moisture, usually after about 2 days. Losses due to steeping vary in composition but generally amount to about 1% of the grain dry matter. This is composed of extraneous matter washed off the grain and any soluble constituents present (Miller, 1969).

Germination

Traditionally, steeped grain is spread to a depth of 25cm on a malting floor, kept moist by sprinkling with water and aerated by turning with a wooden malt shovel. The temperature must be maintained at 15°C, thus air conditioning was a prerequisite for summer malting. Using a floor-malting system such as this, the germination would be stopped after about 6 days. In more modern, automated systems the grains are held at a depth of about 1.5m and water-saturated air is blown through the grains. Matting, caused by tangling of the barley rootlets, is prevented by mechanical turning. Germination is terminated after 3-4 days at a time decided by the maltster, depending on what the malt is to be used for. Well modified malt is required for distilling purposes and less modified malt for lager malt. Respiratory losses incurred during this stage of the process can amount to up to 5% of the grain DM. This represents loss of soluble carbohydrate.

After the malt has been dried, the barley rootlets are removed since they are rich in asparagine which imparts a bitter flavour. These are sold as an animal feed more commonly known as 'Malt Culms'. Malt Culms

represent a further 4% loss of DM. McDonald *et al.* (1981) published the following analysis of the nutritive value of malt culms (g/kg DM):

Crude Protein	271
Crude Fibre	156
Ether Extract	22
Ash	80
DCP	222

Kilning

Kilning is carried out to preserve the malt, and in some cases, to impart the characteristic properties of flavour, colour and friability. A long, cool drying period gives a pale malt of high enzymic activity whereas more rapid, hot drying produces a dark malt of low enzymic activity. By varying the drying conditions the maltster may alter the enzymic characteristics of the malt, however it is the initial stages of drying that are critical. As the malt dries out the heat stability of the enzymes increases so that the final air temperature for drying may be as high as 100°C.

The malt thus provides a source of enzymes to complete the hydrolysis of the starch and fermentable carbohydrate which the yeast can metabolise to alcohol. In order to reactivate the amylase enzymes and complete the hydrolysis of the starch the malt is 'mashed' with hot water.

Mashing

The next stage in the alcohol production process is the 'mashing' of the grains which entails immersing the coarsely milled grains, or grit, as it is now referred to, in hot water. The objective is to produce a 14 to 20 percent sugar solution for fermentation to ethanol. Wetting the malt reacitvates the enzymes thus allowing further degradation of the partially hydrolysed starch. At this point various other sources of starch may be added such as maize rice or wheat. If such adjuncts are included in the mash then the starch must be

available for enzymic breakdown which means that the grain must be processed before being added to the mash tun. Commonly this is done by coarse milling followed by some form of cooking to gelatinise the starch and make it readily available for enzymic attack. Flaking and micronisation of cereal grains will also gelatinise the starch. The proportion of the mash made up by cereal other than barley can be up to 85% in the case of grain whisky whilst malt whiskys are produced entirely from barley. In the brewing industry cereal adjuncts may make up to 50% of the mash depending on the mashing system and the end product required, although the proportion is usually limited to 10%.

When hydrolysis is complete, the liquid phase (or wort) containing the free sugars is drained off whilst the spent grains are retained by the mesh floor of the mash tun. The wort is then pumped to the fermentation vessel or 'washback'. The spent grains are removed from the mash tun. Spent grains are either sold wet directly onto farms, or as a dried product. In the latter case the feed is usually described as a dark grain because of the addition of other waste materials from the fermentation and distilling processes (pot ale syrup or spent wash) to the spent grains before they are dried.

Wort may be produced by the action of an industrial enzyme (bacterial amylase) on a starch source which can then be fermented and distilled in the same way as whisky. Alcohol produced in this way is known as rectified spirit and is used as a constituent of spirits other than malt whisky.

When maize is used for alcohol production there is a much more sophisticated separation of the component fractions of the grain than just described for the malting and mashing of barley grain. The extractions are carried out according to precisely defined conditions giving by-products that vary very little in terms of the final proximate analysis whereas spent grains are a variable commodity, their composition depending on the processing procedures (DeBecze, 1949) and the composition of the original feedstock (Carpenter, 1970).

1.2 Nutritional Characteristics of Spent Grains

The composition of the spent grain reflects that of the original feedstock, however since most of the starch is hydrolysed to fermentable sugars, this and any other soluble materials are washed out in the mash tun leaving the oil, fibre and much of the protein concentrated in the spent grain. The effect of malting and mashing on barley is shown the Table 1.1 (Miller, 1969) It shows the proximate constituents in 100g of barley and the corresponding values from the spent grains.

Table 1.1 Comparative Analyses of Barley and Malt Distillers Grains

	Barley (g)	Malt Distillers Grains (g)
Dry Matter	100	21.3
Crude Protein	9.9	4.2
Crude Fibre	5.3	3.7
Ether Extract	1.8	1.6
Ash	3.1	0.7
Nitrogen-Free Extract	79.9	11.0

Thus malt distillers spent grains are a wet feed, which retain practically all of the ether extract and a sizeable proportion of the fibre and protein present in the parent barley grain. All water soluble fractions, particularly the minerals and the water soluble carbohydrate are present in low quantities. The proximate analyses of spent grain vary widely according to the composition of the barley grain and to the malting and mashing procedures which it has undergone.

The proximate analysis of grain and malt distillers grains have been measured at the North of Scotland College of Agriculture (1984).

Table 1.2 Dry Matter Content and Analysis of Distillery By-Products (g/kg DM unless stated)

	Grain Distillers Grains	Malt Distillers Grains
Dry Matter (g/kg)	275	258
Crude Protein	236	198
Ether Extract	90	82
Crude Fibre	170	173
Ash	28	33
Metabolisable Energy (MJ/kg/DM)	11.3	10.8

The Energy Content of Spent Grains

The concentration of the energy-rich fractions of barley in spent grains, particularly the oil, results in an increase in the gross energy content of the barley grain from a typical value of 18.4 MJ/kg DM in the parent grain, to reported values of 21.5 in the spent grain (MAFF, 1990) However, despite the increase in gross energy content, the metabolisable energy value is lower than that of the original barley, largely because the organic matter digestibility of spent grains is much lower than that of the whole grain. A typical ME value for barley is 13.3 MJ/kg DM, published values for the ME of spent grains vary widely from study to study and according to the type of grain. An average value of 10.2 MJ/kg DM is given (MAFF, 1990).

The variability of the published ME values results from differences in the technique for measuring the OMD and from differences in the oil content of the spent grains. Wainman *et al.* (1984) reported different ME values for comparable samples from the same source as those analysed by ADAS (1982). The differences were accounted for by differences in the methane energy loss and by differences in

measurements of other volatile components. Wainman *et al* measured the methane energy loss to be 0.04 of the GE whereas ADAS used the Blaxter and Clapperton equation (1965) which predicts methane energy loss to be 0.08 of the GE.

El Hag and Miller (1969) measured organic matter digestibility (OMD) under a range of conditions and found the value to vary from 0.52 to 0.65 depending on procedure used (*in vitro* or *in vivo*) and on the inclusion of soluble calcium salts. ADAS (1982) showed that there was a poor correlation between ME and *in vitro* DOMD and that including the ether extract in the correlation improved the accountable variance, suggesting that there is an interaction between ether extract and *in vitro* digestibility. Further studies on distillers grains by El Hag and Miller (1972) showed the sensitivity of the *in vitro* procedure to the nature and quantity of the oil present and to the presence or absence of calcium ions.

OMD measured *in vivo* is similarly affected by the presence or absence of divalent ions in the diet. Miller *et al* (1970) reported that the digestibility of malt distillers grains (MDG) was increased by the addition of calcium chloride and calcium acetate. Lewis and Lowman (1987) showed increases in *ad libitum* intake in steers fed solely on draff if the level of mineral (in particular magnesium) addition was increased. In the same study, Lewis and Lowman reported that offering draff *ad libitum* resulted in significant depressions in OMD when compared to that for maintenance levels of feeding.

When the ME of draff is measured under a standard set of conditions, the variability of the draff is much less than reported in the literature. ADAS (1982) reported a series of trials carried out since 1977 in which the nutritional value of 24 samples of brewers grains was measured. It was concluded that the differences in nutritional value were due to variation in the original feedstock and to differences in the mashing procedure. The mean ME (MJ/kg DM) value for the spent grains was 10.4 (SD 0.5) with a coefficient of variation (CV) of 4.8%. The mean ME for barley is 13.3 (SD 0.5), giving a CV of 3.8% (MAFF, 1990). From this it is apparent that the spent grains

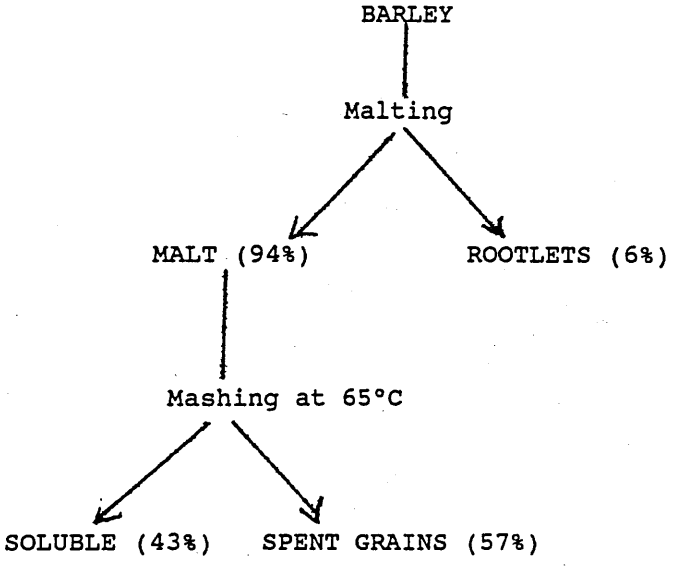
show similar variability to the feedstock barley.

From this same study the CV for the ether extract value for spent grains is 16.9%. This large variation may be due to the concentration effect of mashing magnifying the relatively small differences in the ether extract of the feedstock barley and to differences in the proportion of oil retained in the spent grains after the mashing process. Variation in the oil content of spent grains is a major cause of the variation in the published values of ME. Oil content is the main determinant of the GE of the grains and it has a large influence on the OMD. The relationship between OMD and the oil content of spent grains is complex, depending on experimental factors such as level of feeding and divalent cation concentration in the diet.

The Protein Content of Spent Grains

The nitrogenous constituents of spent grains are those which are less soluble in water, the soluble nitrogen having been washed out in the mash tun. The partition of protein from barley during malting and mashing is given in Figure 1.1, showing data published by Hough (1985)

Figure 1.1 The Partition of Protein During Malting and Mashing



Reported values for the crude protein (CP) content of spent grains are variable because of differences in malting and mashing procedures, and differences in the original cereal grain. Values range from 191 g/kg DM (Miller, 1969) to 297 g/kg DM (National Research Council, 1981). Another source of variation in the CP content of by-products is the addition to the spent grains of other materials Pot Ale Syrup (PAS) or spent wash. The apparent digestibility of the CP of spent grains is much less variable as shown in Table 1.3. Adopting a mean figure of 0.74 would give a good estimate in most cases.

Table 1.3. The Apparent Crude Protein Digestibility of Barley Spent Grains

Reference	CP Digestibility
1908, Kellner	0.73
1955, Dijkstra	0.78
1958, Dijkstra	0.71
1982, ADAS	0.75 (Range 0.64-0.85)
1984, Wainman *et al*	0.75
1984, Wainman *et al*	0.73
1990, MAFF	0.74

Very little of the spent grain protein is soluble, the water soluble fraction having been removed by mashing. The low solubility of spent grain protein was suggested by Crowe *et al.* (1985) to be due to association between the protein and the cellulosic material. Wall *et al.* (1975) reported reduced protein solubility in various solvents when grain had been heated at high temperatures, suggesting that disulphide interactions or covalent bond formation could occur under these conditions. Deltoro and Martinez (1981) investigated the effects of heating brewer's dried grains and concluded that unless the temperature was raised above 120-130°C there was no deleterious effect on the composition of the grains. Since the temperature during mashing does not reach this temperature it is unlikely that the

protein in the spent grains becomes more tightly bound to the cell wall material.

The rumen degradability of draff protein is probably of greater significance in ruminant nutrition. Much of the degradability work has been carried out in the USA where workers such as Klopfenstein and Stock, (1981); Rogers *et al.*, (1986) and Waller *et al.*, (1980) have measured a high protein by-pass value for grain distillers grains. Draff degradability studies for the UK. are few and measurements show considerable variation ranging from 0.54 (Topps, personal communication) to 0.85 (Offer, personal communication) assuming a small particle outflow rate of 0.05 (/hr). The wide variability of degradability values reported reflect differences in processing conditions, in the methods of estimation and their interaction.

The Lipid Content of Spent Grains

The lipid present in barley is concentrated by the mashing process from ether extract values of 16.2 g/kg DM in the parent grain to about 86.0 g/kg DM (range 70 - 118) (MAFF, 1990). The lipid in barley is mainly present in the aleurone layer. Very little of the lipid present in the original barley grain is lost in malting or mashing, although MacLeod and White (1967) have shown that germination under some conditions can cause lipolysis and depletion of fat. Miller (1969) measured approximately 90% of the lipid in barley remaining present in the spent grains, the other 10% being lost during germination and mashing and in the malt culms. Brewers grains tend to have lower lipid contents than the malt distillers grains whilst grain distillers grains are generally higher, ADAS (1982) figures are 77, 86 and 90 g/kg DM respectively.

The concentration of lipid in the spent grains has important effects on its nutritive value in ruminant diets. Palmquist and Jenkins (1984), in a review of fat in lactation rations for cows, noted that fat may be added to diets at 3% of the total ration DM without qualification, but that levels of up to 5% may be beneficial for

highly productive cattle. The effects of fat in ruminant nutrition have been widely researched and will be discussed later in greater detail.

The fatty acid profile of barley and spent grains are very similar and contain a high proportion of unsaturated fatty acids. El Hag and Miller (1972) measured 32.2% saturated and 67.8% unsaturated fatty acids in lipids extracted with 60-80°C petroleum ether. Hyslop (unpublished data) measured a similar profile as shown in Table 1.4.

Table 1.4 Fatty Acid Content of Draff Oil

Fatty Acid	g/kg Fat
C16.0	225.5
C16.1	14.2
C18.0	17.2
C18.1	214.8
C18.2	485.5
C18.3	42.8

The Carbohydrate and Fibre Content of Spent Grains

The carbohydrate constituents of barley, particularly the starch, undergo extensive changes during the production and processing of malt. During the germination phase of malting, the action of gibberellic acid on the aleurone layer causes *de novo* synthesis and secretion of hydrolytic enzymes such as α-amylase. ß-amylase is present in the ungerminated grain in an inactive form. During malting, only approximately 15-18% of the endosperm starch is solubilised, 10-12% is used by the embryo for respiration, and the remainder is converted to simple sugars. The majority of the solubilisation occurs in the mash tun, at higher temperatures where enzymic activity is optimal. The mashing procedure removes about 75 -

80% of the soluble carbohydrate which constitutes approximately half of the dry matter present in the original barley grain (Miller, 1969).

The effect of malting on the barley husk was investigated by Aspinall and Ferrier (1957) who reported that the hemicellulose in barley husk are not modified during malting. However, later studies by Woolard *et al.* (1977) in which free sugars, water-soluble gums and hemicelluloses were isolated, showed that all polysaccharides are modified to varying degrees during malting. They reported that, of the structural carbohydrates, it was the hemicellulose A fraction which undergoes most extensive modifications during malting whilst the hemicellulose B fraction remained unaffected.

In the mash tun considerable amylase activity occurs at the higher temperature (65°C) degrading the starch and some of the pentosans and ß-glucans present in the cell walls. As the temperature is raised still further, all solubilised material dissolves and is extracted leaving the spent grains with the less soluble, more fibrous, constituents. Any soluble carbohydrates remaining in the grains are quickly fermented on storage resulting in slight increases in the structural carbohydrate and the production of lactic acid and volatile fatty acids as the end products of fermentation.

Changes in the carbohydrate major fractions are shown in Table 1.5 (MAFF, 1990).

Table 1.5 Composition of Carbohydrate and Lignin of Barley and Spent Grains

	Feed Carbohydrate Composition (g/kg DM)				
	WSC	Starch	NDF	ADF	Lignin
Barley	37	562	201	64	17
Brewers Grains	13	38	618	263	86
Distillers Grains	5	18	673	294	63

The changes in the composition of the carbohydrate and the increase in the proportion of lignin in the dry matter result in the crude fibre in barley increasing from 50 g/kg DM to 199 g/kg DM for malt distillers grains (MAFF, 1986).

Hyslop (unpublished data) reported that the coefficient of digestibility of the NDF fraction of spent grains was 0.48. The digestibility of the crude fibre in spent grains is relatively low. ADAS (1982) reported a crude fibre digestibility coefficient for brewers grains of 0.44. This is similar to an earlier value reported by Kellner (1908), of 0.40. The ADAS value of 0.44 is the mean of 24 measurements of CF digestibility which ranged from 0.12 to 0.57, the wide range of values possibly reflecting the interaction between lipid content and fibre digestibility.

The fibre digestibility of spent grains is slightly lower than the value reported for cereal straw. ADAS (1988) gave a value of 0.49 for the CF digestibility coefficient of untreated straw compared to 0.75 for alkali treated straw. These values are means of 10 samples including wheat, barley and oat straws. For comparison, the CF digestibility of another fibrous by-product, molassed sugar beet pulp, was reported by ADAS (1982) as 0.90. Thus the fibre digestibility in distillery spent grains is low and similar to that of untreated cereal straw.

The Minerals and Vitamins in Spent Grains

As may be seen from Table 1.1, only about 23% of the ash in barley grains is present in the spent grain after malting and mashing. The mashing procedure removes virtually all water-soluble constituents of the grain including trace elements. Miller (1969) noted that spent grains had low levels of sodium and potassium and that the calcium to phosphorus ratio may be as low as 0.3-0.5.

Watson (1957) recognised the need for mineral supplementation of ruminant diets if spent grains are to be included. Other workers including Black *et al.* (1984) have also identified the need for

mineral supplementation on the basis of compositional data shown in Table 1.6. Lewis and Lowman (1987) reported the low levels of some trace elements and vitamins and designed a complex mineral supplement containing calcium, phosphorus, magnesium, sodium, potassium, trace elements and vitamins when spent grains were used as the sole diet for finishing steers.

Table 1.6 The Mineral Content of Spent Grains

Mineral (g/kg DM)	Malt Distillers Grains	Grain Distillers Grains	Brewers Grains
Calcium	1.7	0.9	3.3
Phosphorus	3.7	2.1	4.1
Magnesium	1.4	0.6	1.5
Sodium	0.9	0.5	0.1
Potassium	0.4	0.2	0.1
Copper mg/kg DM	10	12	19
Manganese mg/kg DM	43	-	31
Zinc mg/kg DM	-	-	73
Cobalt mg/kg DM	0.02	0.04	-
Selenium mg/kg DM	0.02	0.03	-
Total Ash	33	28	42

Black *et al* (1984) and Wainman *et al* (1984).

The mashing process is essentially a water extraction of malted barley or saccharifed cereal grains. It leaves a feedstuff which is low in water-soluble constituents such as minerals and readily fermentable carbohydrate and high in protein, oil and fibre.

The mineral and vitamin content of the grains is low and requires supplementation if they are to be included in large proportions in a ration. Watson (1957) first noted the importance of mineralisation, a finding supported by a more recent experiment carried out by Lewis and

Lowman (1987). It is proposed that the minerals, especially the divalent cations, interact with the oil and influence the digestibility of the fibre fraction.

The low content of readily fermentable carbohydrate also affects the feeding value of the spent grains to ruminants. Fermentable carbohydrate is required by the rumen micro-organisms for microbial synthesis and replication. Feedstuffs which are low in fermentable carbohydrate have low rates of microbial synthesis and a relatively high acetate to propionate ratio, for example, Ac>68% (Thomas and Rook, 1981; Van Soest, 1982).

The remaining constituents are concentrated by the extraction, which equally affects the nutritional value of the grains. The lipid content in spent grains is relatively high giving the feed grains a high gross energy content. The high oil content combined with low levels of fermentable carbohydrate and low digestibility of the fibre make spent grains an unusual ruminant feedstuff because a large proportion of the ME is supplied as lipid. Lewis and Lowman (1987) estimated that this ranged from 0.30 to 0.37 depending on the level of feeding.

The high oil content also influences the digestibility of fibre, both the endogenous fibre and that supplied in other components of the ration, particularly the forage. The interactions between oil, fibre and calcium are not as yet well understood and will be reviewed later (section 1.4). The unsaturated nature of the fatty acids exacerbates this effect. El Hag and Miller (1972) reported that the unsaturated fatty acid are more effective in inhibiting fibre digestion than those which are saturated.

The high lipid content of spent grains limits their use to ruminant livestock, however, it is the high levels of poorly digested fibre which limit their inclusion in monogastric diets since monogastrics have only a limited capacity for fibre digestion. Their use in ruminant diets is also limited as the relatively high fibre content of low digestibility means that their ME is only moderate despite their

high gross energy content. Therefore, in more extensive systems, where moderate performance is acceptable, spent grains may be used extensively. In intensive enterprises, where high growth rates or milk yields are required, their use is more limited.

1.3 The Use of Spent Grains in Animal Feeding

Monogastric Livestock

Monogastric livestock have a limited ability for fibre digestion and, since spent grains have relatively high levels of low digestibility fibre, their use in the diets of pigs or poultry is limited. Their presence in the diet reduces the digestible energy concentration of the diet and may also reduce the digestibility of other dietary constituents such as protein (NOSCA, 1969). This makes spent grains unsuitable for use in poultry and growing and fattening pig diets where appetite relative to requirement is low. However in dry sow diets, where level of feeding is restricted to below appetite, spent grains may be included in the diet provided that the protein requirements are met.

Both malt and grain distillers dark grains have been considered as alternative protein sources in monogastric diets. However the product still has too high a fibre content for it to be the major component of a monogastric diet. The quality of the protein in dark grains is poor and if they are to be used in pig or poultry diets then a high quality protein source should be included if production is to be maintained. Harmon (1974) carried out an investigation into the availability of lysine and tryptophan in dark grains for pigs. It was noted that the tryptophan was biologically available to young pigs but the lysine was not utilised as efficiently as lysine-HCl. Heavier pigs were able to use lysine in the grains more effectively. This supports the limited use of dark grains in dry sow diets. However, in the diets of growing pigs, NOSCA (1984) advise that spent grains should not be included at levels greater than 15% of the diet DM as significant deterioration in growth and feed conversion efficiency occurs above this level.

Similarly, spent grains find only limited use in poultry diets. Jensen (1978) reported that laying hens could be fed up to 20% of the diet dry matter as dark grains without causing adverse effects on performance characteristics. Vandepopuliere *et al.* (1978) reported an experiment in which laying hens were fed a diet containing 43.9% distillers dried grains. They found that the feed conversion efficiency and egg size were reduced, and concluded that this reflected the lower dietary metabolisable energy content of the spent grains.

Ruminant Livestock

Research into the effects of feeding spent grains to ruminant livestock has focused on the animal production aspects rather than on detailed measurement of their digestion and metabolism. However, since the former reflect the latter, observations on production can give valuable clues as to the rumen metabolism and digestibility characteristics of spent grains.

The response of livestock to the dietary inclusion of spent grains reflects the nutritional characteristics of these feeds. Particularly significant in this respect is their high content of fibre, oil and protein which result from the mashing process and their interaction.

Much of the research into assessing the protein value of spent grains has been carried out in the USA. In these experiments spent grains have usually been used to replace conventional protein sources such as soya and has led to the classification of spent grains as a protein source. This conclusion unnecessarily limits the potential use of spent grains in animal feeding. Many experiments have shown the grains to be a valuable source of both energy and protein and the possibility of using them as a sole diet has yet to be fully exploited.

Spent Grains in Dairy Cow Diets

Some of the earliest reports of experiments investigating the effects of feeding spent grains to dairy cows date from the late 1950s. In these early experiments draff was used as a replacement for forage. In 1957 Watson reported that 2.3 kg (DM) of wet distillers grains could replace 1.8 kg of purchased concentrate, provided a mineral supplement was added. Verbeek *et al.* (1958) found that up to 14 kg/d of wet brewers grains could replace silage in dairy cow rations. Maoli and Mazziotti (1960) noted that feeding 10 kg/d of spent grains to dairy cows could increase milk yield by 5% and reduce the amount of forage needed.

More recently draff has been investigated as a replacement for the concentrate part of the diet. The effects on milk yield and milk solids production of feeding draff in this manner have been variable. Schingoethe *et al.* (1983) reported an experiment in which 8 Holstein/Friesian cows fed a basal diet of 3.2 kg/d lucerne hay and *ad libitum* maize silage were offered 13.6 kg/d of corn distillers grains and a reduced amount of the concentrate of lower CP content. Silage intakes were depressed at high levels of inclusion. In this way draff was replacing both the concentrate and the forage components of the diet. Dry matter intake, milk production and rumen ammonia levels were unaffected. This showed that spent grains could be used both as a protein and an energy source.

An experiment was reported by Polan *et al.* (1985), which emphasised the value of draff protein in dairy cow diets. In this experiment wet and dried brewers grains were compared to soya bean meal (SBM). The three feeds were fed at three different levels to supply 14.5, 16 or 17.5% CP. The results showed that milk yield increased with level of CP regardless of source. Both wet and dried brewers grains increased dry matter intake and milk yield compared to SBM. Rumen ammonia and plasma urea measurements showed that the protein in spent grains was less rumen degradable and more efficiently utilised than that in soya bean meal.

On the contrary, other authors have noted reduced milk yields associated with feeding draff. Davis *et al.* (1983) fed pressed brewer's grains with maize silage as a complete diet to 20 lactating Holsteins. The spent grains replaced concentrates to make up 0, 20, 30 and 40% total ration DM. Dry matter intakes, milk yield and fat and protein contents were reduced as spent grain inclusion was increased from 0 to 40% .

Reduced dry matter intakes have been associated with feeding spent grains particularly at higher levels of inclusion (Valentine and Wickes, 1982; Johnson *et al.*, 1987; Hyslop and Roberts, 1989). Hyslop and Roberts (1988) suggested that malt distillers grains should be limited to 15% of the diet DM when used to replace proprietary pelleted concentrates in dairy cow diets if deleterious effects on DM intake were to be avoided. In a further experiment (Hyslop and Roberts, 1989) they reported that malt distillers grains could be included at levels of up to 30% (DM basis) if they were fed as part of a complete diet. In the same experiment when spent grains were offered at higher levels of inclusion, dry matter intakes were reduced but milk production was maintained, resulting in an increased utilisation of ME.

Improvements in feed conversion efficiency (FCE) were also observed by Johnson and Huber (1986), when ammonia treated distillers grains were included at 14% of the diet DM as a source of protein. A further study with ammonia treated distillers grains (Johnson *et al.*, 1987) showed similar increases in FCE. In this experiment the grains constituted 15 or 26% of the diet and were compared to soya bean meal. The results showed that the spent grains depressed dry matter intake without affecting milk yield or composition.

Other reported effects of including spent grains in dairy cow rations were associated with herd fertility. A study carried out at the Lancaster College of Agriculture (Anon, Farmer's Weekly 1989) showed that feeding brewers grains may have deleterious effects on dairy cow fertility. Cattle were offered either 15 kg of concentrate or 6 kg

of concentrate and 15 kg of brewers grains with *ad libitum* silage. Cows fed brewers grains produced on average an extra 1.99 kg/d of milk, however only 45.5% of these cows held to first service compared to 71.4% for cows on the control diet. Cows fed brewers grains also had a predicted calving index that was seven days longer than the control group. Tangl *et al* (1960) also associated feeding brewers grains with reductions in fertility. They noted that the grains had a very low tryptophan content (0.01g/100g) which may be implicated in reduced fertility.

The experiments investigating the effects of feeding draff to dairy cows which have been reported in the literature to date are diverse in nature, and the effects reported variable. The variability is increased further by the different nutritional characteristics of spent grains from different cereals and sources. The effect which has been reported most consistently is the reduction in dry matter intake either of the grains themselves or of the forage component of the diet. This has often been associated with increased efficiency of utilisation of draff ME.

Calculations of the FCE and efficiency of utilisation of ME for milk production should be treated with caution. Many of the experiments are of short term design so that changes in liveweight are often small and subject to large errors. The calculations of efficiency of energy utilisation for lactation must remain questionable and detailed calorimetric studies are needed to investigate the effect of spent grain inclusion on efficiency parameters.

Spent Grains in Beef Rations

As with the dairy cow studies, many experiments have been carried out which show the value of spent grain protein for beef production. Chen *et al.* (1977) showed that the inclusion of small quantities of corn distillers grains, either with or without solubles, improved the nitrogen retention in beef cattle rations. Abrams *et al.* (1983), used either wet distillers grains and urea or soya bean meal and urea as

corn cob ration. They showed that the distillers grains fed in this way had a greater value as a protein source than soya bean meal and urea. Alawa *et al.* (1988) reported that brewers grains could be used as the sole nitrogenous supplement with straw based diets for suckler cows.

Also in common with the dairy cow experiments, other papers have reported depressions in dry matter intake when feeding distillers grains. Broadbent *et al.* (1971) replaced barley in an all-barley diet for Friesian and Ayrshire steers with wet distillers grains at inclusion rates of 0, 25 and 40%. In this experiment, dry matter intakes were reduced as level of inclusion increased. Daily liveweight gains (kg/d) were depressed from 1.22 to 0.95 at 25% inclusion, and further to 0.86 at 40% inclusion.

Improvements in feed conversion efficiency have also been demonstrated. Farlin (1981) reported a 10% increase in FCE when replacing 0, 21, 43 and 64% of corn by wet distillers grains in beef cattle diets. The control diet was 85% corn, 10% hay and 5% urea and mineral supplement. When the spent grains replaced 43% of the corn, the FCE and liveweight gain were increased. At the highest replacement level, feed intake was depressed but as liveweight gains remained the same as for the control diet, a 10% increase in FCE was observed which was similar to that observed at the lower inclusion rate. Firkins *et al.* (1985) observed a linear increase in FCE with inclusion of wet distillers grains when the grains made up 0 to 50% of the diet DM.

Spent Grains in Sheep Diets

Relatively little data concerning sheep meat production from spent grains has been published, most references to sheep and feeding spent grains focusing on more detailed digestibility studies. However, the studies which have included production data show similar trends to those for dairy cows and beef production, namely reduced intake and increased feed conversion efficiency.

Many studies into the evaluation of distillery by-products have been carried out at the North of Scotland College of Agriculture. Miller *et al.* (1970) fed fresh and ensiled malt distillers grains, with and without the addition of salt to mature wether sheep. Although they could report no benefit from salting the spent grains, a practice common on many farms at that time, the results showed that the digestibility and dry matter intakes of the spent grains were increased by the addition of calcium, either as lactate or as carbonate.

Reveron *et al.* (1971) reported an experiment in which malt distillers grains and dried grass pelleted in an 80:20 DM ratio was compared to pelleted dried grass when fed to lambs. Growth rates were 191 and 143 g/d respectively and days to slaughter (45 kg) were 78 and 104 respectively. There was an increase in efficiency of utilisation of ME for growth and fattening from 51% to 63% when spent grains were included, demonstrating their high nutritive value compared to grass.

The results from experiments that have been reviewed here show the variable responses obtained when spent grains are fed to ruminants. The results reflect the nutritional characteristics of the grains, their high lipid, fibre and protein and their low content of soluble carbohydrate and minerals. Of the major constituents of the grains, the protein content has been shown to be beneficial, being equal in quality to that in soya bean meal (Polan *et al.*, 1985). The other components of spent grains are not so beneficial. The high content of fibre in the grains is of lower digestibility than the overall organic matter digestibility (El Hag and Miller, 1972).

The high lipid content and its unsaturated nature have also been shown to reduce the digestibility of spent grains. In 1969 El Hag and Miller showed that extracting the oil from malt distillers grains before incubating them *in vitro* increased the *in vitro* organic matter digestibility (IVOMD) from 0.52 to 0.58, supporting this theory.

In this same study, they also showed that supplementation of the grains with additional calcium could increase the IVOMD from 0.52 to

0.54. This improvement in IVOMD has also been shown *in vivo* by El Hag and Miller and more recently by Lewis and Lowman, 1987. They reported daily liveweight gains of 0.9 kg/d in an experiment where brewers grains were offered as the sole diet to finishing steers (Lewis and Lowman, 1989). However, this growth rate was only achieved if the grains were adequately mineralised.

The most reproducible characteristic of spent grain feeding is the depression in dry matter intake that occurs particularly when grains are included at high levels in the diet. This is manifested either as a reluctance to eat large quantities of spent grains (Valentine and Wickes, 1982) or as a reduction in intake of the other components of the diet, most frequently the forage.

The underlying mechanisms for this reduction in intake may vary between experiments, and several explanations have been suggested. Porter *et al.* (1977) suggested that the high water content of wet brewers grains may have resulted in bulk limitation effects being responsible at least in part for the observed decline in dry matter intakes. A further suggestion worthy of consideration is the palatability of the spent grains. No experiments have been reported assessing the palatability of the grains so this suggestion must remain a matter for speculation.

The most likely explanation for the poor intake characteristics of spent grains is that they are due to an interaction between the different fractions of the feed, in particular the high level of unsaturated oil, the high level of poorly digested fibre combined with the low levels of minerals. All these factors combine to reduce the digestibility and therefore the intake of spent grains.

The effects of high levels of dietary fat on rumen function and the degradation of fibre in the rumen and the interaction of these two factors has been the subject of much research effort. The literature pertaining to these studies is now reviewed.

1.4 The Use of Fat in Ruminant Diets

Perhaps the earliest studies into the effects of feeding supplemental fat are reported by Kellner in 1907 (cited by Sundstol 1974). The literature on this subject was reviewed by Maynard *et al.*, 1941 and Loosli *et al.*, 1944. They observed that high-fat concentrates gave increased milk production and the increase in milk energy output exceeded the dietary energy input. They concluded at that time that inclusion of supplemental fat improved the energetic efficiency of milk production.

Since then, Warner (1960) observed that fats reduced fibre digestibility in beef and sheep rations and rarely gave increased liveweight gains in steers. In a summary of fat in ruminant diets he concluded that inclusion of fat in ruminant diets was not supported by the evidence at that time.

More recently, the metabolism of lipids in ruminants and general lipid nutrition have been reviewed in depth by Palmquist and Jenkins, 1980; Storry, 1981; Harfoot, 1981; Moore and Christie, 1981; Noble, 1981; Palmquist 1984. The most widely reported effect of supplementing ruminant diets with fat is the depression of fibre digestion in the rumen and the disturbance of normal rumen function (Lucas and Loosli, 1944; Brooks *et al.*, 1954; Brethour *et al.*, 1958; White *et al.*, 1958; Grainger *et al.*, 1961; Czerkawski *et al.*, 1966; El Hag and Miller, 1972; Devandra and Lewis, 1974; Palmquist and Jenkins, 1982). Despite this and the other effects such as depressed intake and low-milk-fat syndrome which result, it should be noted that not all studies have reported negative effects of fat supplementation (Palmquist and Conrad, 1978). Palmquist and Jenkins (1980) suggest that factors such as dry matter intake, the type of fat fed and the maintenance of adequate mineral levels particularly calcium may also be important

The Lipid Content of Feeds

The diet of grazing ruminants normally consists of pasture grasses and legumes which may also be consumed in a preserved form, normally either as silage or hay, during winter months. The lipid content of forage crops is generally low a typical figure would be 20 - 30 g/kg DM (MAFF 1990).

Menke (1966) has shown that the lipids of forages are concentrated in the leaf chloroplast which contains 22 g lipid/100 g dry tissue and that these lipids contain high proportions of linolenic acid. Plant material also contains cuticular lipids as waxes and complex lipids but there is no evidence that these contribute significantly to the nutrition of the animal.

The content of lipid and the fatty acid composition of concentrate feeds varies widely depending on the source but are usually present at much higher levels and are more saturated than those found in forages. Sources of lipids included in concentrate feeds are varied and may originate from animal fat, for example tallow, which generally contain highly saturated fatty acids. More commonly, the lipids originate from plants such as cereals or oil seeds which have a much less saturated fatty acid composition.

Lipid Metabolism in the Rumen

Metabolism of lipids in the rumen has been reviewed in detail by Harfoot (1978). There are two main sources of lipid in the rumen, dietary lipid which is ingested with the feed, and microbial lipid which is produced as part of the microbial cells.

Czerkawski 1976 noted that between 5 and 15% of microbial dry matter consisted of lipid and that, since microbial matter could typically make up 230 g/kg of organic matter digested, microbial lipids made a significant contribution to the nutrition of the host animal. This is supported by the presence of branched-chain and odd-numbered chain

length fatty acids in ruminant fat which are produced only by microbial organisms (Keeney *et al.*, 1962). Both bacteria and protozoa in the rumen are capable of synthesising lipids *de novo* although Demeyer *et al.* (1978) suggested direct incorporation exceeded *de novo* synthesis of fatty acids. It seems that microbial synthesis of lipid is dependent, not surprisingly, on the diet of the host. Sutton *et al.* (1975) showed microbial fatty acid synthesis was inhibited when the host was supplemented with cod-liver oil.

Dietary lipids are present in ~~forages and~~ cereal feeds largely in triglyceride form although forages contain significant proportions of more complex lipids. When lipids are ingested and enter the rumen the acyl ester bonds of the triglycerides are quickly hydrolysed by lipolytic bacteria (Hawke and Silcock, 1970). Once hydrolysed from the glyceride ester the free fatty acids could either be incorporated into microbial lipids, form insoluble calcium soaps (Palmquist and Jenkins 1982) or be absorbed onto the surface of food particles or microbial cells.

Harfoot *et al.*, (1973) and Harfoot, (1981) observed that the free fatty acids are quickly adsorbed onto particulate matter and that 80% of biohydrogenation of linoleic acid was associated with food particles. Hawke and Silcock (1969) showed that lipolysis was an essential first step before biohydrogenation could occur, and that this required a free carboxyl group. Viviani and Borgatti (1967) and Hawke and Silcock (1970) noted that biohydrogenation of unsaturated fatty acids was incomplete and observed further that the capacity of rumen contents to biohydrogenate was reduced if food particles were not present.

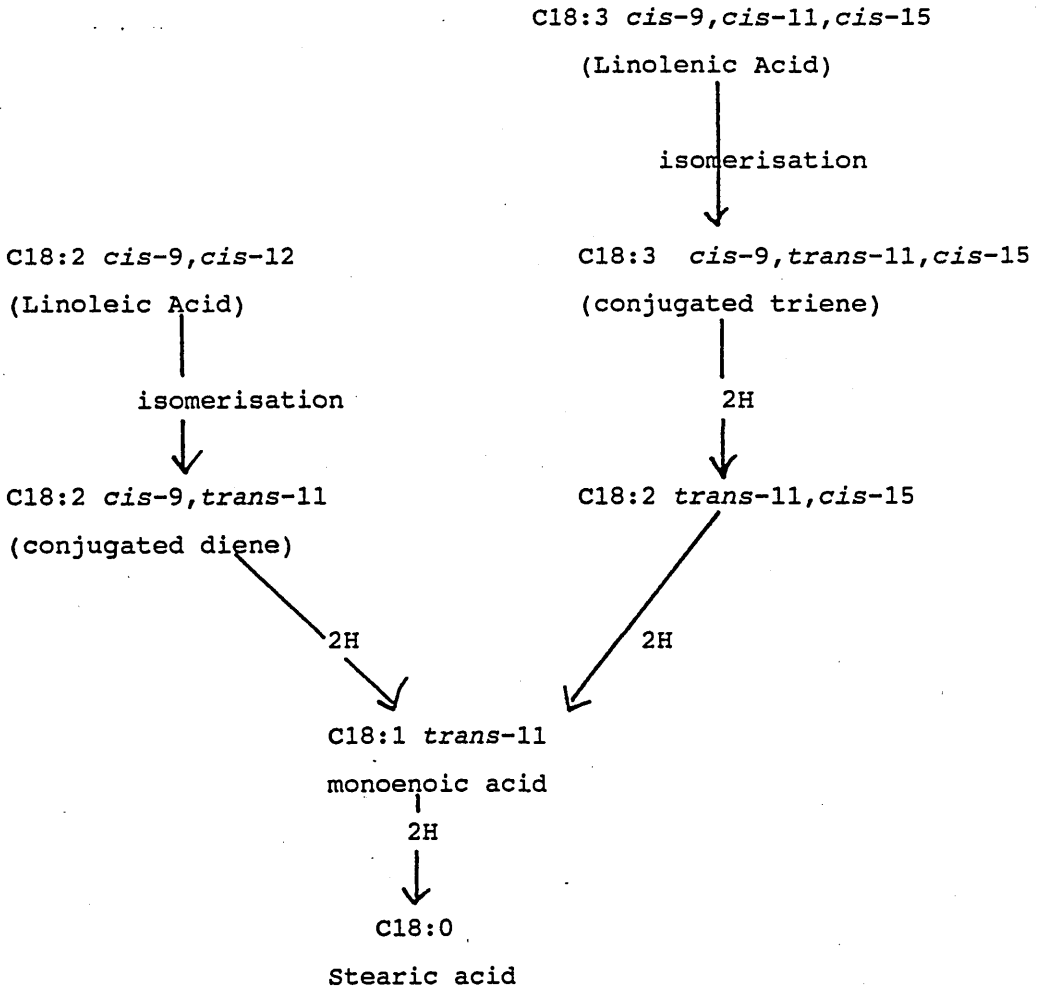
Biohydrogenation has been reviewed by Dawson and Kemp (1970) and Viviani (1970). The end products of biohydrogenation are diverse and the proportions variable. Bickerstaffe *et al.* (1972) measured 80% biohydrogenation of linoleate to stearic acid and 12% to *trans*-11 octadecanoic acid. Moore *et al.* (1969) and Noble *et al.* (1974) have demonstrated differences in the products of biohydrogenation depending on whether esterified or non-esterified fatty acids were the

precursor, suggesting that the initial lipolysis may be the rate limiting step. Fatty acids liberated from triglycerides are quickly adsorbed onto food particles where they are hydrogenated to the saturated acid. Harfoot (1981) suggested that the accumulation of the *trans*-11 fatty acids in rumen contents when linoleic acid was included at high levels *in vitro*, was due to the concentration of linoleic acid exceeding the surface area of food particles.

Harfoot *et al.* (1974) studied the adsorption of fatty acids onto bacterial cells and food particles and observed that adding food particles to the system reduced the degree of absorption onto bacterial cells. He also observed that saturated fatty acids were more strongly adsorbed onto bacterial cells than unsaturated fatty acids. It is possible therefore that the uptake of the end products of biohydrogenation by bacterial cells observed by Harfoot *et al.* (1973) was due to greater physical adsorption rather than metabolic uptake. Henderson observed that long chain fatty acids became inhibitory to the growth of rumen bacteria when their concentrations became greater than could be adsorbed onto the bacterial cells.

By studying the levels of biohydrogenation intermediates and end products, Harfoot (1981) proposed the following metabolic pathway for the biohydrogenation of linoleic and linolenic acids in the rumen:

Proposed Pathway For Biohydrogenation of Linoleic and Linolenic Acids



The Effects of Dietary Fat on Rumen Metabolism

The addition of fat to ruminant diets has been shown to increase the production of propionate relative to acetate produced in the rumen (Steele and Moore, 1968; Nicholson and Sutton, 1971; Ikwuegbu and Sutton, 1982). This effect is thought to be related to the depression in fibre digestion in the rumen also associated with high levels of dietary fat in the ruminant diet (Lucas and Loosli, 1944; Brooks *et al.*, 1954; Grainger *et al.*, 1961; El Hag and Miller, 1972; Palmquist and Jenkins, 1982). It is the reduction in fibre digestibility that is thought to be the cause of reduction in dry matter and organic matter digestibility and therefore depression in intake (Blaxter *et al.*, 1961) and low milk fat syndrome (Lucas and Loosli, 1944; Czerkawski *et al.*, 1966; Palmquist and Jenkins, 1982).

The mechanisms behind the inhibitory effects of dietary fat on fibre digestibility are not clearly understood despite having been the subject of much research effort.

Neiman (1954) reviewed the effects of trace amounts of fatty acids on microbial growth. His main conclusion was that individual fatty acids can either stimulate or inhibit microbial activity, but that it was the relative concentrations of fatty acid and fibre in the diet that determined the degree of inhibition. He also found that, although both saturated and unsaturated fatty acids caused inhibition of fibre digestibility, the effect was greatest with the less saturated fatty acids. He also noted that fatty acids in the *cis* formation were more inhibitory than the *trans* isomers. Later work by El Hag and Miller (1972) on malt distillers grains supported this. In their experiment they removed the lipid from the spent grains and then added individual fatty acids back to the extracted residue before measuring IVOMD. They found that the saturated C16:0 and C18:0 acids were less inhibitory than the unsaturated C18 acids.

Devandra and Lewis (1974) summarised four theories to explain the phenomenon:

1. Physical coating of the fibre by fat

2. Modification of the rumen microbial population from possible toxic effects on certain species.

3. Inhibition of microbial activity from the surface active effects of fatty acids on cell membranes.

4. Reduced cation availability for rumen micro-organisms due to the formation of insoluble complexes with long chain fatty acids.

Devandra and Lewis proposed a model to explain the inhibitory effect of oil on fibre digestibility. They suggested that it was due to a physical coating effect of the feed fibre with the long chain fatty acids which depressed fibre digestibility.

The second theory is supported by an experiment reported by White *et al.* (1958) who observed that cellulose digestibility was still depressed 17 days after fat coated particles had passed out of the rumen. This suggested that the inhibitory effects of fat on microbial activity may be sufficient to change the competitiveness and therefore the populations of different species of rumen bacteria. Some bacteria have been shown to increase in number when the host diet is supplemented with fat and this is usually concurrent with a decease in protozoal numbers (Czerkawski, 1973; Maczulak, 1979).

The adsorption of long chain fatty acids onto microbial cells has been demonstrated (Maxcy and Dill, 1967; Henderson, 1973). Kodiock (1948) suggested that this would change the cell wall permeability and hence the rate at which substances enter and leave the cell. This is a view supported by Czerkawski and Clapperton (1984) who comment on the large surface area-to-volume ratio of bacterial cells and the complex structural lipids in their cell walls. They note that "Therefore, it is not surprising that lipids, particularly polar lipids, such as the unesterified fatty acids should have an effect on microbial metabolism."

Mineral supplementation of high fat ruminant diets has been shown to reduce the inhibitory effects of fat on fibre digestion. *In vitro* studies carried out by Galbraith *et al.* (1971) and Galbraith and Miller (1973) showed that calcium, magnesium, iron, aluminium, zinc, ergocalciferol and cholesterol could all reduce the inhibitory effects of long chain fatty acids on isolated species of bacteria. However, El Hag and Miller (1972) reported that that only calcium and ergocalciferol could successfully restore depressed digestibility in malt distillers grains.

The ability of calcium to reverse the inhibition of cellulose digestion by dietary fat was first noted by Burroughs *et al.* (1950) and later by Brooks *et al.* (1954). They observed that adding lucerne ash to sheep diets containing maize cobs and supplemented with maize oil reduced the inhibition of cellulose digestibility by the supplemental oil. However, it was not recognised until 1958, when White *et al.* showed that lucerne ash could be replaced by calcium, that this was the active ingredient. They concluded that supplemental fat increased the ruminal requirement for calcium. Davison and Woods (1959) observed that addition of magnesium carbonate gave a similar response to addition of calcium carbonate *in vitro* by reversing the inhibition of cellulose digestibility. However, when this was tested *in vivo* (Davison and Woods, 1963) magnesium carbonate was found to be ineffectual.

It is postulated that calcium improves cellulose digestibility in high fat ruminant diets by forming insoluble calcium soaps with the long chain fatty acids, thereby rendering them unavailable to bind to rumen bacteria. This is supported by the studies of Palmquist and his colleagues in the early 1980s. They showed that calcium soap formation in the rumen is slow and incomplete (Jenkins and Palmquist, 1982), probably due to the aqueous medium and they put this theory forward as the reason why calcium supplementation only partially alleviated the inhibitory effects of oil on cellulose digestibility. When tallow fatty acids are added to the diet as the preformed calcium soaps fibre digestibility is restored to control levels (Palmquist and Jenkins, 1982). Ngidi *et al.* (1990) showed that calcium soaps could

be fed to finishing steers in rations containing 85:15 concentrate:forage where calcium soaps of palm fatty acid distillate made up 6% of the concentrate without affecting rumen fermentation.

The supplementation of ruminant diets with calcium soaps as a method of providing high fat rations without disrupting rumen function is now commonly practised. However, it was noted by Palmquist (1984), in an experiment in which dairy cow diets were supplemented with calcium soap of varying degrees of saturation, that very little of the 'protected' unsaturated fatty acids could be measured in the milk fat. It was postulated that calcium soaps of saturated and unsaturated fatty acids had different dissociation patterns and that the unsaturated soaps were dissociating and being biohydrogenated. This theory was later substantiated by *in vitro* studies measuring calcium soap dissociation in rumen fluid (Sukhija and Palmquist, 1990)

Protected Fats in Ruminant Diets

The first protected fats originated from two unrelated technologies of the 1960s. The first was a method of protecting proteins from ruminal degradation and the second, a technique for the production of butter powder.

Many studies, reviewed by Ferguson (1975) have shown that dietary proteins could be modified by heat treatment so as to reduce ruminal degradation, without decreasing the overall protein digestibility. Later studies showed that this could be achieved by formaldehyde treatment. Ferguson and his colleagues showed that this technology could be applied to a wide range of dietary proteins and that this could be used to protect other dietary components, such as starch, from rapid degradation in the rumen.

Hansen (1963) and Snow *et al.* (1967) described techniques for the production of a stable, free flowing butter powder. This was produced by emulsifying butter oil with sodium caseinate and spray drying to produce a fine powder which consisted of butter oil droplets coated in

a thin layer of protein.

Scott *et al.* (1970 a,b) recognised the value of the combination of these technologies. They produced feed supplements in which oil droplets were coated in formaldehyde treated protein which was resistant to the action of rumen micro-organisms at pH 6 - 7 but when exposed to the acidic pH of the abomasum, the formaldehyde-protein bonds were cleaved releasing both the protein and the lipid for digestion and absorption. This increased the supply of polyunsaturated fatty acids reaching the small intestine for absorption and provided a means of manipulating ruminant fat composition.

The use of protected fats in ruminant nutrition has been reviewed by Storry *et al.* (1974) and McDonald and Scott (1977). The use of spray drying followed by formaldehyde treatment proved not to be commercially viable and the use of crushed oil seeds such as sunflower seeds to provide the oil and the protein superseded the former technology.

The benefits of including protected fats in ruminant diets are:

1. The content and yield of total fat in the milk of lactating animals on normal diets can be increased since the depression in *de novo* synthesis of fatty acids in the mammary gland, normally associated with feeding unprotected fats and oils is avoided (Storry, 1974b)

2. Protected lipids can be used to correct low-milk-fat syndrome which is associated with low-forage, high-concentrate diets. Replacing starchy concentrates with a protected lipid in lactation rations normalises rumen fermentation patterns whilst maintaining the energy intake of the animal, and also increases the fatty acid supply which compensates for diminished intramammary synthesis and increased demands of adipose tissue (Storry, 1974)

3. Feeding protected lipids gives the opportunity to manipulate the fatty acid content of ruminant meat (Cook *et al.*, 1970, 1972; Faichney *et al.*, 1972, 1973; Newbold, 1973) and milk fat (Cook *et al.*, 1972 Dryden *et al.*, 1972). This technology has been used to produce ruminant products which are high in linoleic acid. The effects of this and the implications for human health have been reviewed by McDonald and Scott (1977).

Despite the relatively consistent responses of ruminants to diets containing lipid protected by coating with undegraded protein, the production of this material is not economically feasible for several reasons. Consistent quality control of the process to prevent under- and over-protection proved to be difficult, the former causing rumen upset due to the effects of unprotected oil, and the latter resulting in the material not being degraded post-ruminally, reducing the overall digestibility. There have also been difficulties due to the lack of governmental approval of the use of formaldehyde treated products in the diets of lactating animals.

An alternative method of feeding protected fats is to include the lipid as calcium soaps of long chain fatty acids. This method of protecting fats has proved to be unsuitable for less saturated fatty acids because soaps of unsaturated fatty acids dissociate at higher pH than more saturated calcium soaps (Sukhija and Palmquist 1990). This reduces the opportunity to manipulate the composition of ruminant fat by this method, however, the other benefits of feeding protected fat remain.

1.5 The Chemical Structure and Digestion of Fibre in the Rumen

Dietary fibre has been defined as the insoluble structural matter of plants that is resistant to animal digestive enzymes (Van Soest and Robertson, 1976). Other definitions include the soluble substances associated with plant cell walls which include pectin and non-starch polysaccharides or gums (Fonnesbeck and Harris, 1970) even though these substances are not fibrous in the physical sense and are found

largely between adjacent cells. These substances are readily accessible to rumen microbes and are rapidly fermented. In this respect, in ruminant nutrition they resemble storage polysaccharides and, for practical definitions of fibre for ruminant diets, should therefore be excluded. Van Soest (1985) defined fibre as 'insoluble substances from the plant cell wall'. In analytical terms this corresponds to the neutral detergent fibre (NDF).

Plant Cell Wall Structure

Reviews of plant cell wall structure have been published by Northcote (1972), Preston (1974), Colvin (1981) and Dey Brison (1984).

A basic structure common to many types of cell walls of herbaceous plants is one in which cellulose fibrils are embedded in a matrix of pectic substances, hemicellulose, lignin and glycoprotein Bailey (1973) and Theander and Aman (1984).

Structurally, plant cell walls can be divided into the primary and the secondary cell wall, which contain the true structural polysaccharides, and the middle lamella which is composed largely of pectins and fills the space between adjacent cells. The primary cell wall is first formed as the plant cells divide. It consists of cellulose fibrils loosely bound with hemicellulose and structural proteins. As the plant cell matures, more cellulose and hemicellulose are laid down inside the primary wall. As the cell reaches maturity, lignin is deposited, particularly in the corners of the cells, and once this process is complete, the plant cell dies. The proportions of the cell wall constituents depend on their function in the plant, the plant maturity and the plant species.

Hemicellulose

Both the proportion and the composition of the hemicellulose in plant cell walls vary widely taxonomically and with plant maturity. Generally, cereals contain a greater proportion of hemicellulose than

leguminous plants. A typical value for the hemicellulose content of barley straw determined by fibre detergent analysis (Jackson, 1977) is 270 g/kg DM where the cell wall content is 810 g/kg DM.

Hemicelluloses contain residues of xylans, uronides, arabans, glucans, galactans and mannans which exist largely in mixed polymeric form. Commonly, hemicellulose consists of backbone chains of 1-4 linked xylopyranosyl units. The planes of the xylose units lie at about 60° to each other (Van Soest, 1982). Chain lengths range from 50 to 200 xylan units in size and are frequently substituted at the C2 or C3 positions with arabinofuranosyl units or glucuronic acid. The furanosidic linkages are more easily broken than pyranosidic linkages by chemical and digestive action so that the arabinose is more rapidly degraded than the xylan backbone. The rate of degradation of hemicellulose has been correlated to the degree of branching within the polymers. This may be reflected in the arabinose content (Morrison, 1979). Brice and Morrison (1982) noted that, at a constant level of lignification, removal of arabinose side chains increased with the rate of heimcellulose degradation in the rumen.

As a consequence of the substitution of the C2 and C3 positions on the xylan backbones, tight association between adjacent chains is prevented. Generally, the more highly branched hemicelluloses are soluble in acidic or alkaline solutions. For this reason, and because of their low molecular weight, xylans and other hemicelluloses can be extracted by alkali.

Further common substituents of hemicellulose are o-acetyl groups and phenolic groups which are ester linked to the xylan backbone (Hartley, 1972; Bacon *et al.*, 1975). Bacon *et al.* (1975) demonstrated that acetyl groups amounted to between 1 and 2% of the cell wall material of Graminaceous plants. Esterification to these groups has been considered a factor which limits the degradation of hemicellulose. Chesson *et al.* (1982) showed that the phenolic acids ferulic and p-coumaric acid reduced the growth and cellulolytic activity of *Bacteriodes succinogenes* when present at concentrations similar to those found in the rumen.

Cellulose

Cellulose is a linear polymer composed of up to 10,000 glucose molecules linked by β(1-4) glycosidic bonds. Cellulose occurs in a crystalline form made up of fibrils tightly packed together and well ordered in the crystalline regions and loosely packed and disordered in the more amorphous regions. Any one cellulose chain may pass through both ordered and disordered regions. Sihtola and Neimo (1975) noted that inter- and intra-molecular hydrogen bonding was extensive in cellulose and that this was particularly prevalent in the more highly ordered regions.

Hydrogen bonding between sugar units is important in establishing this structure and thus in determining the physico- chemical properties of cellulose (Stone *et al.*, 1969). It is often suggested that the degree of crystallinity, or order, of cellulose determines its degradability. It is established that the cellulose of, for example, cotton is much more highly ordered than that occurring in forage crops, and that it is less susceptible to microbial attack. However, it has been shown by Beveridge and Richards (1975) using X-ray diffraction techniques that the highly ordered and the more amorphous regions of cellulose in the plant cell wall are degraded at a similar rate. In the same study the degree of crystallinity of cell wall material was not increased by incubation in the rumen. This suggests that the crystallinity does not determine degradability.

Lignin

Lignin is a family of related polymers of a three dimensional structure made up of phenylpropane units. Early work into the structure of lignin was carried out by Freudenberg *et al.*, (1955); Freudenberg, (1964) and Freudenberg and Neish, (1968) who showed that phenylpropane glycosides were the precursors for lignification. It was suggested that these molecules diffused into the differentiating areas which were eventually to be lignified, for example, the xylem vessels of the plant, where the glycoside is then enzymically

hydrolysed. This would lead to a build up of p-coumaryl, coniferyl and sinapyl alcohols in the plant cell wall. These molecules are believed to undergo oxidation by randomised depolymerisation in the presence of oxidative enzymes such as peroxidases and laccases forming the complex and indigestible polymers of lignin.

Variations occur in the composition and the properties of lignins from plants from different species (Van Soest, 1982). In Graminaceous plants phenolic acids make up 1 - 2% of cell wall material (El-Basyouni *et al.*, 1964). Hartley *et al.* (1976) identified these acids as p-hydroxybenzoic acid, diferulic acid and the *cis* and *trans* isomers of p-coumaric and ferulic acids. Nimz *et al.* (1981) showed that p-coumaric acid and ferulic acids were linked by their phenolic groups to lignin via ether bonds. They have also shown that most, if not all, of the ferulic acid is present in the cell wall ester-linked to the xylan chains of hemicellulose.

Harris and Hartley (1976) demonstrated that ferulic acid was esterified to the cell walls of mesophyll and non-mesophyll type cells. They showed that p-coumaric acid was present in larger amount in the non-mesophyll type cells and that its proportion in the plant material increased as the plant matured. This has been shown to be related to the decreasing proportion of primary cells.

Silica

Silica is taken up by the plant from the soil in the form of monosilicic acid ($Si(OH)_4$). It is transported up to the shoots and deposited as silica in the plant cell walls during secondary thickening. It is present in the walls in an opaline form ($SiO_2.nH_2O$). The silica content of the cell wall material is dependent on the soil type and on the plant species.

Interactions Between Cell Wall Constituents

Theander and Aman (1984) noted that there were strong indications that hemicellulose, lignin, phenolic and acetyl substituents comprised a vast macromolecular matrix in the secondary cell wall of plants. They also noted that these interrelationships had a great influence on the biodegradability of hemicellulose. Association of cellulose within such a matrix would also impair cellulolytic activity. Chesson and Orskov (1984) concluded that constraints on digestion in the rumen result from the organisation of the various polymers within the cell wall and especially from the cross linkages between structural polysaccharides and lignin.

Lignin is known to establish covalent bonds with the hemicellulose present in the cell wall forming ligno-carbohydrate complexes. These bonds are formed between the different sugar units and the uronic acid of the hemicellulose and the lignin via benzyl ether and benzyl ester bonds respectively (Eriksson *et al.*, 1980).

Phenolic acid monomers found in lignin such as p-coumaric acid and ferulic acid have been found to ester-linked to cell wall preparations (Hartley, 1973). It has been suggested (see Theander and Aman, 1984) that all combined ferulic acid found in bran is ester linked to the terminal arabinofuranosyl residue in xylans. Markwalder and Neukom (1976) reported that diferulic acid found in cereal grain cell wall material was formed by dimerisation of ferulic acids residues of adjacent polysaccharide forming cross linkages. These phenolic acid monomers have also been reported to ester link with the lignin (Higuchi *et al.*, 1967).

Fibre Digestion in the Rumen

Cheng *et al.* (1981) showed that fragments of plant material entering the rumen where extensively colonised by bacteria within one hour. Minato and Suto (1978) showed that there were at least as many bacteria associated with plant material as were free in the rumen

liquor. The bacteria in the rumen are a diverse population of obligate and facultatively anaerobic micro-organisms. Population densities of 10^{10} - 10^{11} have been measured although only a few of these are capable of degrading structural polysaccharides. Chesson and Orskov (1984) listed the major cell wall degrading micro-organisms;

Bacteriodes ruminicola

Bacteriodes succinogenes

Butyrivibrio fibrisolvens

Eubacterium cellulsolvens

Lachnospira multiparous

Ruminococcus albus

Ruminicoccus flavefaciens

Streptococcus bovis

These strains of bacteria are not equally effective in their ability to utilise the different structural polysaccharides. Some strains grow better on oligosaccharides or simple sugars whilst others grow preferentially on highly ordered cellulose. Undoubtedly it is the mixed population with each strain of micro-organism occupying and thriving in a particular niche in the ruminal environment that permits the digestion of complex cell wall polysaccharides. Changes in the diet of the animal from one type of carbohydrate to another may lead to a shift in the rumen population reflecting the preference of a particular cellulolytic bacteria for the new substrate.

Colonisation of fragments of plant material is necessary for the degradation of cell wall polysaccharides. Electron microscopy studies carried out by Akin (1979) and Cheng *et al.* (1977) demonstrated that

degradation of non-mesophyll cells was limited if there was no bacterial attachment. It was also noted (Van Soest, 1981) that this association prevents the loss of bacteria in the liquid out flow from the rumen, which is faster than the small particle outflow, thus allowing microbial populations to establish and remain in the rumen.

Colonisation of plant material is brought about by adhesion of the bacteria via the glycoprotein coat or capsule. Latham *et al.* (1978) showed that removal of the glycoprotein layer or modification by addition of specific antibodies prevents adhesion. Akin *et al.*, (1974); Akin and Amos (1975) and Akin (1976) showed, using electron microscopy, that many different morphological forms of bacteria adhered to cell wall fragments but coccoid bacteria were the most common. Akin *et al.*, (1979) showed that bacteria were less likely to adhere to surfaces that were heavily lignified. This is consistent with the lowered digestibility of plant material with increasing lignin content. This is supported by studies carried out by Latham *et al.* (1978) who showed that bacteria did not adhere to the plant cuticle or the vessel elements.

It has been observed that anaerobic bacteria do not produce extracellular enzymes in significant quantities and that enzymic activity remains associated with the cell wall (Wood *et al.*, 1982). An explanation for this has been found in the sedimentable subcellular membrane-bound vesicles known to be produced by *B. succinogenes*. These vesicles can be seen coating plant cell wall material recovered from the rumen (Forsberg *et al.*, 1981). A range of hydrolytic enzymes have been identified from rumen bacteria including cellulases, xylanases, hemicellulases and arabinofuranosidase. Extracellular hemicellulases have been shown to degrade isolated hemicellulose and hemicellulose-lignin complexes but have limited activity on intact cell walls. The increased loss of hemicellulosic material when cellulase activity was increased suggests that a synergistic relationship may exists between bacteria producing cellulase and hemicellulase. Dehority (1973) noted that this was true when intact grass cell walls were degraded by a combination of *B.ruminicola* and *R. flavefaciens*. He found that the dry matter loss was greater with a

combination of the two strains than with either strain alone.

The degradation of fibrous material does not follow the kinetic characteristics normally associated with enzyme-mediated reactions. Since the substrate is, by definition, insoluble, the rate limiting factors are the particle surface and the accessibility of hydrolysable linkages. Association of the structural polysaccharides with lignin causes additional limitations to microbial degradation of fibre.

The digestion of fibre by rumen micro-organisms has been described by Chesson (1986) as a surface phenomenon since there is no evidence of degradative enzymes penetrating into the cell wall structure. The size of molecular aggregates of extracellular enzymes released by the micro-organisms responsible for fibre degradation, coupled with the low porosity of lignified cell walls, therefore limit the extent of fibre digestion. This is supported by analysis of cell wall material from barley straw before and after a 72h incubation in the rumen (Chesson *et al.*, 1983). It was shown that there was little change in the fibre composition or content except for a limited accumulation of the phenolic component. This was explained by a build up of phenolic material at the surface of the fibre particles during digestion restricting the action of degradative enzymes on the digestible parts of the straw.

The protective mechanism of surface build up of phenolic material is not the only way in which lignin limits fibre digestibility. Digestibility is also reduced by the bonding of structural polysaccharides to lignin. This is supported by the low correlation of digestibility with lignin content between plant species whereas the correlation within a species is much higher. This occurs because of differences in the carbohydrate-lignin linkages between different plant species. The cleavage of alkali-labile linkages which include some of those bonds restricting cell wall degradation can lead to large increases in fibre digestibility.

A further mechanism for the reduced cell wall digestibility when the cell wall is highly lignified is the inhibition of attachment of

rumen micro-organisms to the cell wall (Richards, 1976; Akin, 1979).

The rates of digestibility of isolated structural polysaccharides have been studied. However, since these polymers never occur in this form in plant cell walls, and there is always a possibility of modification during extraction processes, the results cannot be extrapolated to predict their rate of degradation in the intact cell wall. Jackson (1977) suggested that the crystallinity of cellulose is a limiting factor in polysaccharide degradation. However, Beveridge and Richards (1975) showed that there was no preferential degradation of the more amorphous, less crystalline cellulose. The loss of dry matter from a cellulose preparation *in sacco* was linear with time and the degree of crystallinity was not increased during the course of degradation. Isolated hemicelluloses are generally fermented more quickly than isolated cellulose. The rate of hemicellulose degradation may be influenced by the degree of substitution of the xylan molecule (Brice and Morrison, 1982).

As degradation of plant material proceeds its hemicellulose content is often seen to increase. This has been interpreted as an indication of the association of hemicellulose with lignin. Chesson and Orskov (1984) however interpreted this as the preferential degradation of the less lignified cells which are generally lower in hemicellulose, and the increase in proportion of lignified cells which are usually associated which higher levels of hemicellulose.

Van Soest and Jones (1968) and Van Soest (1970) have demonstrated that the presence of silica in the cell walls limits the degradability of the polysaccharides in the rumen. Van Soest (1970) showed that the removal of silica from rice straw by treatment with neutral detergent leads to significant increases in the *in vitro* organic matter digestibility.

Morris and Bacon (1977), Bacon (1979) and Bacon and Gordon (1980) have shown that acetyl groups account for 1 to 2% of cell wall material of graminaceous plants. They also showed that the concentration of acetyl groups was greater in cell walls isolated from faecal material

than that from the diet, indicating that they are less digestible than the other cell wall constituents.

Jones (1978) suggested that some metal cations may bond or complex to the carboxyl or hydroxyl groups of the uronic acids. Such associations may limit cell wall degradability.
The rate of fermentation of plant material is also affected by the hydratability and the ion exchange capacity of the plant material. It is thought that these affect the attraction and attachment of micro-organisms to plant particles (Van Soest *et al.*, 1984) which themselves have considerable ion exchange and hydration capacities. The adsorption of other components of the diet such as lipids and minerals reflect the surface properties of the fibre. It has been suggested (Van Soest and Robertson, 1984) that constituents of the fibre such as uronic acid, the phenolic groups of lignin and the nitrogenous substances all contribute to its adsorptive properties.

1.6 The Effect of Alkali Treatment on Plant Cell Walls

Alkali treatment of plant fibre has been shown to increase its digestibility for ruminant animals. Most of the work relates to the alkali treatment of straw, and has been reviewed by Jackson (1977) and Capper *et al.*, (1977).

Strongly alkaline conditions, such as 18% aqueous sodium hydroxide, have been shown to swell cellulose (Gardner and Blackwell, 1974) and it was proposed that this occurs by breaking of the hydrogen bonds which exist between the cellulose in its crystalline state. Whistler and Bemiller (1958) noted that the normal fit of cellulose molecules to each other is so close that only fragments of 15 -18 monosaccharide units could be dissolved in 15% sodium hydroxide solution. Swelling of cellulose can also be brought about by ammonia (Zeronian, 1985).

The hydrogen bonds and the ester-linkages in hemicellulose can also be cleaved by the action of alkali. Polysaccharides are also broken down

by the peeling reactions observed by Whistler and Bemiller (1958). These reactions commence from the reducing end of the polysaccharide. The monosaccharide unit are released and form aliphatic acids such as lactic, glycolic or saccharinic acid.

Chesson and Orskov (1984) noted that a number of the internal linkages in lignin were susceptible to relatively mild hydrolysis particularly the a-aryl and a-alkyl ether bonds. Grierer (1985) suggested that, although such bonds do not occur commonly in lignin molecules, the effect on the molecular size and three-dimensional structure could be of importance. This is supported by Sarkanen (1982) who noted that mild hydrolysis of of the lignin from wood caused a reduction in molecular weight of the lignin fragments. Tarkow and Feist (1969) suggest that such a fragment would be capable of passing through pores in the cell wall matrix and could be liberated from the fibre particle.

Other cell wall components such as cutin and suberin are also liable to alkali cleavage of ester linkages within the molecule (Ternrud, 1987). Hartley (1981) noted that silica in the cell wall could also be solubilised by the action of alkali.

The Effect of Alkali on Fibre Degradation in the Rumen

Perhaps of greater importance than the effects of alkali on the individual components of the plant cell wall is the effect it has on the interactions between cell wall constituents and how this affects fibre digestibility in the rumen.

Morris and Bacon (1976) showed that the hemicelluloses of Graminaceous plant cell walls are esterified with acetic acid and that the acetyl groups impeded degradation. Feist *et al.* (1970) suggest that such linkages would also be alkali labile. This is supported by Jackson (1977), Evans (1979) and Chesson (1981) who all note that treatment of plant cell wall material with alkali caused the loss of acetyl and phenolic groups, and the solubilisation of some hemicellulose and silica and the hydrolysis of some bonds between hemicellulose and

lignin.

Feist *et al.* (1970) showed that the intermolecular linkages between the uronic acid groups of hemicelluloses and cellulose are hydrolysed. Chesson *et al.* (1983) showed that few of the ring hydroxyls of glucuronic acid and arabinose residues were found to carry alkali labile linkages. Xylose residues however, were extensively substituted on carbon atoms 2 and 3, 40% of all residues having one or more substituents linked by alkali labile bonds. They went on to demonstrate that the majority of these bonds were not accounted for by acetyl and phenolic acid ester links and suggested that these represented bonding to polyphenolic material.

Since plant fibre is not a homogenous material and can contain variable amounts of polysaccharides and polyphenolic substances in complex matrices which are held together by many different types of bonding, the effects of alkali treatment are difficult to elucidate accurately. The problems are further compounded by factors such as differences in the source of fibre which change with plant species and maturity.

Calcium Hydroxide Treatment

The effects of calcium hydroxide on straw and other fibrous feed residues has been reviewed (Owen *et al.*, 1984). It was noted that calcium hydroxide did not react so quickly and that consequently it was less effective than sodium hydroxide in upgrading low quality fibre. In practical terms, the use of calcium hydroxide has many of the characteristics of the 'ideal' chemical listed by Owen *et al.* Apart from increasing the intake and/or the digestibility of the feed, treatment with the chemical should be cost effective (the cost of treatment should be less than the improved nutritive value of the feed). It should be readily available, ideally naturally occurring. It should leave non-toxic residues in the feed and be non-polluting to soils and water courses and it should be non-hazardous to handle.

Although the action of calcium hydroxide on fibre is slow, (Nwaduke, 1979; Klopfenstein and Owen, 1981), it can be increased by heating. Klopfenstein, (1984) noted that $Ca(OH)_2$ treatment of maize cobs with additional water and at 45°C caused an exothermic reaction, which reached 70°C and increased cob digestibility *in vitro* from 0.43 to 0.54. When the treated cobs were fed to lambs dry matter intakes were increased from 1000 g/d to 1455 g/d. Similarly Djajanegara *et al.* (1985) reported that treating wheat straw with calcium hydroxide increased both the intake and the digestibility of the straw when fed to sheep.

A similar response was observed with malt distillers spent grains. As a final year project for an honours degree the effects of calcium hydroxide on the intake and digestibility of spent grains was investigated (Barker, unpublished data). Increases in digestibility *in vitro*, *in sacco*, and *in vivo* and increased intake by sheep were observed when grains were treated at ambient temperature with $Ca(OH)_2$. Digestibility and intakes were increased further when the treatment was carried out at 60°C. It was suggested that the responses were due to two factors; upgrading the fibrous fraction, and the formation of insoluble calcium soaps with the unsaturated oil present in spent grains.

1.7 Introduction To Experimental Studies

Studies reviewed in the preceding section suggest that the use of draff in ruminant diets is limited by its moderate digestibility and poor dry matter intake leading to variable (frequently poor) animal performance. These problems reflect the chemical characteristics of spent grains, particularly the high levels of slowly-degraded fibre and of unsaturated oil.

The primary aim of this research project was to investigate the use of $Ca(OH)_2$ to ameliorate the problems associated with feeding high levels of draff in ruminant diets.

A programme of work is described to make a detailed evaluation of the consequences of $Ca(OH)_2$ treatment of barley spent grains. The effects on the following parameters were investigated:

chemical composition,

storage characteristics,

rumen degradation,

digestibility in whole ruminant gut

ad libitum intake

animal performance.

Initially, chemical and *in sacco* measurements were used as indices of the effects of $Ca(OH)_2$-treatment on draff. The effects of different treatment parameters and their interactions on the nutritional characteristics of draff were studied. More detailed studies of degradation of the feed in the rumen were carried out using *in vitro* and *in sacco* techniques. The digestibility of the major fractions of the feed were measured *in vivo* and the effects on intake and growth were evaluated in finishing lambs.

The field of study diversified, investigating the effects of $Ca(OH)_2$-treatment of maize spent grains with concurrent *in sacco* studies to give information on the rumen degradation characteristics.

Novel uses for the $Ca(OH)_2$-treated barley spent grains were investigated, such as their use as silage effluent absorbents and a role as a fat carrier in ruminant feeds. Detailed studies of the changes in carbohydrate composition that occur with $Ca(OH)_2$-treatment are also described.

The final section describes two small experiments investigating the long-term storage characteristics of $Ca(OH)_2$-treated spent grains. The results and potential applications of the findings are discussed.

CHAPTER TWO

ANALYTICAL METHODS

All procedures were carried out using analytical grade chemicals obtained from BDH (BDH Chemicals Ltd, Poole) unless otherwise stated. All determinations were carried out in duplicate, the final value taken as the mean of the two measurements. If measurements varied by more than 5% of the final value then measurements were repeated.

2.1 Analysis of Feed and Faecal Samples

2.1.1 Oven Dry Matter Determination

A known weight of sample was dried in a forced draught oven at 60°C. When a constant weight had been reached the dry weight of the sample was expressed as g DM/kg fresh weight.

2.1.2 Organic Matter Determination

Approximately 3g of sample was placed in a preweighed (to the nearest mg), dry silica crucible. The crucible and contents were dried at 60°C to a constant weight and were reweighed. The dry crucible and contents were then placed in a muffle furnace at 500°C overnight. The crucibles were allowed to cool in a dessicator and were then reweighed. The organic matter was expressed as g OM/kg DM.

2.1.3.1 Total Nitrogen Determination

The total nitrogen content of feed and faecal samples was carried out on dried milled samples and was determined in triplicate by the Kjeldahl method according to the procedure described by Egan *et al.* (1981)

Reagents

Digestion Mixture.

The digestion mixture was made up by mixing slowly with cooling the following reagents:

40 g selenium oxide in 100 ml distilled water

2 litres of concentrated sulphuric acid

Buffer.

The following reagents were dissolved in 2 litres of distilled water:

5 g NaOH

3.74 g anhydrous Na_2HPO_4

31.8 g $Na_3PO_4.12H_2O$

10 ml $NaHCLO_3$ (10 - 14 % av. Cl).

Caustic Phenol.

Caustic phenol solution was prepared by dissolving the following reagents 1.6 litres of distilled water:

2.4 g NaOH

0.1 g sodium nitroprusside

20g phenol

Ammonia Standards.

Ammonia standards ranging from 0.05 to 0.250 g/l were made up from a stock solution containing 4.7168 g of ammonium sulphate in 1 litre of 10% sulphuric acid. The standards were made up by dilution with 10% sulphuric acid.

Procedure

Digestion.

A known weight (between 0.1 and 0.15 g)of dried, milled sample was weighed to the nearest mg into a 75 ml glass digestion tube. Using a dispenser, 8 ml of digestion mixture and 3 x 1 ml of hydrogen peroxide (100 vol) were added. The tubes were then placed on a block digester (Tecator Ltd. Bristol) and taken through the following temperatures:

Temperature °C	Time (minutes)
155	16
195	9
200	15
250	20
349	60

The tubes were allowed to cool and were diluted with distilled water up to a volume of 75 ml. The diluted digests were mixed and allowed to stand overnight before sampling and analysing using the Indo-phenol blue method (2.1.3.2).

2.1.3.2 **Analysis for Ammonia-N**

The ammonia released by the digestion was measured colorimetrically using the Indo-phenol blue method.

Aliquots of 0.1 ml from the digests and standards were transferred into 50 ml test tubes. Caustic phenol (8.0 ml) followed by buffer solution (20 ml) was added to each tube. The tubes were mixed thoroughly and were allowed to stand at room temperature for 2 hours exactly.

The absorption of the solutions was measured at 585 nm using a spectrophotometer (SP8-500, Pye-Unicam Ltd. Cambridge, England). A regression equation was calculated from the absorbencies of the standards and was used to estimate the concentration of ammonia present in the samples.

2.1.3.3. **Determination of True Protein**

Triplicate samples of dried, milled test material were weighed (to the nearest 0.1 mg) into large boiling tubes. Deionised water (25 mls) was added to each tube which was then clamped over a microburner. The contents were boiled gently for 10 minutes and allowed to cool. Then, in turn, 10 mls of each of the following solutions was added:

Copper sulphate solution (60 g $CuSO_4.5H_2O$/l)
Sodium Hydroxide solution (12.5 g/l)

The tubes were mixed thoroughly and carefully boiled for a further 10 minutes. When cool, the contents and deionised water washings were transferred to a large centrifuge tube. This was centrifuged for 20 minutes at 2,500 rpm. The supernatant was decanted off and all the residue was transferred by washing with deionised water to a 75 ml digestion tube. The nitrogen in the samples was then measured as described for previously using the Indo-phenol Blue technique (2.1.3.2).

2.1.3.4. **Determination of Urea-Nitrogen**

Urea nitrogen was determined by difference. The ammonia-nitrogen of the sample was measured as described in Section 2.1.3.2 with and without the addition urease to the sample. The method was carried out as described in the Sigma Diagnostics Urea Nitrogen kit (Sigma Diagnostics, P.O.Box 14508, St Louis, MO 63178, USA).

2.1.4 Determination of Water Soluble Carbohydrate

Extraction

The feed to be analysed was dried and milled through a 1 mm screen. A subsample was taken (0.2 g to the nearest mg) and placed in a screw-topped bottle with 200 ml of distilled water. The bottle was attached to a shaking machine and shaken for 1 hour. The contents of the bottle were then filtered through Whatman No. 1 filter paper. The filtrate, except for the first few mls which was discarded, was retained for immediate determination of soluble carbohydrate.

Reagents

Anthrone Reagent

Anthrone reagent was prepared by adding, with stirring, 760 mls of concentrated sulphuric acid (98% w/w H_2SO_4) to 330 mls of distilled water. After cooling, 1 g of thiourea and 1 g of anthrone were dissolved in the acid. The reagent was stored in a refrigerator.

Glucose Standards

Standard solutions of glucose with concentrations ranging from 0 to 0.2 mg/ml were prepared by diluting a stock solution (0.8 mg/ml) of glucose immediately prior to use.

Procedure

Water extract (2 mls) was pipetted into a glass test tube followed rapidly by 10 mls of anthrone reagent. The mixture was shaken, loosely stoppered, and placed in a boiling water bath for 20 minutes. When cool the absorbance was measured at 620 nm using a spectrophotometer (SP8-500, Pye-Unicam Ltd. Cambridge, England). A regression equation for concentration of carbohydrate and absorbance at 620 nm was prepared from the glucose standards and was used to calculate the concentration of carbohydrate in the water extracts.

2.1.5 Determination of Neutral Detergent Fibre (NDF)

Reagents

Neutral Detergent Fibre Solution

Five litres of NDF solution was prepared by dissolving 93g of EDTA (disodium salt) and 34g of sodium tetraborate in distilled water with heat and constant stirring. When this had dissolved 150 g of sodium dodecyl sulphate was added with 50 mls of 2-Ethoxyethanol. In a second beaker 22.8 g of disodium orthophosphate (anhydrous) was dissolved. When all reagents were dissolved, the solutions were mixed together and diluted with distilled water to 5 litres. The pH of the solution was checked to be between 6.9 and 7.1.

Procedure

Approximately 500 mg of dried, milled sample was weighed (to the nearest mg) into a sintered glass crucible (Porosity 4) and was flushed through with 50 mls of petroleum ether (boiling range 40° - 60°C) using a Buchner flask. To remove any residual ether the crucible was then placed in an oven at 60°C for 10 minutes. When dry, the sample was transferred to a wide-necked 500 ml conical flask using a soft brush and 100 mls of NDF solution was added. The flask was fitted with a pear-drop condenser and the mixture was gently boiled for exactly 1 hour. The contents of the flask were filtered through a clean, preweighed sintered glass crucible (Porosity 1). The flask was washed with hot water and filtered the same sintered crucible. The residue was washed with hot water until detergent free and then rinsed with acetone. The crucibles were then placed in an oven at 100°C overnight, allowed to cool and were then reweighed. The residues were then ashed in a muffle furnace at 500°C for at least 3 hours, cooled and reweighed. The NDF content was calculated as follows:

$$\frac{\text{Crucible + fibre (mg) - Crucible + ash (mg)}}{\text{Sample weight (mg)}} \times 100 = \text{NDF\%}$$

2.1.6 Determination of Acid Detergent Fibre (ADF)

Reagents

Acid Detergent Fibre Solution

20 g of Cetyltrimethylammonium bromide was dissolved in 1 litre of 0.5M sulphuric acid

Procedure

A 2 g subsample of dried, milled sample was weighed, to the nearest mg, into a 500ml wide necked conical flask. This was fitted with a pear-drop condenser and was refluxed gently for 60 minutes. The hot contents of the flask were then filtered through a preweighed sintered crucible (porosity 1) using a Buchner flask. The flask was rinsed with hot water to ensure quantitative transfer of the residue to the crucible. The residues were washed with hot water until the filtrate was colourless. The crucibles were dried overnight in an oven at 100°C, cooled and reweighed. The residues were ashed in a muffle furnace at 500°C for at least 3 hours, cooled and reweighed. The ADF content of the sample was calculated as described for the NDF.

2.1.7 Lignin Analyses

Lignin was measured using the acetyl bromide technique as described by Morrison (1972).

2.1.8 Gross Energy

The gross energy of feed and faecal samples was determined on dried milled representative samples. The dried material was dampened slightly to allow compression into a cohesive pellet. A dry matter determination was carried out on the pelleted material and the pellet was placed in a preweighed bomb crucible and weighed. The pellets

were completely combusted in an adiabatic bomb (Parr 1241, Parr Instruments, Illinois) which recorded the heat of combustion. The gross energy per unit weight of dry matter could than be calculated.

2.1.9 Ether Extract and Acid Hydrolysed Ether Extracts

The extraction of lipid material soluble in 40 - 60°C petroleum ether was carried out in accordance with The Feedingstuffs (sampling and analysis) (Amendment) Regulations, 1985.

2.2 The Measurement of Calcium Soap Formation

The majority of the data concerning the measurement of the composition of the nutrients in feedstuffs is based on the proximate analysis of foods, a system originally devised by Henneberg and Stohmann. In this system fats and oils are measured using a repeated extraction with petroleum ether. This British Standard method is given in 'The Feeding Stuffs (Sampling and Analysis) (Amendment) Regulations 1985. This outlines two procedures, one in which the sample is extracted using 40-60°C petroleum ether, and the other which requires hydrolysis of the sample with hydrochloric acid before an identical ether extraction. The latter method is applicable to feeds in which the fats or oils cannot be extracted by petroleum ether alone.

In order to measure calcium soap formation as a proportion of the total lipid present in a sample of feed, it is necessary to make two measurements; firstly the total oil content of the feed, and secondly, the soap (or non-soap) content. Each of these methods have been developed from modifications of existing lipid extraction procedures to give reproducible measurements of total lipid and soap formation in feeds containing high levels of insoluble fatty acid soaps.

Measurement of Total Fat

Measurement of the fat content of feeds which have high levels of calcium soaps was shown to be unreliable using the British Standard method, despite extracting the sample with ether prior to acid hydrolysis and a further ether extraction. This is demonstrated in the following results given in Table 2.1. Feeds of a known fat content were prepared in duplicate, with and without $Ca(OH)_2$ treatment, and were analysed using the acid hydrolysed ether extract technique to try to establish the total oil and the proportion of the oil present as calcium soap. Samples were extracted with 40-60°C petroleum ether initially, refluxed in 3M HCl, filtered, dried and ether extracted again.

Table 2.1. Extraction of Fat Supplemented Feeds +/- $Ca(OH)_2$ Treatment Using British Standard Methods.

Sample (Known oil %)	Ether Extract (g/kg DM)	Total Oil (g/kg DM)
10%	37	109
10% + $Ca(OH)_2$	29	98
20%	128	198
20% + $Ca(OH)_2$	88	167
40%	301	367
40% + $Ca(OH)_2$	67	216
50%	433	470
50% + $Ca(OH)_2$	209	398
60%	457	505
60% + $Ca(OH)_2$	318	451

The results show that the total oil measurement is lower for the fat supplemented feeds which had been treated with $Ca(OH)_2$. This is due to the formation of calcium soaps. In the samples with the highest

levels of oil the differences in the total oil measured is greater. In the untreated samples a larger proportion of the oil is extracted in the initial ether extraction. When the samples were subsequently acid hydrolysed the protected oil is liberated from the soaps and may be lost during the filtration stage. This theory was tested by repeating the procedure and washing the filtrate with petroleum ether and returning the solvent + oil to the extraction vessel. This gave a marginal increase in the recovery of the oil. The extraction of lipids has been reviewed by Christie (1987). He discusses the use of various solvents or solvent combinations as extractants for lipids and outlines the method used by most lipid analysts, namely a modified 'Folch' procedure using chloroform-methanol. This method was modified as described by Grace and Body (1979) using an acidified mixture of chloroform and methanol and was the method used to extract the total oil in feeds with high levels of saponified oil.

Table 2.2 Extraction of Lipid from Feeds Containing High Levels of Calcium Soaps using Chloroform-Methanol Extraction with and without Addition of HCl.

Sample (Known % oil)	Non-Acid Extract g/kg DM	Acid Extract g/kg DM
20%	188	196
20% + $Ca(OH)_2$	143	192
40%	377	394
40% + $Ca(OH)_2$	341	396

Refluxing with non-acid chloroform-methanol did not recover total oil and showed that acidification of the solvents was required for quantitative extraction of calcium soaps. Comparison of these results (given in Table 2.2) with those for the British Standard method show that this gives more quantitative recovery of total lipid. This method was therefore used throughout as the standard measurement of the total

oil in feeds.

Measurement of Calcium Soaps

Since insoluble fatty acid soaps are variably extracted with different solvents, extraction with petroleum ether with correction for the amount of soap extracted was chosen as the reference method. In this method the feed sample was extracted in the routine manner, using the Soxtec apparatus, and the oil that was extracted was ashed in a muffle furnace and then analysed for calcium. Using the weight of total oil, the weight of ether extract and the weight of calcium in the ether extract, it is possible to calculate the proportion of the oil that was present as soap in the feed sample. This method makes the following assumptions;

1. That all the calcium measured in the ether extracted oil is combined with the oil as soap.

2. That the difference between the total oil measurement and the ether extract is due to insoluble fatty acid soaps.

Both of these assumptions require further investigation. The first may not hold if there is a large excess of free calcium in the feed sample, and the second assumption is also open to speculation as differing views have been expressed by different authors as to the composition of the difference between acid and non acid hydrolysed extracts.

The total calcium soap present in a feed was calculated as follows;

$$T - E + \frac{(E \times \text{mg Ca/g } E \times \text{ave. mol. wt. of fatty acids})}{40}$$

Where T = Total Oil (acidified chloroform-methanol extract)

E = Ether Extract

This was then expressed as a percentage of the total oil to give the

percentage soap formation.

2.3 Mineral Analyses

All mineral analyses were carried out by the analytical services unit, Gibbs Yard, SAC (Auchincruive).

2.3.1 Plasma Calcium and Magnesium

Levels of these minerals in plasma samples were determined by dilution with lathanum oxide buffer and measurement using inductively coupled plasma atomic emission spectrometry.

2.3.3 Phosphorus

Plasma phosphorus levels were determined using Ames Sera Pak.

2.3.4 Copper

Copper measurements in feed samples were carried out using a dry ash preparation by placing in a muffle at 480°C overnight and then dissolving in 0.6N HCl. Levels of copper could then be measured by inductively coupled plasma atomic emission spectrometry.

2.4 Digestibility Measurements

2.4.1 *In vitro* Organic Matter Digestibility (IVOMD) Determination

The IVOMD of feed samples was determined according to the method developed by Tilley and Terry (1963) and modified by Alexander and McGowan (1966).

The sample was dried at 60°C and milled through a 1mm screen. Two 500mg subsamples were weighed directly into glass tubes. One litre of rumen liquor was obtained from each of three fistulated sheep and then filtered as quickly as possible through 2 layers of muslin. The rumen liquor was swept with CO_2 and added to four times its volume of McDougalls buffer (1948) previously saturated with CO_2 and at pH 6.9. Ammonium sulphate solution (1 ml of a molar solution) was added per 50 ml of rumen liquor/buffer mixture. The tubes were swept with CO_2 and then capped with rubber bungs fitted with bunsen valves. The tubes were then incubated in a water bath at 38.5°C and the pH was adjusted electrometrically to 6.9 after 24 hours. The digestion was carried out over a period of 48 hours during which the tubes were gently swirled from time to time to ensure mixing of the contents.

To terminate the incubation, 1.5 ml followed by a further 2.5 mls of 6M HCl as added to each tube. Aqueous pepsin (5 mls containing 0.12g of 1:10,000 pepsin from Zimmerman and Hoppes, Milton Keynes, England) was injected into each tube after the electrometric adjustment of the pH to 1.2. The tubes were incubated for a further 48 hours at 38.5°C, again with gentle swirling from time to time.

After 48 hours the incubation was terminated by filtering the contents of the tubes in the presence of a filter-aid (hyflo supercel) through fibreglass filterpaper (Whatman GF/A). Filter papers and residues were dried at 100°C, cooled and weighed and then placed in a muffle furnace at 480°C for 16 hours and cooled and reweighed. A parallel estimate of the total OM of the original sample was carried out. The procedure was also carried out on control tubes which contained only rumen liquor and buffer. The digestibility coefficients of the samples were calculated as follows:

$$\text{IVOMD \%} = \frac{\text{Sample OM (mg)} - \text{Residue OM (mg)} - \text{Control OM (mg)}}{\text{Sample OM (mg)}} \times 100$$

2.4.2 *In Sacco* Digestibility

The *in sacco* digestibility of feeds was determined by incubating feeds in polyester bags in the rumen of sheep. Mature Suffolk cross wethers fitted with permanent rumen cannulae were used. The sheep were maintained on a diet of 200 g/d Ewebol concentrate and 800 g/d chopped meadow hay fed in two feeds at 9.00 am and 5.00 pm. Each sheep could accommodate up to 5 bags at one time. The bags were made of synthetic polyester fibre (Sericol Group Ltd, London) with a pore size of 40 - 50 um sewn into bags measuring approximately 10 x 21 cms with a rounded base.

Between 3 and 5 grammes of feed was placed in each dried, preweighed bag and was weighed to the nearest mg. The neck of the bag was twisted and then tightly bound with nylon string. The long ends of the string were passed through polythene tubing (approx. 15 cms) and then through a central hole in a rubber bung designed to fit the rumen cannulae of the sheep. The strings from all bags were tied together and to a small piece of polythene tubing to prevent it passing back through the hole.

Bags were incubated in the rumen of the sheep for different times ranging up to 48 hours, usually 0 time (0 h) bags were included as one of the times. These bags were washed without incubation in the sheep.

After incubation the bags were rinsed down under the cold tap, detached from the tubing and bung, and placed in an automatic washing machine on the cold rinse cycle. After this the bags were dried in an oven at 60°C until they had reached a constant weight (usually after 48 hours). The bags were reweighed and the digestibility of the residual dry matter was calculated.

The digestibility of any other fraction of the feed such as NDF or OM was required then this was determined on a sample of the original feed and on the residues remaining in the bags after incubation.

2.4.3 Measurement of Cotton Strip Digestibility

Cellulose digestion was measured by incubating 1.5 x 12 inch strips of unbleached calico in the rumen for 24 hours. Three strips were incubated in each sheep every day. The cotton strips were cut from the cloth using pinking shears to minimise loss of DM by fraying. The strips were then placed in an oven at 60°C for a few hours, allowed to cool and then weighed to the nearest mg. Each strip was tied at the mid point with nylon string. The strings were passed up polythene tubing to prevent twisting during incubation, and through a hole in the middle of a 35 mm diameter rubber bung which fitted securely in the cannula of the sheep. After incubation the strips were hand washed in a warm dilute solution of non-biological washing powder according to the manufacturer's recommendations. The strips were thoroughly rinsed in cool water and were dried for a minimum of 48 hours at 60°C. The strips were then reweighed and the DM digestibility was determined.

2.4.4 Measurement of Neutral Detergent/Cellulase Digestibility

NCD digestibilities were determined as described by M.A.F.F. (1989)

2.5 Lipid Analyses

2.5.1 Extraction of Lipids from Rumen Samples

Lipids from the milled samples were extracted by weighing approximately 200 mg (to the nearest 0.1 mg) into a round bottomed flask. Several glass beads were added to prevent bumping. 15 mls of methanol was added and was refluxed gently for 15 minutes. This was cooled and 30 mls of chloroform was added. The mixture was refluxed again for a further 20 minutes. this was allowed to cool and then filtered through Whatman 41 filter paper into a measuring cylinder. Flask and filter paper were washed with 2:1 chloroform/methanol

mixture. Potassium chloride (0.88%) was added to 1/5th of the volume of solvents. The measuring cylinders were shaken gently and the contents were allowed to separate into two layers overnight. The upper aqueous layer was discarded and the lower chloroform layer containing the lipid was placed in a preweighed round bottomed flask. The chloroform was evaporated off under vacuum at 45°C and once completely dried the flask was reweighed to give the weight of lipid extracted. The lipid was then redissolved in 5 mls of chloroform for storage. All samples were derivatised and analysed by GC as described in section 2.5.4.

2.5.2 Extraction of Plasma Lipids

The samples were centrifuged at 3,000 rpm for 15 minutes and the supernatant plasma samples were taken off and stored at -20°C until required for further analysis. The lipid from the plasma samples was then extracted by the method of Nelson and Freeman (1959) and was stored in chloroform in glass vials.

2.5.3 Separation of Plasma Lipids into Lipid Classes

The plasma lipids were separated into the separate lipid classes using a thin layer chromatographic (TLC) technique as described by Noble *et al.*, (1990). Following visualisation and identification the different classes of plasma lipids were eluted from the silica gel.

2.5.4 Derivatisation and Gas Chromatoghraphic (GC) Analysis

Separated lipid fractions were transmethylated by refluxing with methanol:toluene:sulphuric acid (20:10:1) in the presence of pentadecanoic acid as described by Christie *et al.*, (1970). Blank determinations were carried out to account for branch chain fatty acids. The methyl-esters of the fatty acids were analysed by gas chromatography using the method described by Noble *et al.* (1990).

2.5.5 **Milk-fat Extraction**

The lipid from milk samples was extracted by taking a 10 ml subsample in to a large glass stoppered tube. To this 10 mls of chloroform and 20 mls of methanol was added. This was homogenised for 30 seconds using a laboratory mixer emulsifier (Silverson Machines Ltd. Waterside, Chesham, Bucks.). A further 10 mls of chloroform was added and the mixture was filtered through Whatman 41 filter paper. 10 mls of 0.88% KCl was added, the tube was stoppered and shaken gently. The aqueous and chloroform layers were allowed to separate overnight. The top aqueous layer was discarded and the chloroform layer was dried under vacuum using a rotary evaporator. The dry lipid was then dissolved in 1 ml of hexane before methylation and GC analysis.

2.5.6 **Milk-fat Methylation and Gas Chromatographic (GC) Analysis**

The extracted milk fats were methylated prior to GC analysis using the following method. Approximately 25 ul of melted fat sample was added to 200 ul of heptane in a 5 ml screw capped test tube and thoroughly mixed. 10 ul of methanolic potassium hydroxide was added and the mixture was vortex mixed for a further 20 seconds. 1.8 ml heptane was added and the mixture was vortex mixed again. The mixture was allowed to separate completely into two separate layers, 2ml of the upper layer was then put into an autosampler vial for injection onto the GC.

The methylated samples were analysed by gas chromatography using an AI 93 Gas Chromatograph fitted with a flame ionisation detector (A.I. Cambridge Ltd., Cambridge, UK). The column was 2m X 2mm i.d. glass packed with 10% SP-2330 on Chromosorb W-AW 100-120 mesh (Supelco Inc., Bellefonte,Pa, USA). The analysis conditions were as follows: 60°C. for 2 min. then a temperature rise of 20 °C./min. up to 200 °C. then held for 10 min. The injector and detector temperatures were both 250°C. The carrier gas was nitrogen at a flow rate of 20 ml/min. and the hydrogen and air flowrates were 25 and 250 ml/min. respectively. 1ul of sample was injected. The data was collected on a Shimadzu CR3A integrator (Dyson Instruments Ltd., Tyne & Wear).

2.6 Carbohydrate Analyses

2.6.1 Hydrolysis with 72% Sulphuric Acid

A known weight of sample was taken (between 15 and 25 mg) and 1ml of 72% H_2SO_4 was added. This was stirred using a glass rod and thoroughly mixed over a one hour period at room temperature. 11.5 mls of distilled water was added with 1 ml of meso-inositol solution (2 mg/ml) as an internal standard. This mixture was then heated at 100°C for 16 hours, cooled, neutralised with barium carbonate and then filtered. A small amount of Dowex 50w x 8 (H^+) resin was added to the filtrate and the solution was then taken off and evaporated to dryness.

2.6.2 Hydrolysis with 2M Trifluoroacetic Acid

A known weight of sample was taken (between 10 and 20 mg) and was placed in a Teflon capped glass vial. To this was added 1 ml of 4M TFA and 1 ml of meso-inositol solution (2 mg/ml) to act as an internal standard. This was heated overnight at 100°C and allowed to cool. The solution was removed to a stoppered test tube and evaporated to dryness.

2.6.3 Derivatisation

To the dry hydrolysed samples 0.5 ml of 5% hydroxylammonium chloride solution in dry pyridine was added. This was heated at 100°C for 15 minutes and then allowed to cool. Acetic anhydride (0.5 ml) was then added and the mixture was heated at 100°C for a further hour. After cooling, 2 ml of chloroform was added with 5 ml of 2M NaOH. The tubes were shaken carefully but vigorously and the pH of the aqueous layer was checked for alkalinity with indicator paper. The aqueous layer was then discarded and the chloroform layer was washed again by shaking with 5mls of 1M HCl. The acidity of the aqueous phase was checked

with indicator paper. The aqueous layer was removed and the chloroform layer was washed with 5 mls of distilled water. The aqueous layer was removed again and the chloroform layer was dried using a little anhydrous sodium sulphate. The chloroform solution was removed to a fresh test tube and was dried in a stream of air.

2.6.4 Gas Chromatographic Analysis

The gas chromatographic analysis was carried out on a Packard 439 instrument fitted with a flame ionisation detector. The column (2m x 2mm i.d.) was 5% OV 225 on Chromosorb W AW DCMS (100-120 mesh) and operated isothermally at 210°C with nitrogen (40ml/min) as the carrier gas. The injector and detector were maintained at 250°C. the sample was dissolved in 200ul of chloroform and a 5ul sample was injected directly onto the column. The data were collected on a Spectra Physics 4290 integrator.

2.6.5 Ultra Violet Absorption Spectra

The optical density of samples was measured over wavelengths ranging from 250 - 400 nm using a scanning spectrophotometer (Shimadzu 420). Approximately 10 mg of the soluble substances were dissolved in 10 mls of distilled water and diluted to give readings within the range of the instrument.

2.7 Miscellaneous Analyses

2.7.1 Analysis of Rumen Liquor Volatile Fatty Acid Content

Reagents

Preservative Solution

Preservative solution was prepared by dissolving 15 g of Metaphosphoric Acid (flake - approx. 60% HPO_3) in 100 ml of distilled water.

Internal Standard

756 mg of oxalic acid and 160 mg of 2 - ethyl n - butyric acid was dissolved in 100 ml of distiller water.

VFA Standard Solution

Stock Solution

Stock solution was prepared by making the following quantities up to 50 ml in a volumetric flask:

Acetic Acid	3.00 g of 1M
Propionic Acid	3.70 g of 1M
Butyric Acid	4.40 g of 1M
Isobutyric Acid	0.440 g of 0.1M
Valeric Acid	0.510 g of 0.1M
Isovaleric Acid	0.510 g of 0.1M

Working Standard

The working standard was prepared by mixing 5 ml of acetic and 2 ml of each of the other stock solutions in a 100 ml volumetric flask and making it up to the 100 ml mark with distilled water.

Procedure

Rumen liquor was removed from the sheep via the cannula using flexible polythene tube fitted to suction equipment as described by Alexander and McGowan (1969). Using a 10 ml wide-mouthed pipette, 8 ml of liquor was immediately taken and mixed with 2 mls of 15% metaphosphoric acid in a stoppered plastic centrifuge tube. Samples were centrifuged at 3000 rpm at 4°C for 10 minutes. The supernatant was frozen at -20°C in a 7 ml Bijou bottle awaiting analysis.

Samples were defrosted and, if necessary, spun down again to remove any particulate matter at 1000 rpm. Into a 1.5 ml microtube 0.75 ml of liquor and 0.75 ml of internal standard solution was pipetted. 0.75 ml aliquots of the working standard solution were treated in the same way. The microtubes were centrifuged again and the supernatant placed in an auto sampler vial ready for GC analysis. GC analysis was carried out as described in Section 2.7.4 with the addition of 1 ml of 0.1M NaOH to neutralise the metaphosphoric acid.

2.7.2. Measurement of BOD_5

BOD_5 was measured by incubating 20 mls of effluent diluted in 580 mls of distilled water, in a waterbath at 20°C, in darkness, for 5 days. Levels of oxygen were measured using an oxygen electrode (model 8012-100, EIL, Hanworth Lane, Chertsey, Surrey, UK). BOD_5 were estimated allowing for the oxygen levels in a blank incubation flask.

2.7.3 Water Extraction of Silage Samples

Water extracts of the core samples were made by shaking 20g of silage in 100 mls of distilled water for 1 hour followed by filtration through a coarse muslin.

2.7.4 Gas Chromatographic Analysis of Water Extracts of Silage Samples

The extracts were analysed by GC for ethanol, lactic acid and VFAs according to the method of Fussell and McCalley (1987). The samples were prepared as follows:- 5 mls of water extract was mixed with 1 ml of pivalic acid solution (0.22 % w/v) and 4 mls of oxalic acid solution (0.945% w/v). The mixture was centrifuged at 4°C and 3,000 rpm for 10 minutes. The supernatant was placed in an autosampler vial and 1 ul was injected injected into a glass GC column (2m x 2mm packed with Graphpac GC 60 -80 mesh containing 0.3% Carbowax 20M and 0.1% H_3PO_4 (Alltech Associates, Carnforth, UK)). The measurement was carried out isothermally at 175°C using N_2 as the carrier gas at a flow rate of 24 ml/min. An electronic integrator was used to quantify peak areas.

2.7.5 Milk Constituents

The proportions of milk solids were analysed by the Dairy Chemistry Department, SAC, Auchincruive, Ayr. The preserved milk was analysed for butterfat, protein and lactose. Butterfat was measured using the Gerber Method (BS 696). Milk protein was measured using AOAC Semi-Micro Kjeldahl Method and lactose was measured polarimetrically according to the method described by Grimbleby (1956).

2.7.6 Assessment of Silage Fermentation

The titration technique of Moisio and Heikonen (1989) was used to provide a rapid assessment of the progress of fermentation when investigating the ensilage of NID with grass. The method depends on measurement of the buffering curve over the range pH 2 to 5 of juice expressed from the silage. This allows prediction of the following parameters; lactic acid + formic acid, acetic + propionic + butyric acids, total nitrogen, amino acid nitrogen and residual sugar.

CHAPTER THREE

OPTIMISATION OF $Ca(OH)_2$ TREATMENT OF SPENT GRAINS

3.1 Introduction

The use of calcium hydroxide to improve the nutritional value of fibrous feeds is not a novel concept. It has been used to treat such feeds as wheat straw, maize cobs and maize stalks (Patterson *et al.*, 1980). Calcium hydroxide has several advantages over other alkalis such as sodium hydroxide, more commonly used to upgrade fibrous feedstuffs, in that it is cheaper, less corrosive and safer to handle. Additionally, the calcium residues left in the feeds are less toxic to the animal than high levels of sodium or ammonia which could result from sodium hydroxide or ammonia treatments. However, the major disadvantage of using calcium hydroxide is that it has been shown to be less effective at upgrading fibrous feedstuffs, requiring longer for the upgrading reactions to occur. Whereas sodium hydroxide has been shown to achieve maximum benefit in the treatment of straw within minutes of treatment, (Junker, 1976), Klopfenstein and Owen, (1981) and Asadpour (1979) reported that calcium hydroxide required 10 - 14 days to react at ambient temperatures.

The optimal treatment conditions required for calcium hydroxide treatment of particular feedstuffs have not been established in all cases. Since the mode of action of different alkalis on fibrous materials is assumed to be similar, the levels of application and treatment conditions have also been assumed to be similar to those for sodium hydroxide treatment. Treatment conditions most commonly used range up to 50 g $Ca(OH)_2$/kg DM and in all reported cases the treatment has been applied cold.

One solution to the slow reaction of calcium hydroxide is to increase the treatment temperature. Klopfenstein (1984) reported that treatment of maize cobs with water (to 60 % moisture) and 50 g $Ca(OH)_2$/kg DM at 45°C in a large insulated container produced an exothermic reaction in which the temperature increased to 70°C and

then slowly decreased. The maize cobs were fed to lambs and treatment was shown to increase dry matter intake from 1000g/d to 1455 g/d. *In vitro* dry matter digestibility was also increased from 0.43 to 0.54.

A further requirement for calcium hydroxide treatment is moisture. Patterson *et al.* (1980) treated maize cobs, maize stalks and wheat straw with 50 g $Ca(OH)_2$/kg DM at 20, 40 and 60% moisture for a minimum of 15 days. They reported that the highest digestibilities and intakes by lambs were at 40% moisture for the wheat straw and maize cobs. It was concluded that at <40% moisture there was insufficient water in which the chemical could react. At higher moisture levels the residues fermented when ensiled, the fermentation acids neutralising the alkali before the reaction could reach completion.

Treating spent grains with calcium hydroxide has two potential benefits in terms of ruminant digestion: firstly, the upgrading action of the alkali on the fibrous fraction of the feed, and secondly, under alkaline conditions the triglyceride present in the grains may be hydrolysed to free fatty acids which, in the presence of divalent cations, can form insoluble fatty acid soaps.

The aims of the following series of experiments were:-

1. To optimise conditions for $Ca(OH)_2$-treatment of Malt Distillers Grains (MDG).

2. To investigate the effects of NaOH-treatment of MDG.

3. To investigate the effects of $Ca(OH)_2$-treatment of other spent grains.

Alkali treatment was carried out by mixing the wet grains (either fresh or grains which had been frozen and allowed to thaw) thoroughly with alkali in an industrial food mixer. Sodium hydroxide was applied as a 40% solution and calcium hydroxide was applied as industrial grade lime (Limbux, ICI). After mixing, the grains were sealed in polythene bags and held at various temperatures for various times

depending on the treatment conditions. Grains were dried at 60°C and milled through a 1mm screen for analysis or left whole for *in sacco* digestibility measurements.

The effect of alkali treatment on the digestibility of spent grains in ruminants was measured using the *in sacco* and *in vitro* techniques given in Section 2.4. The action of alkali on the fibre fraction of the grains was measured using neutral detergent fibre (NDF) measurements (section 2.1.5) and measurements of the water soluble carbohydrate (WSC) content (section 2.1.4), and on the lipid fraction by measurement of the percentage soap formation (section 2.2). Measurements were made initially using MDG and were repeated on a smaller scale using other sources of spent grain such as Brewer's Grains and Wheat Dreg (the wet by-product from a grain distillery). Calcium hydroxide treatment was also applied to other spent grains prepared in the laboratory, including wheat, barley, rye, maize and oats.

3.2 Optimisation of $Ca(OH)_2$ Treatment of Malt Distiller's Grains

Two experiments are described in this section, the first, a 64 plot, 4 x 4 x 4 factorial design experiment which investigated the effects of treatment time and temperature and level of $Ca(OH)_2$ on MDG digestibility. The second was carried out on the basis of the results from the first and investigated only the effects of treatment time on the $Ca(OH)_2$ treatment of MDG.

Experiment 1

3.2.1 Methods

A 64 plot (4 x 4 x 4) factorial design experiment using four levels of $Ca(OH)_2$ application (0, 40, 80 and 120 g/kg DM), four treatment temperatures (20, 40, 60 and 80°C) and four treatment durations (24, 48, 72 and 96 hours) was carried out. The requirement for moisture in the treatment process was not investigated as the MDG were of

approximately 25% DM and water was therefore not considered to be a limiting factor. The greatest effects of moisture on alkali treatment have been shown when the fibrous residues are of <40% DM (Patterson *et al.*, 1980)

Final pH was recorded immediately after treatment was complete, the grains were dried and soap formation was measured. *In vitro* (IVOMD) and 0h and 24h *in sacco* digestibility (ISOMD) measurements were carried out on all 64 treatments. NDF and WSC measurements and NDF digestibility *in sacco* (ISNDFD) were carried out on the following 10 treatments, selected to give the full range of each treatment parameter:

Table 3.1 Samples Selected for Measurement of WSC and NDF and NDF *In Sacco* Digestibility.

g $Ca(OH)_2$/kg DM	Time (hours)	Temperature °C
80	24	20
80	24	40
80	24	60
80	24	80
80	48	60
80	72	60
80	96	60
0	24	60
40	24	60
120	24	60

Results were analysed statistically by analysis of variance using the EDEX 6H statistical package. An example of the statistical analysis carried out is given in Appendix 1.

3.2.2 Results

The results showing the effect of treatment on the mean values for pH, soap formation and digestibility *in sacco* and *in vitro* are given in Table 3.2.

Table 3.2 Effect of Treatment Conditions on pH, Soap Formation and Digestibility

	Final pH	% Soap Formation	In Sacco 0h OMD	In Sacco 24h OMD	In Vitro OMD
g $Ca(OH)_2$/kg DM					
0	4.01^a	27.06^a	0.246^a	0.634^a	0.513^a
40	7.25^b	40.82^b	0.247^a	0.662^b	0.545^b
80	10.09^c	50.97^c	0.507^b	0.836^c	0.647^c
120	11.06^d	63.91^d	0.574^c	0.863^d	0.652^c
Time (Hours)					
24	8.49^a	46.59	0.376	0.760	0.603
48	7.97^b	45.45	0.361	0.748	0.589
72	8.10^b	42.87	0.404	0.735	0.576
96	7.84^b	47.85	0.433	0.751	0.588
Temperature (°C)					
20	8.92^a	45.14^{ab}	0.309^a	0.737^a	0.591^{bc}
40	8.21^b	42.70^a	0.371^b	0.732^a	0.583^{ab}
60	7.88^c	51.02^b	0.408^c	0.740^a	0.604^c
80	7.40^d	43.90^a	0.487^d	0.785^b	0.574^a

Means in the same column for the same parameter with different superscripts differ significantly at $p<0.05$

pH

$Ca(OH)_2$ level had the greatest effect on the final pH with each value being significantly different ($p<0.05$) from the others. Final pH was significantly reduced by each increment of treatment temperature ($p<0.05$) but extending the treatment time beyond 48 hours had no significant effect.

The analysis of variance of these results showed that there were significant interactions between level of $Ca(OH)_2$ and treatment temperature ($p<0.05$), and between treatment time and level of $Ca(OH)_2$ ($p<0.001$) for final pH. The effect of these interactions on the final pH can be seen in Figures 3.1a and 3.1b. Final pH was generally inversely related to temperature for the same level of $Ca(OH)_2$ but the effect was greater at higher $Ca(OH)_2$ levels. When the grains were treated without $Ca(OH)_2$ the final pH increased slightly with increasing treatment time. At 40 g $Ca(OH)_2$/kg DM the pH drifted down to neutrality after 96 hours, whilst at the 80 and 120 g $Ca(OH)_2$/kg DM levels the pH the reductions in pH were 2.38 and 2.15 pH units respectively.

Soap Formation

Soap formation was not significantly affected by treatment time or by temperature, but increased significantly ($p<0.05$) with level of $Ca(OH)_2$. The low level of $Ca(OH)_2$ treatment gave soap levels of 40.8% which was increased to 63.9% at the highest level (120 g $Ca(OH)_2$/kg DM). The differences between the total oil and ether extract measurements for the unlimed draff and the ether extract and the acid hydrolysed extract for the unlimed samples was thought to reflect the extraction of complex polar lipids by the acid procedure. There were no significant interactions between treatment conditions affecting soap formation.

Figure 3.1a Effect of $Ca(OH)_2$ and Temperature on Final pH

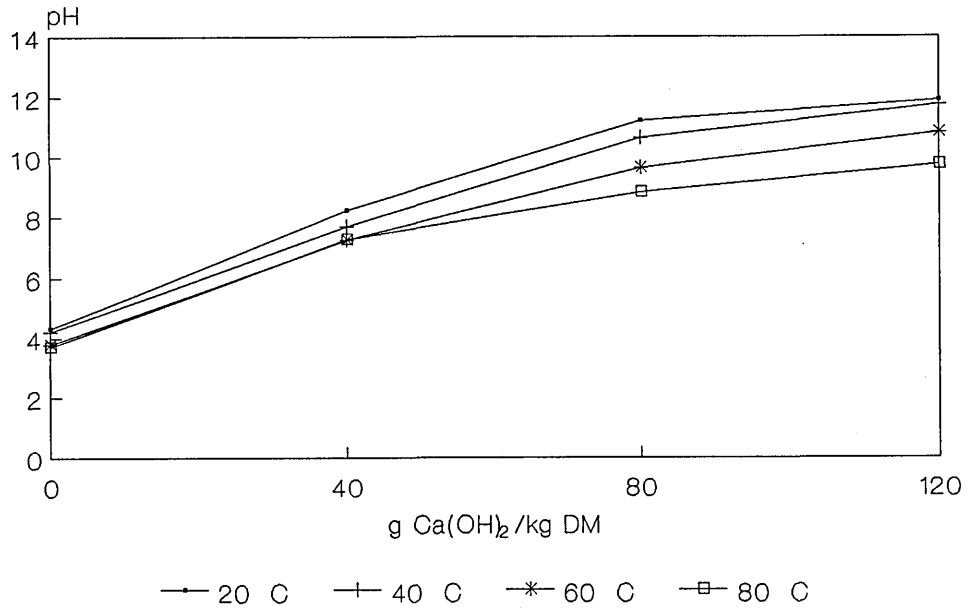
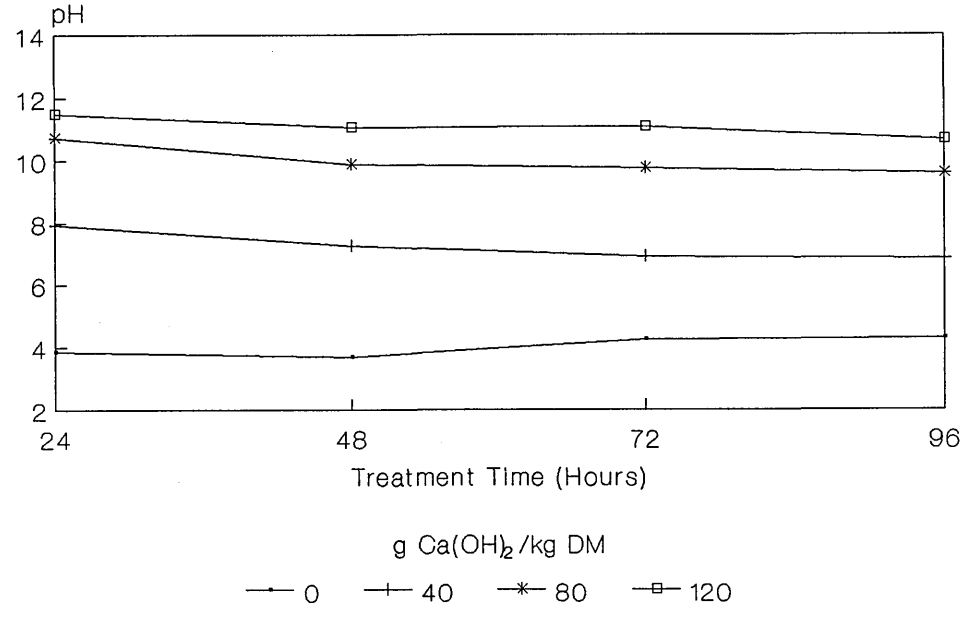


Figure 3.1b Effect of $Ca(OH)_2$ and Time on Final pH



Digestibility

Figure 3.2 shows the effect of the various treatment parameters on 0h and 24h ISOMD on MDG. The greatest effects of treatment were due to the level of $Ca(OH)_2$ application. The 0h ISOMD was more affected by level of $Ca(OH)_2$ and treatment temperature than the 24h ISOMD. Treating the spent grains with 120 g $Ca(OH)_2$/kg DM increased the 0h ISOMD from 0.25 to 0.57 and the 24h ISOMD from 0.63 to 0.86. This suggests that treatment with $Ca(OH)_2$ increased the digestibility of spent grains by preferentially solubilising (or particularising) the more digestible fraction of the spent grains. A consequence would be that the residual insoluble organic matter that remained in the bag after small particles and soluble matter had been lost, was less digestible over 24 hours than the insoluble residue of the untreated material.

There was a significant interaction between the level of $Ca(OH)_2$ and the treatment time on the 0h ISOMD losses which is shown in Figure 3.3. In general there was a sharp increase in the 0h OMD when the level of $Ca(OH)_2$ increased from 40 to 80 g/kg DM but the increase between 80 and 120 g/kg was less. This pattern of response was common to all treatment times, but the effect increased with time. The 0h ISOMD was greatest at 30 g $Ca(OH)_2$/kg DM and 96 hours treatment time when digestibility was 0.67.

Figure 3.4 shows the effect of treatment temperature and level of $Ca(OH)_2$ on the IVOMD of spent grains. Both level of $Ca(OH)_2$ and treatment temperature significantly ($p<0.05$) increased IVOMD. The interaction between these parameters was also significant ($p<0.05$). The IVOMD of the grains was reduced at higher temperatures which may have been due to the formation of resistant linkages (Crowe *et al.*, 1985) in the insoluble fraction of the grains.

These results can be summarised:

1. Soap formation, 24 hour ISOMD and IVOMD were affected only by level of $Ca(OH)_2$.

Figure 3.2 Mean In Sacco
Organic Matter Digestibility

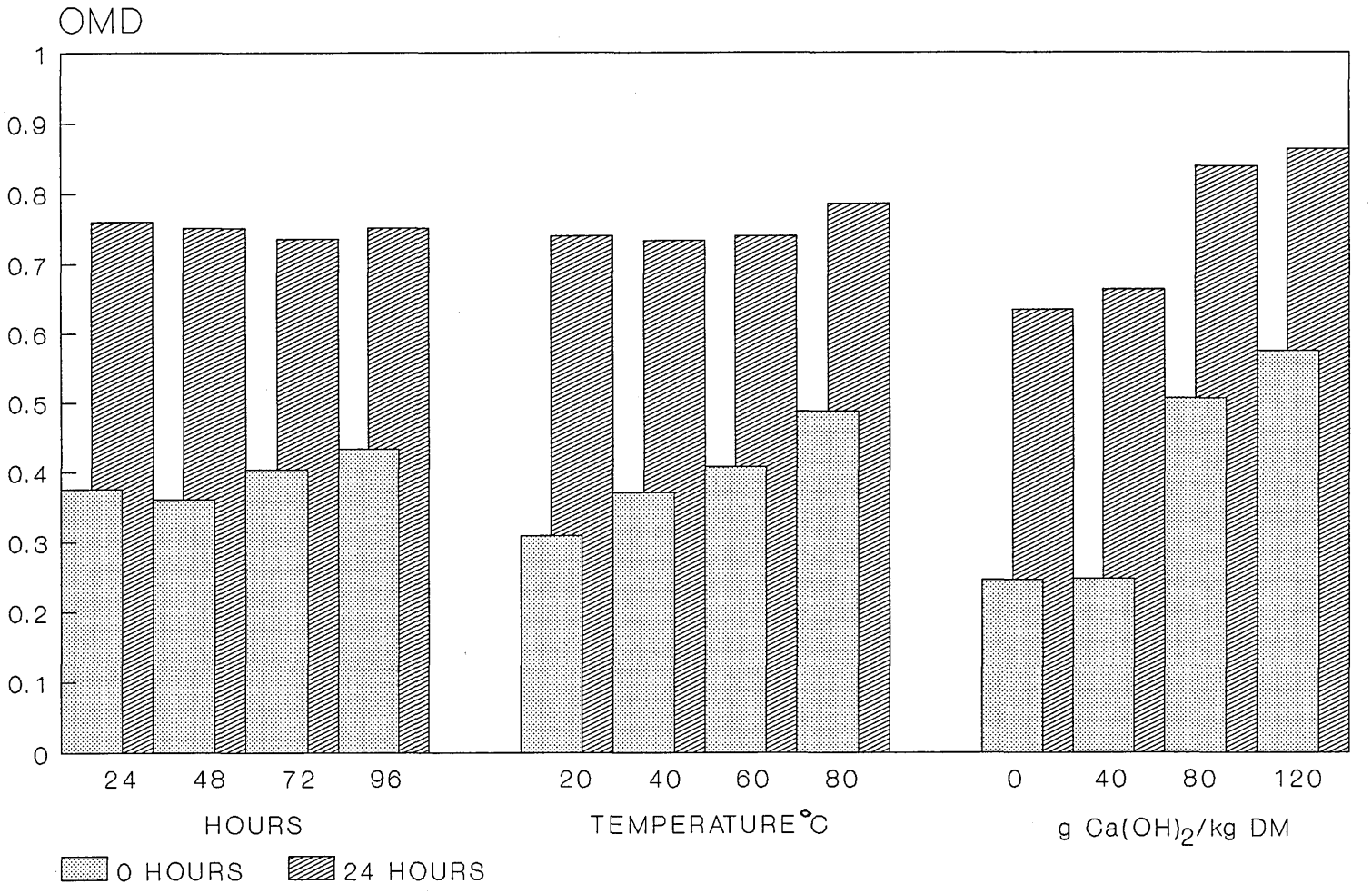


Figure 3.3 Effect of $Ca(OH)_2$ and Time on 0 h In Sacco OMD

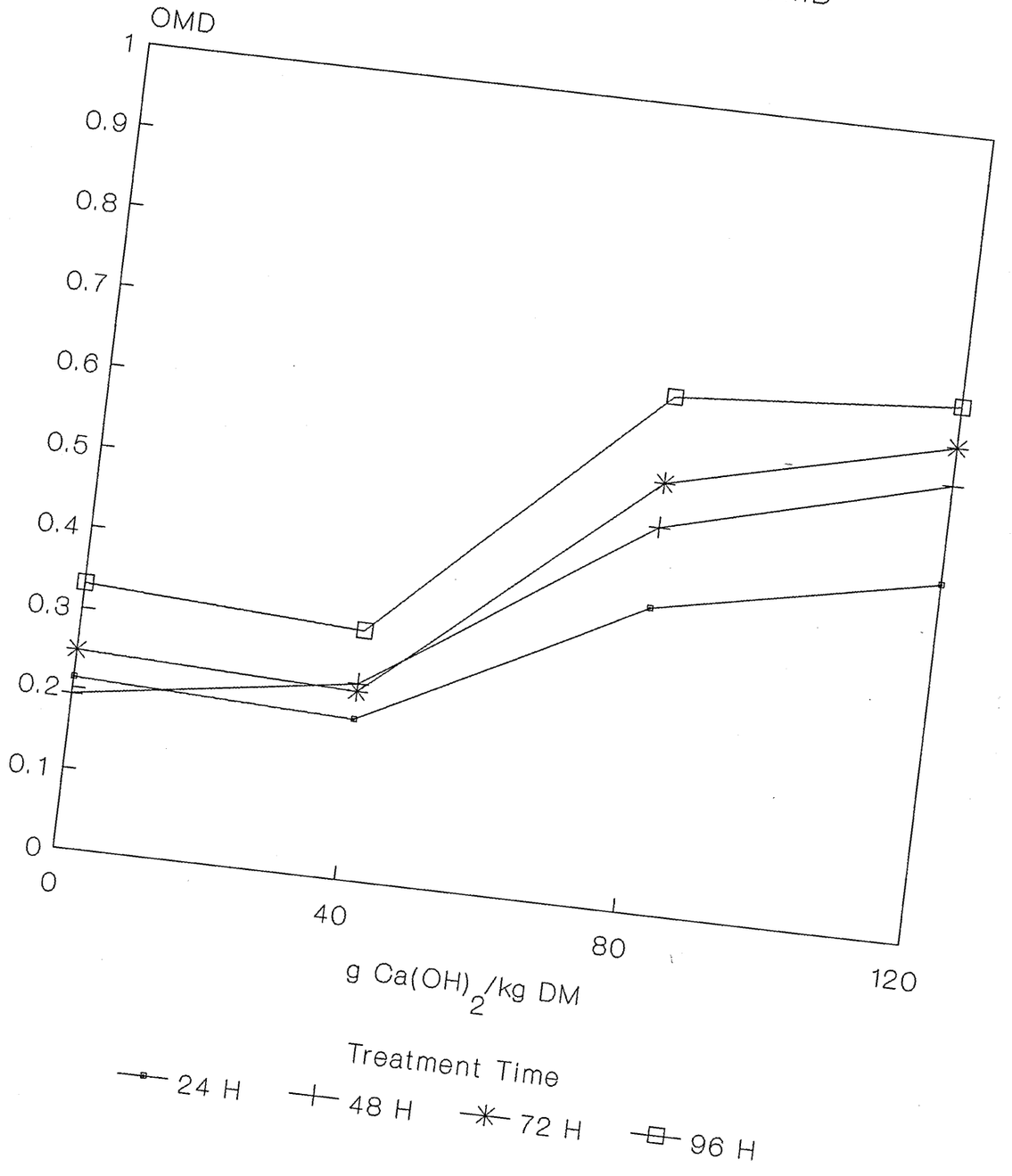
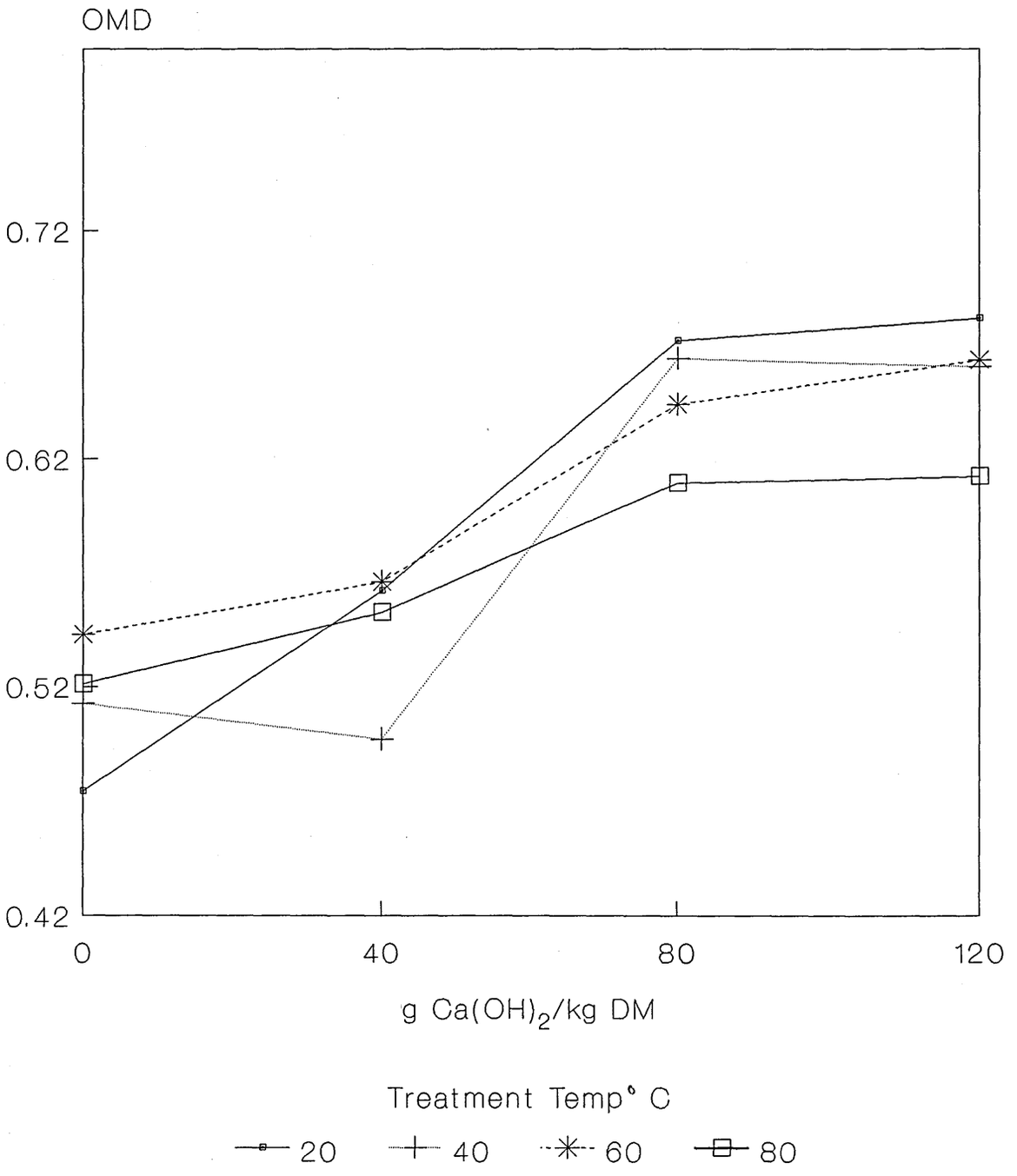


Figure 3.4 Effect of $Ca(OH)_2$ and Temperature on IVOMD[2]



2. The 0 hour ISOMD losses were affected by level of $Ca(OH)_2$ and treatment temperature and by their interaction.

3. The greatest increases in digestibility occurred when the level of $Ca(OH)_2$ was increased from 40 to 80 g/kg DM. The increase from 80 to 120 g/kg DM gave smaller responses. The optimal level of $Ca(OH)_2$ was therefore taken as 80 g/kg DM.

The values for measurements of WSC and NDF content and ISNDFD made only on selected samples are given in Table 3.3. The effects of treatment on WSC and of NDF contents are shown in Figures 3.5a and 3.5b. They show the effects of the different treatment parameters on the fibre and soluble carbohydrate fractions. The main effects were due to treatment temperature and level of $Ca(OH)_2$. Increasing either treatment temperature or level of $Ca(OH)_2$ reduced the NDF whilst increasing the WSC contents. Treatment time had no effect on NDF or WSC. The increase in WSC and reduction in NDF explain, in part, the increase in 0 h ISOMD observed.

The effect of treatment on the 0h and 24h ISNDFD is given in Table 3.3 and shown in Figure 3.6. Comparison with Figure 3.2 for the OMD shows that the effects of level of $Ca(OH)_2$ on NDF and OM are similar. Both the 0 h and the 24 h digestibilities for OM and NDF were increased with level of $Ca(OH)_2$, the greatest increase occurring between 40 and 80 g $Ca(OH)_2$/kg DM. The similarity of the *in sacco* OM and NDF digestibility responses suggests that the effect of treatment on the OM as a whole was largely determined by the effect on the NDF fraction. The response of 0h ISNDFD to treatment suggests that the action of $Ca(OH)_2$ was to particularise the more digestible parts of the fibre. With the NDF the 0h loss could not be due to solubilisation since soluble material would not be measured by the NDF technique. However, an increased content of small particles would lead to increased 0h losses from the bags due to leakage through the 40um pores of the bag material. This suggests that the increase in 0h ISOMD observed was due to particularisation and not just solubilisation of the OM. Further investigation into the fate of this fraction lost from the nylon bag in the rumen is required to describe more fully the

Figure 3.5a Effect of $Ca(OH)_2$, Treatment Temperature and Time on NDF Content

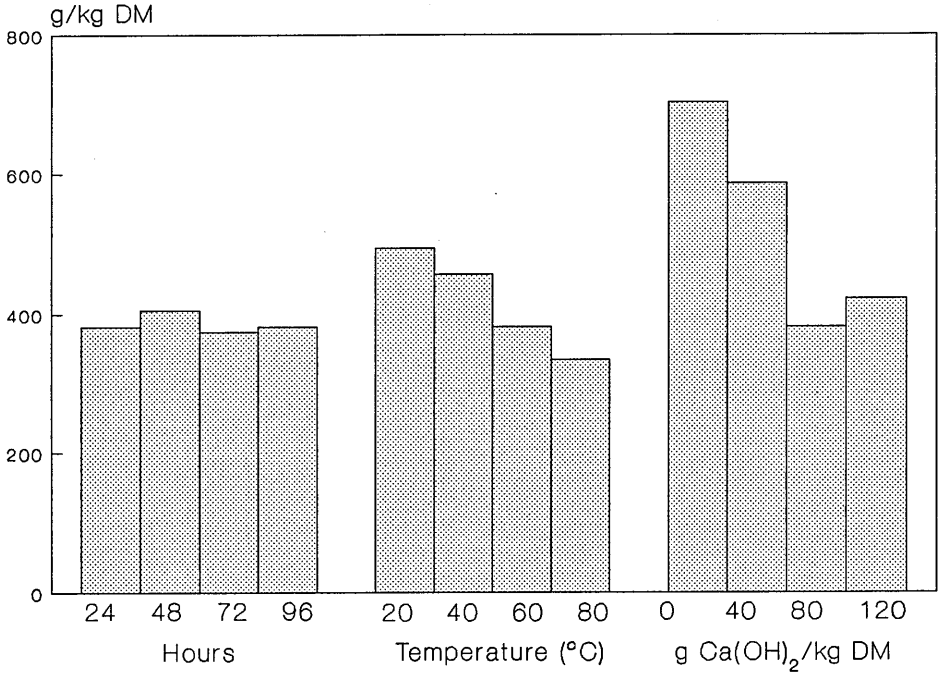


Figure 3.5b Effect of $Ca(OH)_2$, Treatment Temperature and Time on WSC Content

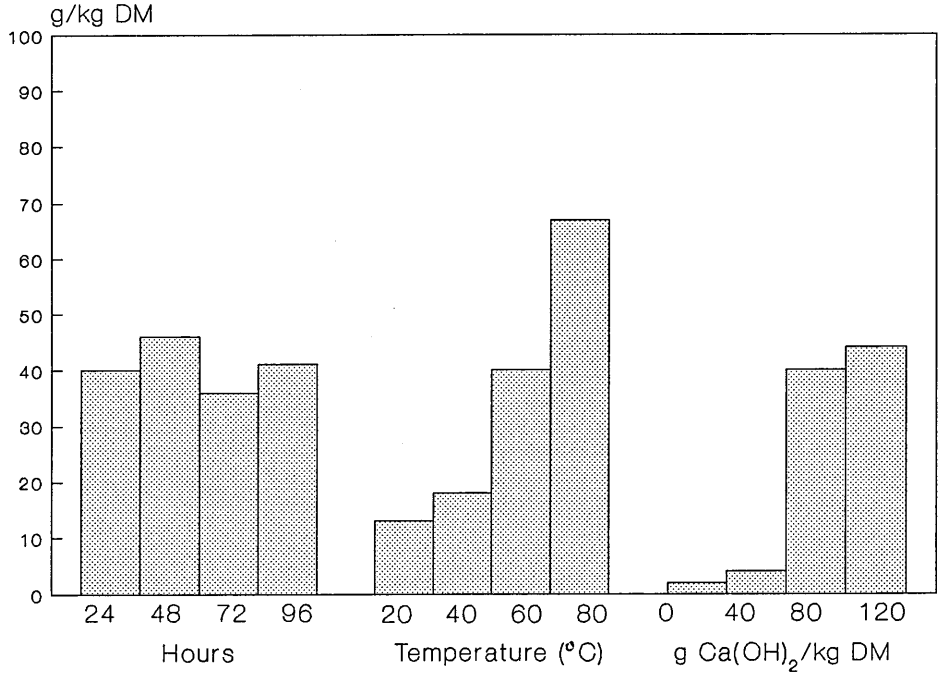
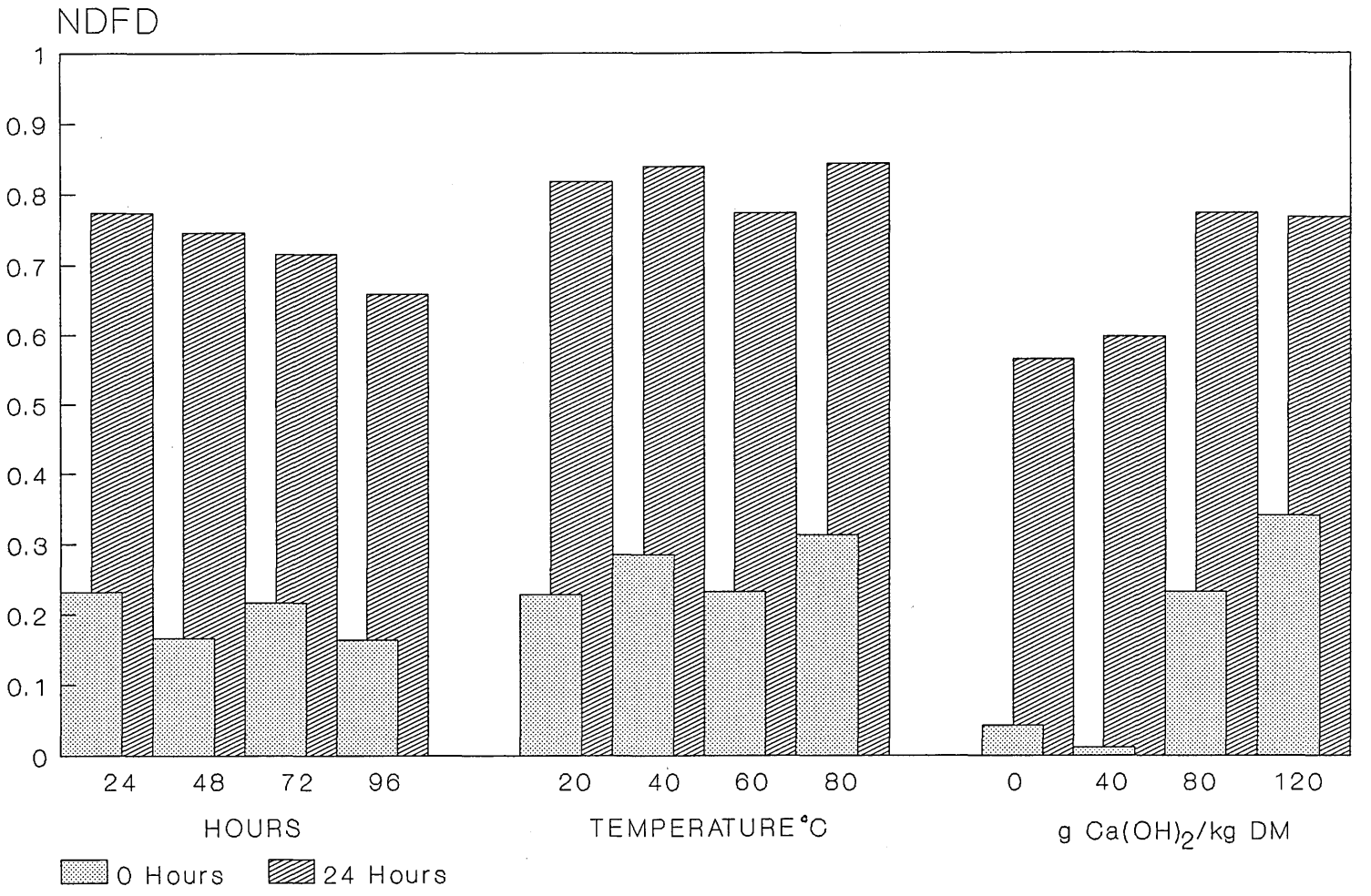


Figure 3.6 Mean In Sacco
NDF Digestibility



rumen degradation of $Ca(OH)_2$-treated MDG.

Table 3.3. Effect of Treatment Conditions on WSC and NDF Content and *In Sacco* NDF Digestibility (ISNDFD)

	WSC	NDF	ISNDFD	
	g/kg DM	g/kg DM	0h	24h
g $Ca(OH)_2$/kg DM				
0	2	704	0.043	0.565
40	4	586	0.012	0.597
80	40	422	0.232	0.774
120	44	382	0.341	0.768
Time (Hours)				
24	40	422	0.231	0.774
48	41	382	0.166	0.746
72	54	374	0.217	0.715
96	56	418	0.164	0.658
Temperature (°C)				
20	13	494	0.228	0.818
40	18	457	0.285	0.840
60	40	422	0.231	0.774
80	67	334	0.313	0.844

The response of ISNDFD to other treatment conditions differed from ISOMD; ISNDFD was unaffected by treatment temperature and decreased slightly with treatment time. This effect was unexplained since treatment time had no significant effect on any other digestibility measurements.

Correlation coefficients between measurements from the selected samples and different treatment parameters were calculated, the significant correlations are shown in Table 3.4. It shows that NDF content was negatively correlated with the level of $Ca(OH)_2$ whilst the latter was positively correlated with the WSC content. A possible

explanation of this could be that $Ca(OH)_2$ treatment was releasing monosaccharides from the hemicellulose which is normally measured as NDF. The NDF content correlated negatively with NDF digestibility (both 0 and 24h). Increasing the level of $Ca(OH)_2$ thus reduces the NDF content, increases the WSC content and increases the digestibility of the residual NDF which remained in the spent grains after treatment.

Table 3.4 Correlation of Treatment Conditions and NDF Content, WSC Content and *In Sacco* NDF Digestibility.

	NDF	0h ISNDFD	24h ISNDFD	WSC
$Ca(OH)_2$	-0.801**	0.841**	0.726**	0.642*
NDF		-0.761**	-0.660*	-0.754**
0H NDF			0.884***	0.614*
24H NDF				0.512

* p<0.05, ** p<0.01, *** p<0.001

The analysis of the selected samples and measurement of their ISNDFD showed:

1. NDF content was reduced and WSC was increased with level of $Ca(OH)_2$ and treatment temperature. Treatment time (over the times measured) had no effect on NDF or WSC content.

2. 0 h and 24 h ISNDFD was unaffected by treatment temperature, but increases with level of $Ca(OH)_2$. This effect was most marked when the level of $Ca(OH)_2$ was increased from 80 to 120 g/kg. 24 h ISNDFD decreased slightly with treatment time.

Experiment Two

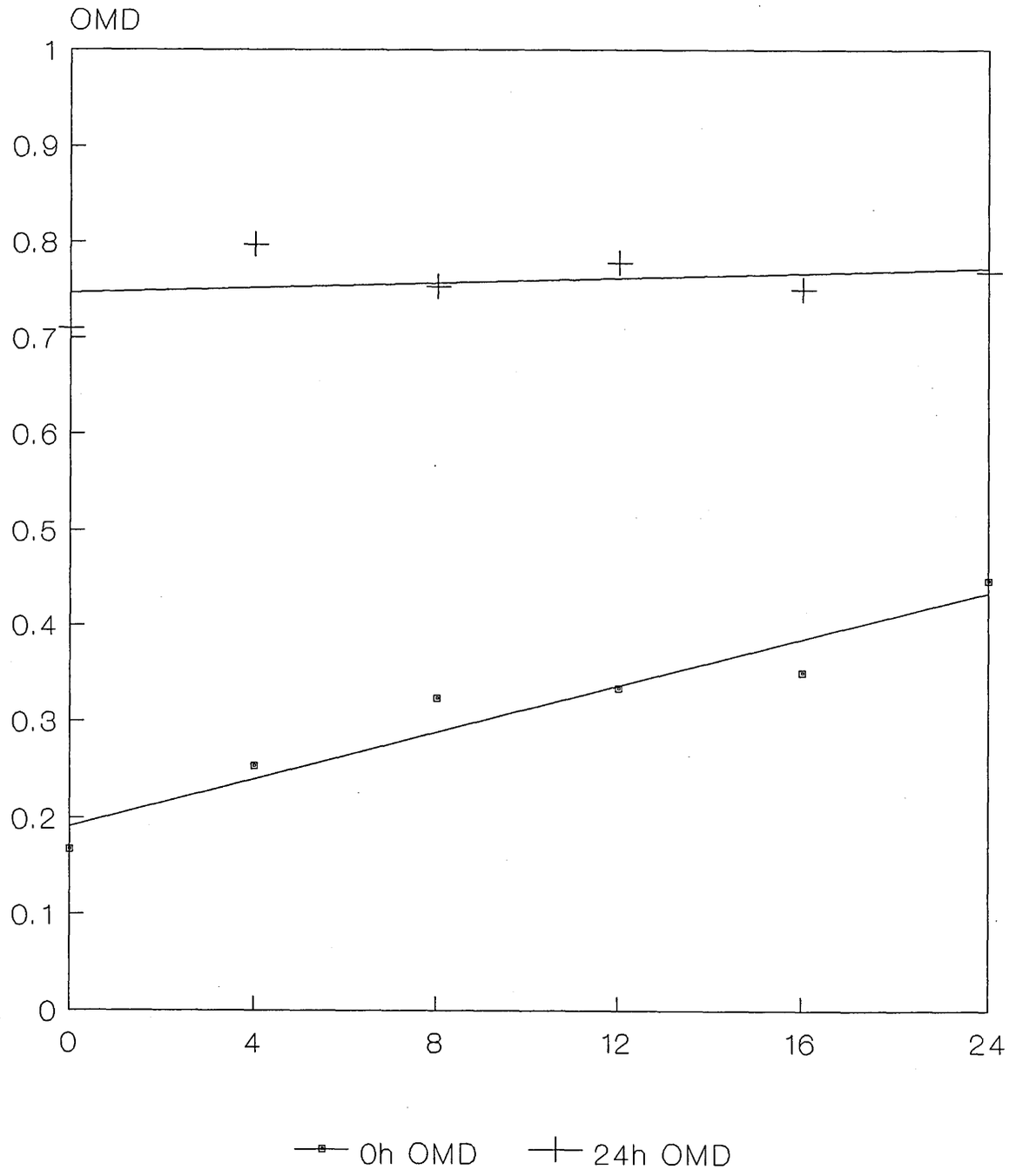
The treatment times used in experiment 1 did not affect the 24h ISOMD or the IVOMD significantly ($p<0.05$). This observation made it necessary to conduct a second experiment using shorter treatment times (0, 4, 8, 12, 16 and 24 hours) to identify when the increase in digestibility occurred. This was carried out in a similar manner to Experiment 1 but using shorter treatment times. Grains were treated with 80 g $Ca(OH)_2$/kg DM at 60°C for 0, 4, 8, 12, 16 and 24 hours. Final pH was recorded and the samples were analysed for OM, NDF and ether extract. Digestibility measurements included IVOMD, ISOMD and ISNDFD at 0h and 24h.

Table 3.5 The Effect of Treatment Time on Malt Distiller's Grains Treated with 80 g $Ca(OH)_2$/ kg DM at 60°C.

Treatment Time (h)	Final pH	Ether Extract (g/kg DM)	ISOMD 0 h	ISOMD 24 h	IVOMD
0	11.22	66.3	0.169	0.710	0.561
4	9.90	63.1	0.253	0.798	0.631
8	9.50	61.0	0.324	0.753	0.633
12	9.33	62.0	0.334	0.778	0.635
16	8.85	65.7	0.350	0.750	0.604
24	8.58	55.5	0.447	0.768	0.609

The results in Table 3.5 show that increasing the treatment time beyond 4 hours had little effect on the parameters measured with the exception of the 0h ISOMD which is shown in Figure 3.7. The ether extract was not affected by treatment time suggesting that soap formation under these conditions was instantaneous. This is contrary to the findings of Jenkins and Palmquist (1980) who observed that formation of calcium soaps of fatty acids was slow in an aqueous medium. This could be due to the alkaline conditions in $Ca(OH)_2$-

Figure 3.7 Effect of Treatment Time on In Sacco OMD



treated spent grains which would favour soap formation. Results for this experiment not shown in Table 3.8 but included in Appendix 2 are NDF content and NDF *in sacco* digestibility. These measurements similarly showed no difference due to increasing the treatment time.

The percentage of the spent grains washed from the bags without rumen incubation increased linearly with the time that the feed was held at 60|C. Regression analysis gave the following equation:-

$$0h\ ISOMD = 19.77 + 1.106T \quad (Adjusted\ R^2\ 82.5\%)$$

Where T = treatment time (hours)

3.2.3 **Discussion**

The effects of treatment conditions on different chemical indicators of the response varied, for example, although OM and NDF *in sacco* measurements showed a similar response to level of $Ca(OH)_2$, the effects of treatment temperature were different for each. Also notable was the different responses of the 0 h and the 24 h ISOMD to various treatment parameters. This suggests that the increase in digestibility of MDG was not brought about by the effects of $Ca(OH)_2$ on a single fraction of the feedstuff, but rather that the response was multifactorial, and dependent on several different reactions occurring, each responding in a different manner to the various treatment conditions.

The present study showed that the level of $Ca(OH)_2$ was the most influential factor in upgrading MDG. Increasing the level of $Ca(OH)_2$ gave significant increases in all digestibility parameters, the greatest increases occurring between the 40 and 80 g/kg DM treatments. Junker (1976) investigated the influence of different treatment parameters on the IVOMD of NaOH treated straw and also found that level of alkali had the greatest impact.

Comparison of the increases in digestibility obtained when $Ca(OH)_2$ treatment had been applied at ambient temperature, Abrams *et al.* (1983), with the responses measured in the present experiments demonstrates the benefit of treatment at elevated temperatures. The effect of heating the $Ca(OH)_2$ treated grains was to reduce the reaction time, although in experiments 1 and 2 the IVOMD and the 24h ISOMD were not significantly affected by treatment time beyond 4 hours. Substantial improvement in draff digestibility occurred when the draff was dried immediately after $Ca(OH)_2$ treatment (0h holding time) compared to values obtained without $Ca(OH)_2$ addition (experiment 1). This suggests that the reaction causing the digestibility to be increased was relatively rapid at 60°C and can occur to a significant extent during the drying phase of the treatment.

Unlike other digestibility coefficients, the 0h ISOMD was increased linearly with treatment time (see Figure 3.7). This suggests that two separate reactions are occurring, one which increases the digestibility of the OM and a second which increases the level of soluble/small particle material. This is supported by Theander and Aman (1984) who suggest that there are two neutralising reactions that occur during alkaline treatments. In the first the hemicelluloses are partly solubilised and the cellulose becomes swollen. Due to the cleavage of ester linkages and the formation of new phenolic groups, where lignin-ether bonds are broken, acetic and phenolic acids are released. In the second reaction, which occurs more slowly, the alkali is slowly neutralised by the 'peeling reactions' of polysaccharides (Whistler and BeMiller, 1958). In this reaction sugar units are lost from the reducing end of the polysaccharide and form acids such as lactic, glycollic and saccharinic acid.

The results from experiment 2, in which treatment time was increased from 0 to 24 hours, suggest that it was the former of these two reactions which was mainly responsible for the increase in the IVOMD and 24 hour *in sacco* digestibility and was largely complete within 4 hours at 60°C. Increasing the treatment time from 4 to 24 hours increased the solubility of the OM due to the peeling reactions but not the digestibility. The loss of sugar units and the subsequent

formation of the acid products from such reactions does not increase the digestibility of the grains suggesting that the polysaccharides from which they are lost are already readily fermentable by rumen bacteria.

A further effect of the $Ca(OH)_2$ on the spent grains was to reduce the proportion of the oil which was extractable by 40°C - 60°C petroleum ether. This was thought to be due to hydrolysis of the triglycerides in the draff oil under the alkaline conditions followed by saponification of the free fatty acids due to the presence of high levels of Ca^{2+}. Calcium soaps of fatty acids have low solubility in most solvents including water and petroleum ether. This result is not supported by the findings from the experiment in which $Ca(OH)_2$ replaced NaOH for treatment of spent grains (Section 3.5) in which the level of calcium hydroxide had no effect on soap formation. The levels of calcium hydroxide used in that experiment ranged from 0 to 40 g $Ca(OH)_2$/kg DM and were therefore much lower than those used in this experiment. The average soap formation (%) in the spent grains treated with a mixture of sodium and calcium hydroxide was 21.5, whilst soap formation in this experiment ranged from 27.1 to 63.9%. The mean soap formation measured in the grains treated with 40 g $Ca(OH)_2$/kg DM was 40.8. This suggests that the inclusion of sodium hydroxide in the treatment of spent grains reduces the amount of calcium soap formation. This is possibly due to the preferential formation of sodium rather than calcium soaps.

3.3 The Effect of Sodium Hydroxide on Malt Distiller's Grains

The experiment described in the section investigates the effect of NaOH-treatment on the chemical composition and digestibility of MDG and the effect of partially substituting NaOH for $Ca(OH)_2$.

3.3.1 Methods

MDG were treated as described in section 3.1 with NaOH at levels of 0, 12.8, 27.2, 38.4 and 51.2 g NaOH/kg DM. Another batch was treated with $Ca(OH)_2$ at levels of 16, 24, 32 and 40 g/kg DM and sufficient NaOH to give a pH of approximately 12. Treated grains were held at 60°C for 24 hours. Analyses included WSC, ether extract and total oil, NDF, IVOMD, and ISOMD and ISNDFD at 0 and 24 hours. Analyses were carried out as described in Chapter 2.

3.3.2 Results

1. Effect of NaOH on MDG

pH

The initial pH values measured immediately after addition of sodium hydroxide and final pH measured after 24h at 60°C, are shown in Figure 3.8. The greatest reductions in pH over the 24 hour holding period occurred when the initial pH was 10.69 (at a treatment level of 27.2 g NaOH/kg DM). Above and below this level of alkali the difference between initial and final pH were less.

NDF and WSC

Figure 3.9 shows the effect of sodium hydroxide treatment on WSC and NDF content. It shows that NaOH-treatment reduced the content of NDF and increased the level of WSC in the spent grains. In the untreated grains, the NDF content was 740 g/kg DM. This was reduced to 595 g/kg DM at the 51.2 g NaOH/kg DM level and still further to 372 g/kg DM at the highest treatment level. Whilst the NDF content was reduced with

Figure 3.8 Effect of NaOH
on Initial and Final pH

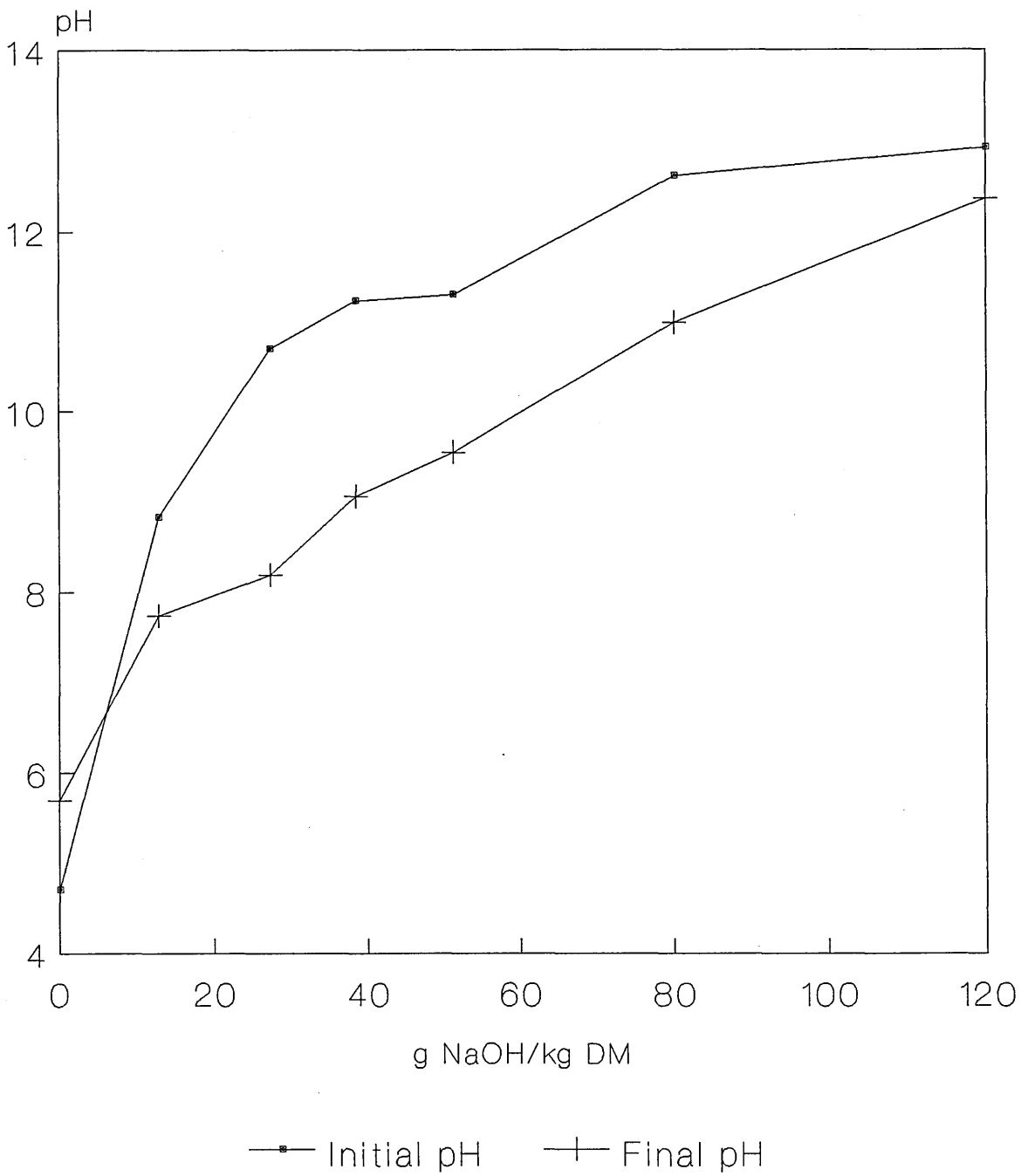
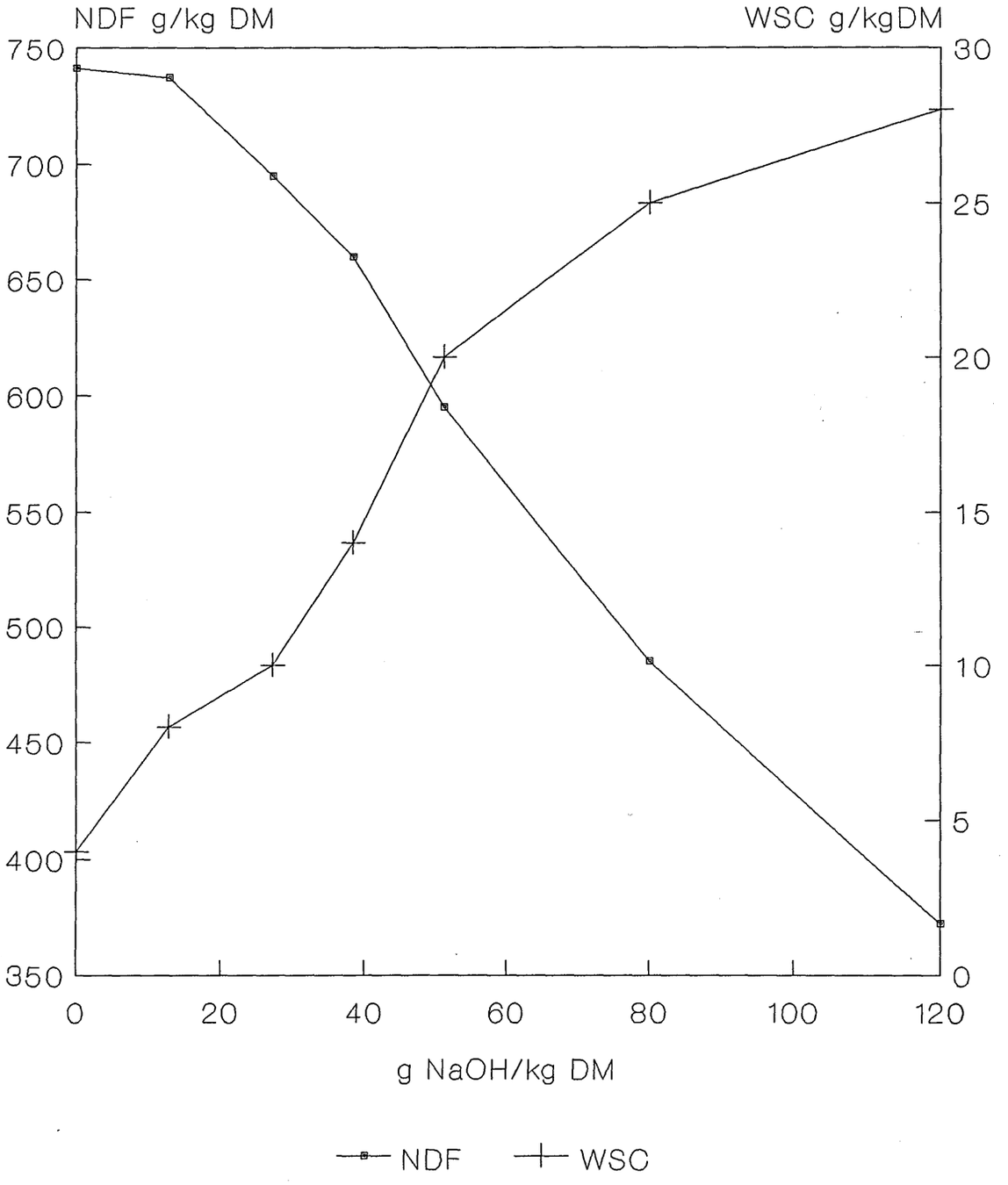


Figure 3.9 Effect of NaOH on
Spent Grain NDF and WSC Content



increasing concentrations of sodium hydroxide, the WSC content was increased from 4 g/kg DM in the untreated material to 20 g/kg in the 51.2 g NaOH/kg treatment and to 28 g/kg at 120g NaOH/kg DM.

Soap Formation

The ether extract and total oil values were slightly reduced at the higher levels of sodium hydroxide treatment. However if this was expressed on an organic matter basis then there was no significant effect of treatment ($p<0.05$) The total oil was 97.5 g/kg DM and the ether extract was 88.6 g/kg DM. This suggests that sodium hydroxide treatment has little effect on the formation of insoluble soaps.

Digestibility

Table 3.6 The Effect of Sodium Hydroxide Treatment on the Digestibility of MDG Held at 60°C for 24 Hours.

NaOH (g/kg DM)	IVOMD	ISOMD 0h	ISOMD 24h	ISNDFD 24h
0	0.372	0.184	0.586	0.539
12.8	0.390	0.195	0.614	0.544
27.2	0.454	0.197	0.646	0.579
38.4	0.472	0.241	0.706	0.639
51.2	0.485	0.266	0.752	0.732
80.0	0.509	0.259	0.775	0.753
120.0	0.157	0.306	0.736	0.756

The *in sacco* and *in vitro* OMD results are given in Table 3.6. The digestibility of NaOH-treated MDG both *in vitro* and *in sacco*, increased with increasing levels of alkali up to 80 g NaOH/kg DM. At the highest level (120 g NaOH/kg DM) the IVOMD was depressed, probably because the high residual levels of alkali from the feed overcame the buffering system used in the *in vitro* assay leading to an increased pH

with resultant depression in microbial activity. A small reduction in the digestibility at the highest NaOH level occurred in the 24h ISOMD. This was much less than observed *in vitro* presumably as a result of the superior buffering capacity of the rumen.

The *in sacco* digestibility of the NDF fraction of the treated material was increased from 0.54 for the control to 0.76 in the 120 g NaOH/kg DM treatment, in line with the increase in ISOMD. The effects of treatment on IVOMD, ISOMD ISNDFD are shown in Figure 3.10. The *in sacco* disappearance of OM and NDF follow similar curves, showing small increases below a level of 30g NaOH/kg DM, greater increases between 30 and 60 g NaOH/kg DM, and very little further effect on digestibility above this level. The 0h ISOMD at the highest level of NaOH was not depressed as other digestibility parameters were. Since 0h disappearance does not depend on microbial activity, only on the solubility of the feed, this result was as expected. The IVOMD response to increasing levels of NaOH was greatest when the level of NaOH was between 20 and 30 g/kg DM, above this level the response was much smaller. At 120g NaOH/kg DM the IVOMD was drastically reduced. As suggested earlier this was probably due to the effect of residual alkali on the *in vitro* system. The changes in the digestibility of the fibre content of spent grains coupled with the reduction in the quantity of NDF present in the grains represents a large increase in the digestibility of fibre fraction and the DM.

2. Effect of NaOH + $Ca(OH)_2$ on MDG

The results shown in Table 3.7 demonstrate that the ratio of calcium to sodium hydroxide had little effect on digestibility. The initial pH in each treatment was approximately 12 and the final pH was slightly higher for the treatment with the highest level of $Ca(OH)_2$ than for the other three treatments. There was no effect of treatment on the ether extract values or on soap formation. Ether extract and total oil levels remained at approximately 76.5 and 97.5 g/kg DM respectively for all treatments.

Figure 3.10 Effect of NaOH
on Spent Grain Digestibility

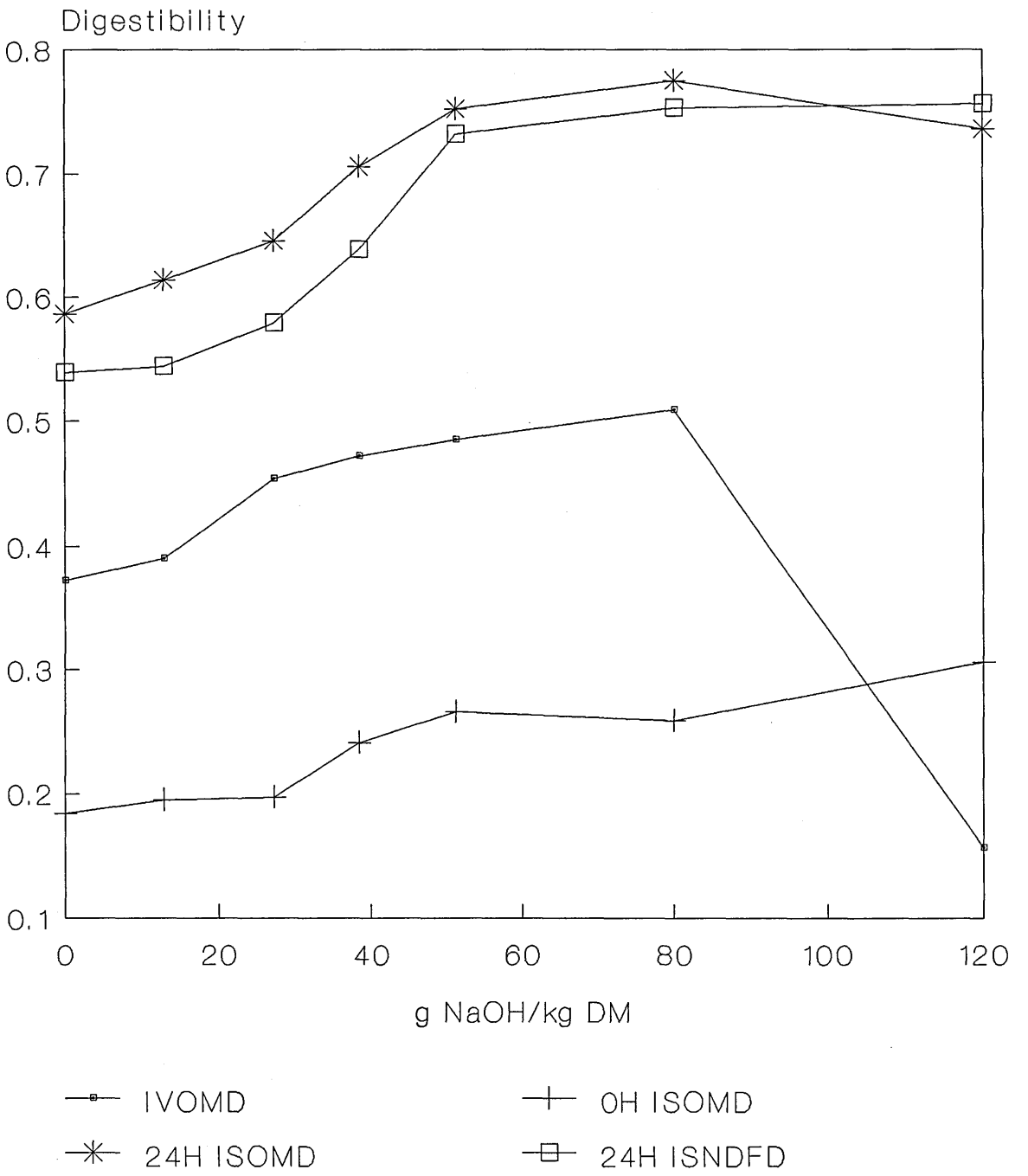


Table 3.7 The Effect of a Combination of Sodium Hydroxide and Calcium Hydroxide on pH and Digestibility of MDG

$Ca(OH)_2$ (g/kg DM)	NaOH	Final pH	In Sacco OMD 0h	24h	IVOMD
16.0	16.0	8.2	29.4	71.7	61.3
24.0	14.4	8.2	26.6	71.1	59.1
32.0	14.4	8.2	25.6	68.3	58.5
40.0	12.8	8.7	29.2	76.0	60.3

3.3.3 **Discussion**

Several groups have researched the effect of mixtures of $Ca(OH)_2$ and NaOH on fibrous residues (Rounds *et al.*, 1976; Wilkinson and Gonzalez-Santillana, 1978; Klopfenstein, 1978). Waller and Klopfenstein (1975), showed that a mixture of 30g $Ca(OH)_2$ and 10g NaOH/kg DM was as effective as treatment with 40g NaOH/kg DM. Owen *et al.* (1982) demonstrated that a mixture of NaOH and $Ca(OH)_2$ 45 and 15 g/kg DM respectively was as effective as ammonia in upgrading wheat straw.

The potential advantages of using mixtures of $Ca(OH)_2$ and NaOH is that the disadvantage of each alkali when used on its own (slow reaction time for $Ca(OH)_2$, and high residual Na^+ concentration for NaOH), may be avoided. The results from this small-scale experiment suggest that using heat to reduce the reaction time and NaOH to give an initial pH of 12 allows the reaction to go to completion. When these result are compared with where NaOH was the only alkali used, 40g $Ca(OH)_2$/kg DM + 12.8g NaOH/kg DM gave a similar response *in sacco* to 80g NaOH/kg DM (0h, 0.29 vs. 0.26 and 24h 0.78 vs. 0.76 respectively).

In vitro, the mixture of alkalis gave a higher OMD than NaOH alone (0.61 vs. 0.51). This suggests that substituting some of the NaOH by $Ca(OH)_2$ reduces the inhibition of rumen microbial function observed when NaOH is the sole alkali. A possible explanation for this could

Figure 3.11a Effect of NaOH and $Ca(OH)_2$ on Spent Grain 24h In Sacco OMD

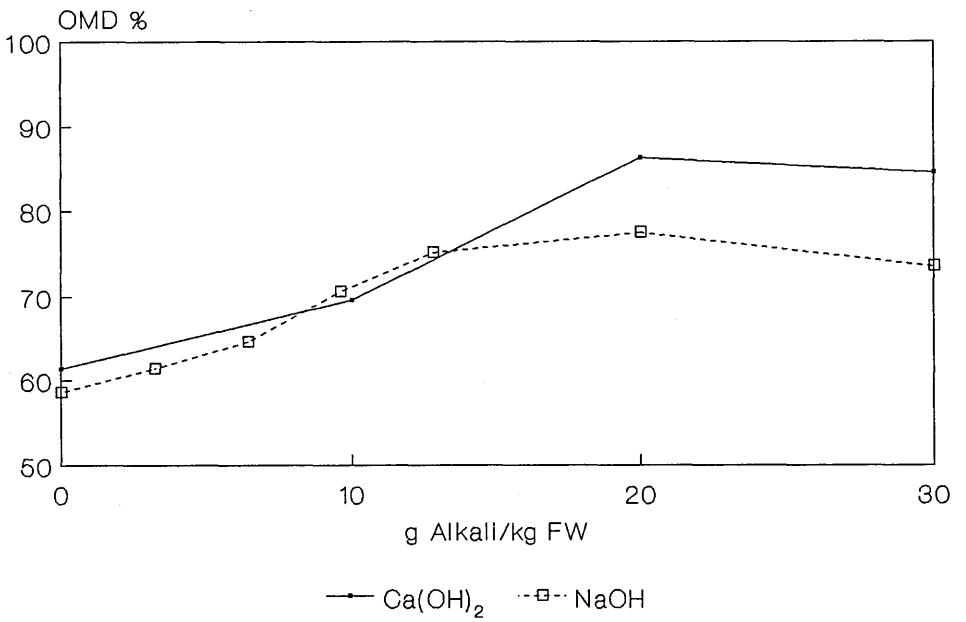
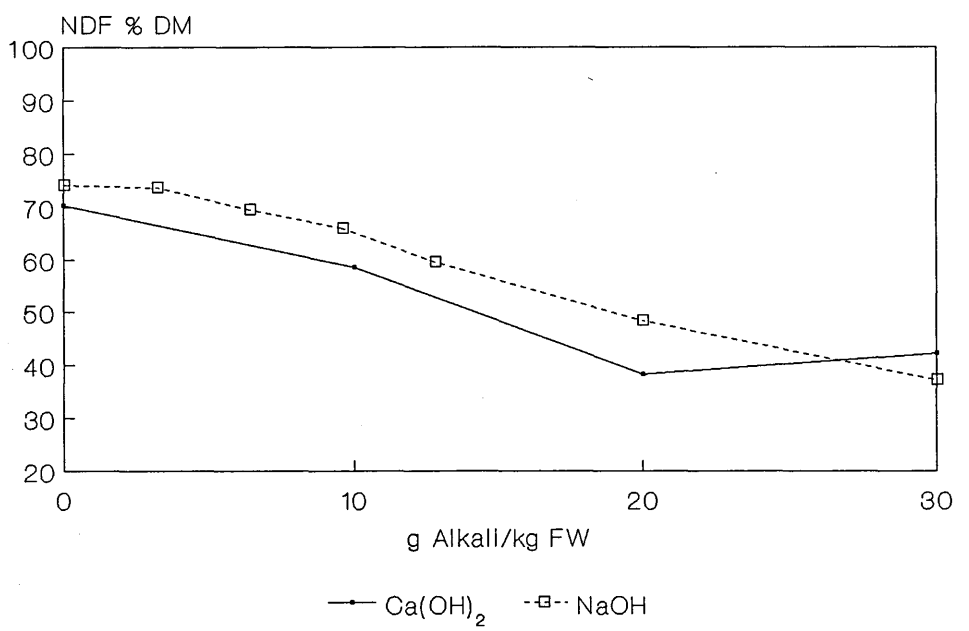


Figure 3.11b Effect of $Ca(OH)_2$ and NaOH on Spent Grain NDF Content



have been the formation of insoluble calcium soaps of the fatty acids which may have reduced the inhibitory effect of the oil on rumen microbial function. However this was not supported by the ether extract value which was not affected by the addition of calcium hydroxide. This suggests that, over the range of concentrations tested, the proportion of oil in soap form was unaffected by the ratio of $Ca(OH)_2$:NaOH.

The comparison of these results suggests that the partial substitution of $Ca(OH)_2$ for NaOH can give the similar digestibility responses to those observed using NaOH as the sole alkali, yet results in a feed of lower sodium content and higher pH.

When MDG were treated with NaOH the differences between the initial and final pH values were greatest between 25 and 50 g NaOH/kg DM. The pH reduction was less at NaOH levels below this range suggesting that there was insufficient alkali to complete the reaction. At levels above this range, the reduction in pH was also less than the maximum, probably due to there being a high level of residual alkali. If the spent grains were to be fed to ruminant livestock the high pH of these treatments would limit the amount that could be fed. Studies *in vivo* by Stigsen (1975) showed that animals fed NaOH-treated straw disclosed relatively high amounts of net base in their urine. This was counteracted by neutralising the straw with hydrochloric acid. Other studies (see Kristensen, 1981) have shown the damaging effect of excessive sodium intake leading to kidney overload and low blood Ca and Mg levels.

The effect of sodium hydroxide on MDG was similar to that reported for other fibrous feeds. Modes of action of chemical treatment of fibrous feeds have been described by Waller (1976). The major effect was to solubilise, and thereby render highly digestible, some of the hemicellulose whilst the levels of cellulose and lignin remain unaffected. Thus the level of NDF was reduced whilst the ADF (although not measured here) could be expected to remain approximately equal to that found in the untreated grains. A further part of the digestibility increase was caused by the fact that the residual

cellulose and hemicellulose had a higher IVOMD (Klopfenstein, 1976). Tarkow and Feist (1968) suggested that the rate of cellulose and hemicellulose digestion was increased by alkali treament due to swelling of the cellulose fibrils caused by disruption of the hydrogen bonds.

The reduction in the NDF content at higher levels of NaOH treatment (741 g/kg for the untreated MDG down to 372 g/kg for the 120 g NaOH/kg DM) suggests that approximately half of the NDF was present as alkali labile hemicellulose. The increases in the WSC content do not fully account for the reduction in the NDF content. This suggests that the solubilised material consists of only a small proportion of reducing sugars and that it was mainly soluble oligo- or polysaccharides that are released from the fibre by the action of alkali. No measurements of the lignin or phenolic components of the fibre were made, however lignin contents are not generally reduced by chemical treatment (Klopfenstein *et al.*, 1972; Rexen and Thomsen, 1976; Ololade *et al.*, 1970). This suggests that the increase in fibre digestibility was due to the breaking of bonds between lignin and hemicellulose or cellulose without removal of the lignin.

In laboratory scale experiments on the NaOH treatment of straw carried out by Rexen *et al.* (1975) to establish the relationship between treatment pressure, temperature and level of NaOH, it was found that increasing any of these parameters increased the IVOMD. However in contrast to this, Junker (1976) reported that increasing the treatment temperature from 60°C to 150°C did not increase IVOMD. In the same experiment it was observed that the reaction time and pressure had no effect on IVOMD, but that level of sodium hydroxide had the greatest impact, digestibilities ranging from 0.58 at 2% NaOH to 0.77 at 10% NaOH. Several equations defining the relationship between the level of NaOH (g/100g) and IVOMD(%) have been derived for factory and on-farm treatment of chopped straw;

$IVOMD = 43.9 + 5.28x - 0.23x^2$ Wilson and Brigstocke (1977)
$IVOMD = 50.1 + 4.02x$ Rexen and Vestergaarde Thomsen (1976)
$IVOMD = 42.3 + 7.01x - 0.34x^2$ Wilkinson and Gonzales Santillana (1978)

From the data given in Table 3.6 the following equation was derived;

$IVOMD = 38.62 + 1.74x$ where x = g NaOH/100g DM and R^2 = 79%

The effect of NaOH on the *in sacco* 0h and 24h digestibilities are shown in Figure 3.3 and can be defined using the following equations;

$$0h\ ISOMD = 18.70 + 1.03x \qquad R^2 = 86.4\%$$
$$24h\ ISOMD = 62.08 + 1.43x \qquad R^2 = 58.4\%$$

At the highest level of treatment (120 g NaOH/kg DM) the digestibility of the grains *in vitro* and *in sacco* was depressed. This was probably due to the effect of high levels of Na^+ or OH^- or both in the *in vitro* system and acting locally in the micro-environment of the nylon bag *in sacco*. Values for this level of NaOH have therefore been excluded from the relationships.

The increase in the 0h bag OM losses at increased levels of treatment was partly due to the increase in the soluble fraction of the feed, and probably also due to small particle loss. Lewis and McDonald (1989) also observed higher than expected 0h *in sacco* losses from bags containing untreated draff. It is thought that this was due to small particle loss as draff is, by virtue of the mashing procedure, low in all soluble constituents. From the present study, it cannot be established if the action of alkali on draff was to increase the soluble component or the proportion of small particles but it is likely that both processes are involved.

The response coefficients for the effect of NaOH on the IVOMD of MDG are lower than those shown above for straw. Coefficients for straw are approximately between 4 and 5, that for spent grains was 1.74. This difference may reflect the different contents and structure of the fibre for the two materials. The IVOMD results for the sodium hydroxide treated spent grains show relatively small increases in digestibility due to treatment (0.39 to 0.51 at 80 g NaOH/kg DM). This was less than has been observed when other forages have been

upgraded in this way, a typical response for straws has resulted in a doubling of the IVOMD from around 0.30 to 0.60 when levels of 100 - 120 g NaOH/kg DM were used (Nicholson, 1984). Generally the response has been greatest where the forage treated has been of a lower quality which may explain the lower response to alkali for spent grains compared to straw.

Several studies suggest that there are differences between *in vitro* and *in vivo* digestibility responses especially when levels of NaOH exceed 4-5% of the DM (Singh and Jackson, 1971; Mowat and Ololade, 1970; Klopfenstein *et al.*, 1972). Levels of NaOH up to 12% have been evaluated in this experiment and increases in IVOMD and *in sacco* OMD have been observed in all except the highest levels of treatment. It is doubtful whether the higher levels of treatment used here would give increases in *in vivo* digestibility. Nicholson (1984) observed that responses obtained in IVOMD corresponded to increases in *in vivo* digestibility quite well at treatment levels of between 4 and 6% NaOH. Above this level *in vivo* digestibility, dry matter intake and daily liveweight gain tended not to increase further or in some cases decreased while the IVOMD continued to increase.

A further reason for the smaller increase in the IVOMD may be due to the higher level of oil in spent grains compared to other forages. The *in vitro* system is particularly sensitive to the levels of lipids included in the feedstuff. The oil in spent grains has a particularly inhibitory effect on rumen bacteria because of its unsaturated nature. The effect of alkali on the lipid in spent grains would be to hydrolyse the triglycerides present and liberate free fatty acids. Sodium soaps of the fatty acids may be formed, however since these soaps are water soluble, they would still be available to the rumen bacteria and would, therefore still give an inhibitory effect on cellulolysis and digestion. Free fatty acids or their sodium salts are more inhibitory to microbial function than their unhydrolysed triglycerides (El Hag and Miller, 1972). This may be an important factor in the depression of IVOMD observed at the highest level of NaOH when hydrolysis of oil would be expected to be greatest.

3.4 Calcium Hydroxide Treatment of Other Spent Grains

The effects of calcium hydroxide on the fibrous fraction and on the digestibility of spent grains was investigated in a range of spent grains which were produced both on an industrial scale and also on a laboratory scale. Spent grains produced industrially included Brewer's Grains, a by-product from the brewing industry and Wheat Dreg. The latter is a by-product from a grain distillery, produced by filtering the spent wash from all the different processes in the distillery to form a spent grain with approximately 350 g/kg dry matter.

Spent grains were produced in the laboratory by coarse milling of the whole grains, treatment with an amylase enzyme followed by hot water extraction to simulate the industrial mashing process.

3.4.1 Methods

Laboratory Preparation of Spent Grains

The effects of $Ca(OH)_2$-treatment on wheat, barley, oats, rye and maize spent grains prepared on a laboratory scale was compared. A 200g sample of each type of grain was coarsely milled and added to boiling water and maintained at boiling point for 2 hours. The boiled grains were then filtered through muslin and rinsed with warm water. The residual grains were then mixed with 1 litre of water at 37°C with approximately 10g of an amylase enzyme (Amylase (40 EU/mg) BDH cat no. 39004). They were then left for 48 hours in a sealed container at 37°C. The grains were again filtered through muslin and rinsed with warm water and then dried at 100°C for 24 hours.

A 30g sample of each type of spent grain was taken, sufficient water was added to give a dry matter of approximately 280 g/kg, and then they were thoroughly mixed with 3 g $Ca(OH)_2$ (to give a concentration of 100g $Ca(OH)_2$/kg DM). Measurements included pH after treatment, NDF, and ISOMD at 0h and 24h.

Treatment of Brewers Grains and Wheat Dreg

Brewer's grains were treated with 0, 40, 80 and 120 g $Ca(OH)_2$/kg DM at 60°C for 24 hours. Similarly, a sample of Wheat Dreg (Grants Distillery, Girvan) was treated under the same conditions with 0, 28.5, 57.1, 85.7 and 114.3 g $Ca(OH)_2$/kg DM (0, 10, 20, 30 and 40 g $Ca(OH)_2$/kg FW).

The final pH of both types of grains was recorded prior to drying. Grains were analysed for ether extract and NDF content and the *in sacco* 0 and 24 hour OMD was measured as described in Sections 2.1.10, 2.1.5, 2.4.2 respectively.

3.4.2. **Results**

Table 3.8 The Effect of $Ca(OH)_2$ Treatment of Five Different Spent Grains

Grain	+/- $Ca(OH)_2$ 100g/kg DM	NDF (g/kg DM)	ISOMD 0h	ISOMD 24h
Oats	-	841	0.028	0.150
	+	490	0.112	0.566
Barley	-	842	0.028	0.441
	+	332	0.368	0.841
Wheat	-	757	0.020	0.420
	+	390	0.309	0.705
Maize	-	822	0.030	0.472
	+	287	0.636	0.929
Rye	-	994	0.033	0.317
	+	855	0.242	0.632

The results of the NDF determinations and the ISOMD measurements are shown in Table 3.8. All grains showed large increases (a mean increase of 0.375) in the 24 hour *in sacco* digestibility after $Ca(OH)_2$ treatment. Accordingly, all grains showed reduced levels of NDF after treatment although this effect was less marked for the rye grains. The NDF levels measured were higher and the reductions in the NDF content were greater for the laboratory prepared barley and wheat spent grains than had been observed in those grains obtained from distilleries. This could have been caused by the more vigorous washing and extraction procedures employed in the laboratory compared to the industrial mashing process normally used to produce wort and spent grains.

For maize, the ISOMD of the $Ca(OH)_2$-treated grains was increased to a level greater than that measured concurrently for the unprocessed ground maize grain (0.64 vs. 0.83 respectively). Similarly, the barley spent grains had an approximately equal ISOMD after $Ca(OH)_2$ treatment (0.70) as for the whole, unprocessed grain (0.71).

Table 3.9 The Effect of $Ca(OH)_2$ Treatment of Brewer's Grains.

$Ca(OH)_2$ (g/kg DM)	Final pH	Ether Extract (g/kg DM)	NDF (g/kg DM)	ISOMD 0h	ISOMD 24h
0	4.30	87.7	630	0.087	0.549
40	7.28	86.7	619	0.113	0.619
80	9.18	54.1	427	0.505	0.790
120	10.41	28.6	327	0.656	0.817

The results given in Table 3.9 show that the response of Brewer's Grains to treatment with $Ca(OH)_2$ was similar to that shown by MDG. The ether extract content of the grains decreased with increasing levels of $Ca(OH)_2$ indicating that soap formation had occurred leaving the oil present in the grains less soluble in petroleum ether. The

NDF content was inversely related to $Ca(OH)_2$ level suggesting that the $Ca(OH)_2$ cleaves alkali-labile linkages in the fibrous fraction of the spent grains. The 0h ISOMD values support this, showing large increases in losses from nylon bags without incubation in the rumen. Bags incubated in the rumen for 24 hours also showed increased losses of organic matter as the level of $Ca(OH)_2$ treatment was increased.

Table 3.10 The Effect of $Ca(OH)_2$ Treatment on Wheat Dreg

$Ca(OH)_2$ (g/kg DM)	Final pH	Ether Extract (g/kg DM)	NDF (g/kg DM)	ISOMD 0h	ISOMD 24h
0	3.46	9.74	576	0.180	0.636
28.5	5.88	8.17	565	0.224	0.724
57.1	8.04	8.03	538	0.227	0.716
85.7	9.48	7.23	470	0.395	0.832
114.3	10.40	5.81	342	0.366	0.815

The results from analysis of the Wheat Dreg are given in Table 3.10. They show that the improvements in *in sacco* digestibility with $Ca(OH)_2$ treatment are of the same order to those observed for the Brewer's and the MDG. Without $Ca(OH)_2$ treatment Wheat Dreg was more digestible than Brewer's Grains yet after treatment with $Ca(OH)_2$ the difference disappeared as the *in sacco* OMD of both spent grains after 24 hours increased to approximately 0.80. Results obtained from the laboratory production of spent grains of oats, wheat, barley, maize and rye gave similar responses (as shown in Table 3.8), $Ca(OH)_2$ treatment increased the *in sacco* digestibility, and reduced NDF values.

The results from these experiments show that other spent grains react in a similar way to that observed for MDG. The major effect of $Ca(OH)_2$-treatment are to increase the digestibility of the grains (as measured *in sacco*). It is reasonable to assume that the IVOMD would also be increased as observed with MDG. The results show that the causes of this effect are twofold; firstly the NDF content was reduced, which because NDF is the least digestible component of the

OM, increases the OMD. Secondly, although this factor is less important in determining the *in sacco* OMD, the formation of calcium soaps reduces the inhibitory effects of oil on microbial function.

It is possible that the different types of grain would require different optimal treatment conditions. However, since the main subject of this research project is MDG further investigations into this possibility are not justified at this point although may be worthy of further studies.

3.5 **Discussion**

This series of small experiments have demonstrated that for the alkali treatment of MDG, calcium hydroxide was more effective at than sodium hydroxide under the treatment conditions described. Figures 3.11a and 3.11b shows the comparative effects of the two alkalis on 24h ISOMD and on NDF content respectively. The $Ca(OH)_2$ gives greater increases in the ISOMD (and IVOMD) than the NaOH. This result was not expected for two reasons; firstly, NaOH is a stronger alkali than $Ca(OH)_2$, and secondly, comparisons of the two alkalis in upgrading fibrous residues have generally shown NaOH to be superior. Since industrial grade $Ca(OH)_2$ was used for treatment and its purity was not known the titratable alkalinity of each alkali was measured by titration with hydrochloric acid. This showed that the titratable alkalinity (defined as the weight of HCl required to neutralise 100g of the base) was 93.8 and 62.2 g/100g for NaOH and $Ca(OH)_2$ respectively.

A possible factor in the ISOMD response of spent grains to $Ca(OH)_2$-treatment compared to NaOH-treatment may be the formation of calcium soaps. Although in these experiments the concentration of oil in the rumen as a whole was not high, since the sheep were fed a standard diet of concentrates and hay, the oil concentration within the micro-environment of the nylon bag may have been sufficient to inhibit local cellulolytic activity. In order to digest the spent grains the bacteria must first colonise the fibre particle and will therefore experience high levels of oil present in the grains.

The optimal treatment conditions, taken as those conditions which give the greatest response for the least vigorous conditions, were taken as 24 hours at 60°C with 80 g $Ca(OH)_2$/kg DM. The reaction time could be reduced further if a higher temperature was used, however the practical constraints of sealing a large bulk of material in polythene at a high temperature meant that 60°C was the preferred temperature. Apart from the production of higher levels of solubles there was no advantage in using a higher temperature or prolonging the treatment time, as neither 24 h ISOMD or IVOMD increased with temperatures above 60°C or treatment times of longer than 24 hours. The effect of additional of $Ca(OH)_2$ on 24 h ISOMD and ISNDFD at levels >80 g/kg DM are smaller than those observed when $Ca(OH)_2$ increases from 40 to 80 g/kg DM. 80 g $Ca(OH)_2$/kg DM was therefore chosen as the optimal level of $Ca(OH)_2$, since it was a compromise between maximal effect and minimal levels of ash in the final feed.

The duration of the heating period can provide a useful method for altering the proportion of the draff fibre that is solubilised. This introduces the possibility of manufacturing feeds of quite different rumen fermentation characteristics from the same by-product. Given prolonged heating and a high level of $Ca(OH)_2$ application (> 80 g $Ca(OH)_2$/kg DM) up to 50% of the feed organic matter was lost instantaneously from a nylon bag. Whilst this loss may not be entirely of soluble material and may include small particles, it is likely that it would be quickly fermented by rumen micro-organisms. Less vigorous treatment conditions would produce a feed which was fermented much more slowly in the rumen but yet still retains a substantially higher digestibility than the untreated grain.

The reduction in the NDF content and the increase in the soluble content of the MDG may increase their potential use as a monogastric feedstuff. The high proportion of poorly digested fibre is one of the major limitations to the inclusion of spent grains in pig and poultry diets and upgrading the fibre fraction may increase its digestibility in monogastric diets. However, the high level of ash in the nutritionally improved draff (NID) as a feedstuff would partially negate the increase in DE given by treatment, giving the feed only a

moderate ME. This would limit its potential use in rations for highly productive stock, but may be suitable for less energy-dense diets.

These treatment conditions have been shown to be effective in upgrading many different types of spent grain, including those from industrial sources and those prepared in the laboratory. The high level of moisture present in the grains makes them ideally suited to alkali treatment. Sundstol *et al.* (1978) observed that the IVOMD of ammonia treated straw could be increased when the moisture content was increased. Similarly, Patterson *et al.* (1980) showed that moisture content was critical for the $Ca(OH)_2$ treatment of maize cobs and found the optimal DM to range from 55 to 60%. The dry matter content of spent grains as they leave the mash tun is approximately 23 - 25%. Occasionally grains are centrifuged or pressed to remove excess water, however they rarely exceed 40% DM. The wet nature of the grains means that extra water would not have to be included in a treatment process to optimise upgrading. The high moisture content of spent grains is of particular benefit when they are to be treated with $Ca(OH)_2$ which has a relatively low solubility in water (solubility coefficient of 0.185g in 100ml of water at 0°C).

Apart from the superior effect of $Ca(OH)_2$ over NaOH under the conditions described, there are other advantages to $Ca(OH)_2$-treatment:

1. $Ca(OH)_2$ is less corrosive and safer to handle than sodium hydroxide and other alkalis used to upgrade feedstuffs.

2. $Ca(OH)_2$ is cheaper and likely to be more readily available locally than other alkalis. This could be of benefit particularly in developing countries.

3. The residual Ca^{2+} is less toxic to the animal than Na^{+}.

4. The formation of calcium soaps in feedstuffs such as spent grains which are relatively high in oil. This reduces the toxic effects of unsaturated oils on rumen bacteria. This benefit would not occur with sodium hydroxide treatment.

CHAPTER FOUR

THE EFFECTS OF ALKALI TREATMENT ON THE DIGESTION OF MALT DISTILLER'S GRAINS

In Chapter 3 the effects of calcium and sodium hydroxide treatment of spent grains on *in sacco* and *in vitro* digestibility and on the fibre and lipid fractions were reported. These results illuminated several areas of study which required further investigation:-

1. To characterise the ruminal degradation of NID

2. To investigate the contribution made by soap formation to the increase in digestibility.

3. To measure the effect on digestibility *in vivo*

4.1 Characterisation of the Digestion of NID in the Rumen

In chapter 3 it was noted that *in sacco* losses of OM and NDF were increased when spent grains were treated with alkali even without incubation in the rumen (0 time incubations). The increase in 0 h ISOMD was greater (by a factor of 1.63) than the increase in ISOMD after incubation in the rumen for 24 h. The *in sacco* technique assumes that all material that is lost from the bag is digested. This assumption is unlikely to be true for the large 0 h losses observed with NID, particularly if, as suggested, part of this loss was due to leakage of small particles. Although the *in sacco* technique can describe how a feed is physically degraded in the rumen, it yields no information on the digestion characteristics of the fraction that is instantaneously lost from the bag.

A better indication of the degradation of the treated grains in the rumen is provided by measuring the rate of gas production by rumen microbes incubated with the grains *in vitro*. This measurement more accurately reflects the rate at which the material is digested by

rumen micro-organisms by measuring the rate of formation of the end products of fermentation. However, measurement of gas production is inappropriate for estimation of degradation over periods exceeding 6 hours as end product inhibition of fermentation would be anticipated. For a more complete characterisation of ruminal digestion of NID the *in sacco* degradation curves measured over 72 hours should be combined with observations of the rates of gas production measured over 6 hours. Only the latter method can describe the fate of the solubilised and particulate material lost from the bag at 0 hours.

The technique of measuring the rates of gas production from feeds incubated with rumen liquor *in vitro*, although crude, has been used by many researchers and has been developed into the standard *in vitro* technique more commonly used to assess the digestibility of feeds. In 1943, Quin incubated various carbohydrates with rumen liquor in gas-tight flasks, and measured gas production manometrically. The *in vitro* method developed through the work of McDougall (1949), Burroughs *et al.* (1950), Bentley *et al.* (1954) Johnson *et al.*, (1958) and Tilley and Terry (1963) and provided a method to predict quantitatively the digestibility of a feed *in vivo*. The technique no longer depended on the short term measurement of gas production as end product inhibition would make this method inappropriate for feeds which are fermented more slowly.

A more refined apparatus for the collection and analysis of gas produced *in vitro* was described by Czerkawski and Breckenbridge (1983) who recognised the requirement for a simplified technique which could give some indication as to the nature of the fermentation reactions, and in which analysis of the products could be carried out at the end of the incubation. This technique used glass syringes with Luer fittings and a two way plastic stop cock to allow collection of the gas for analysis. The glass syringes were charged with strained rumen contents, artificial saliva (McDougall, 1948) and substrate and then maintained at 39°C in a waterbath. Gas was collected at 30 minute intervals over an incubation time of between 3 and 5 hours. The gas was analysed directly by GC.

For the purposes of this experiment it was assumed that it was not necessary to analyse the composition of the gas. Since the composition of the substrates did not vary widely, the composition of the gas produced was not expected to vary significantly. Total gas production was therefore used as the measure of fermentation of substrate.

Methods

1. *In Vitro* Gas Production

Samples of rumen contents were obtained before the morning feed from sheep offered 300g of sheep concentrates in two feeds and *ad libitum* hay. The rumen liquor was quickly strained through muslin, diluted by half with artificial saliva (McDougall, 1948) and maintained anaerobically by flushing with CO_2. Triplicate 100 ml glass syringes fitted with Luer fittings and plastic stop cocks were charged with 60 ml of the rumen liquor/artificial saliva mixture and 500 mg of dried, milled substrate. All air was removed from the syringes which were then inverted several times to mix the contents. The syringes were immersed in a waterbath at 39°C and the volume of gas produced was measured and released at half hourly intervals.

Gas production rates were found to be constant for at least the first six hours of incubation but tailed off thereafter. The volume of gas produced in the first six hours was taken as the standard measurement. In order to adjust the production figures for differences that may have occurred from day to day (for example, due to differences in the rumen liquor), a sample of molassed sugar beet pulp (MSBP) was incubated with each batch to serve as a control. Values for the volume of gas produced in 6 hours from the MSBP were then used to normalise values for other feeds so as to allow for between-batch variation.

Incubations were carried out on samples derived from MDG as shown in Table 4.1. The soluble fractions referred to were prepared as described in Chapter 8.

Table 4.1. Pre-treatments of Malt Distillers Grain Samples Incubated *In Vitro* for Gas Production Measurements.

Alkali	Level (g/kg DM)	Temperature °C	Holding Time (h)	Fraction
NaOH	40	60	24	Whole Feed
				Soluble Fraction
				Insoluble Fraction
$Ca(OH)_2$	80	60	24	Whole Feed
				Soluble Fraction
				Insoluble Fraction
				Ether Extracted
$Ca(OH)_2$	80	60	0	Whole Feed
			4	Whole Feed
			8	Whole Feed
			12	Whole Feed
			16	Whole Feed
			24	Whole Feed
Untreated				Whole Feed
				Ether Extracted

2. *In sacco* Degradation.

The *in sacco* measurements were carried out using duplicate bags containing MSBP or NID incubated in three fistulated wether sheep. The animals were fed 300g of concentrates in two feeds and *ad libitum* chopped hay. The bags were prepared and washed as described in section 2.4.2 and were incubated in sheep for 0, 3, 5, 8, 16, 24, 48 or 72 hours. The OM of the feed and undigested residues was measured as described in section 2.1.2, and these measurements were used to calculate the proportion of OM lost from the bags and the a, b and c

terms for equation defining the rate and potential extent of digestion in the rumen (Orskov and McDonald, 1979).

Results and Discussion.

1. Gas Production

The results are given in Table 4.2

Table 4.2. In Vitro Gas Production (mls Gas Produced in 6 Hours) from Samples of MDG incubated with Rumen Liquor.

	Whole Feed	Soluble Fraction	Insoluble Fraction	Ether Extracted
Untreated MDG	31.35	68.16	19.49	56.33
NaOH-treated MDG	31.57	35.67	17.84	-
$Ca(OH)_2$-treated MDG	45.72	86.33	22.63	61.12
Sugar Beet Pulp	71.42	87.39	-	-

SE = 3.638

The volume of gas produced after 6 hours incubation *in vitro* was less for NID than for MSBP. This result was not expected since the content and rates of fermentation of the soluble fractions of NID and MSBP were similar despite differences in their chemical composition. In MSBP this is largely present as WSC (200 g/kg DM, MAFF, 1990), whereas for NID, WSC accounts for only approximately 20 % of the soluble fraction (40 g WSC /kg NID DM, Chapter 3). However, in Chapter 8 it was shown that the soluble fraction contained high levels of two highly substituted arabinoxylans. Brice and Morrison (1982) observed that highly branched polysaccharides were degraded more rapidly in the rumen than those with a lower degree of substitution. The gas production observations from the present experiment suggest that such polysaccharides are fermented as quickly by the rumen micro-

organisms as the disaccharides of the molasses present in MSBP. The explanation for the lower rates of gas production for NID compared to MSBP may be due to differences in the rates of fermentation of the insoluble fractions. The insoluble fibre of MSBP must be much more rapidly fermented than that from NID, but measurements are needed to confirm this.

The volume of gas produced from the whole feed was increased by $Ca(OH)_2$ treatment of the spent grains whilst NaOH treatment had no effect. This suggests that the $Ca(OH)_2$-treated grains would be fermented more quickly in the rumen than untreated or NaOH-treated grains. The increase in rate of gas production from the $Ca(OH)_2$ treated grains can be explained inpart by the formation of calcium soaps of fatty acids (CSFA) making the unsaturated oil unavailable to the rumen microbes and thus reducing the inhibitory effect of the unsaturated oil in spent grains. Formation of sodium salts of long chain fatty acids does not lower their toxicity to rumen micro-organisms (El Hag and Miller, 1972).

A further explanation for the reduced response with NaOH-treatment could be due to differences in the soluble fractions. The low value observed for the NaOH treated soluble fraction suggests that sodium hydroxide treatment is in some way producing substances in the soluble fraction which are inhibitory to rumen microbial activity. There are two possible explanations for this:-

1. There is excessive residual NaOH which has been washed out in the soluble fraction and concentrated by freeze drying, increasing the pH of the *in vitro* system above optimal for microbial activity.

2. Sodium hydroxide treatment solubilises substances from the cell wall which have an inhibitory effect on microbial fermentation. Jackson (1977), Evans (1979) and Chesson (1981) list the release of acetyl and phenolic groups from plant cell wall material as one of the effects of alkali treatment. Akin and Rigsby (1985), Chesson *et al.*, (1982)

and Akin *et al.*, (1988) have noted that mmolar concentrations of phenolic acids may be sufficient to influence the activity of cellulolytic micro-organisms. In the preparation procedure the soluble fractions were freeze dried which would have concentrated any phenolic substances solubilised by NaOH treatment. Additionally, in the *in vitro* system such substances could accumulate since there is no removal of the breakdown products from cell wall degradation as there is *in vivo*.

To investigate the first possibility, the pH of the rumen liquor was measured after 6 hours incubation with sodium hydroxide-treated grains. The pH was found to be unchanged throughout the 6 hour incubation regardless of the presence or type of alkali suggesting that inadequate pH control was unlikely to be the cause of the low NaOH response.

Whatever the reason for the poor fermentability of the NaOH-treated grain solubles these results suggest that the modes action of $Ca(OH)_2$ and NaOH on spent grain fibre are not the same. Both alkalis solubilise substantial proportions of the spent grains but the large difference in the *in vitro* digestion of the soluble fraction suggests that the two alkalis liberate different substances from the cell wall material.

The effect of alkali on the initial rate of fermentation of the insoluble fraction of the spent grains appears to be negligible. This is supported by Bacon *et al.* (1981) who reported that the effects of alkali on straw were reduced after neutralisation and washing. It has been shown that alkali treatment in the presence of water facilitates the diffusion of alkali-labile materials out of the cell wall matrix. The solubilised hemicelluloses can then be reabsorbed onto the fibre. This phenomenon is seen in the alkaline pulping process (Ahlgren *et al.*, 1969). Model studies on the absorption of xyloglycans to cellulose have been made (Valent and Albersheim, 1974). This loose attachment of polysaccharide would facilitate colonisation by providing a readily fermentable starting substrate for the degradation

of insoluble cell wall material.

The incubation of the ether-extracted MDG showed that the $Ca(OH)_2$-treated grains were more fermentable than the untreated. Comparison of these measurements represents the improvement in *in vitro* digestibility due to upgrading the fibre fraction. The removal of the ether extract increases the gas production from both $Ca(OH)_2$-treated and untreated grains. The increase in gas production due to ether extraction was greater for the untreated than for the $Ca(OH)_2$ treated grains. This suggests that there is less inhibitory, ether-extractable oil in the $Ca(OH)_2$-treated grains than in the untreated grains and supports the theory of reduced microbial inhibition by oil due to the formation of calcium soaps of fatty acids (CSFA) with $Ca(OH)_2$ treatment.

Table 4.3. Effect of Treatment Time on In Vitro Gas Production

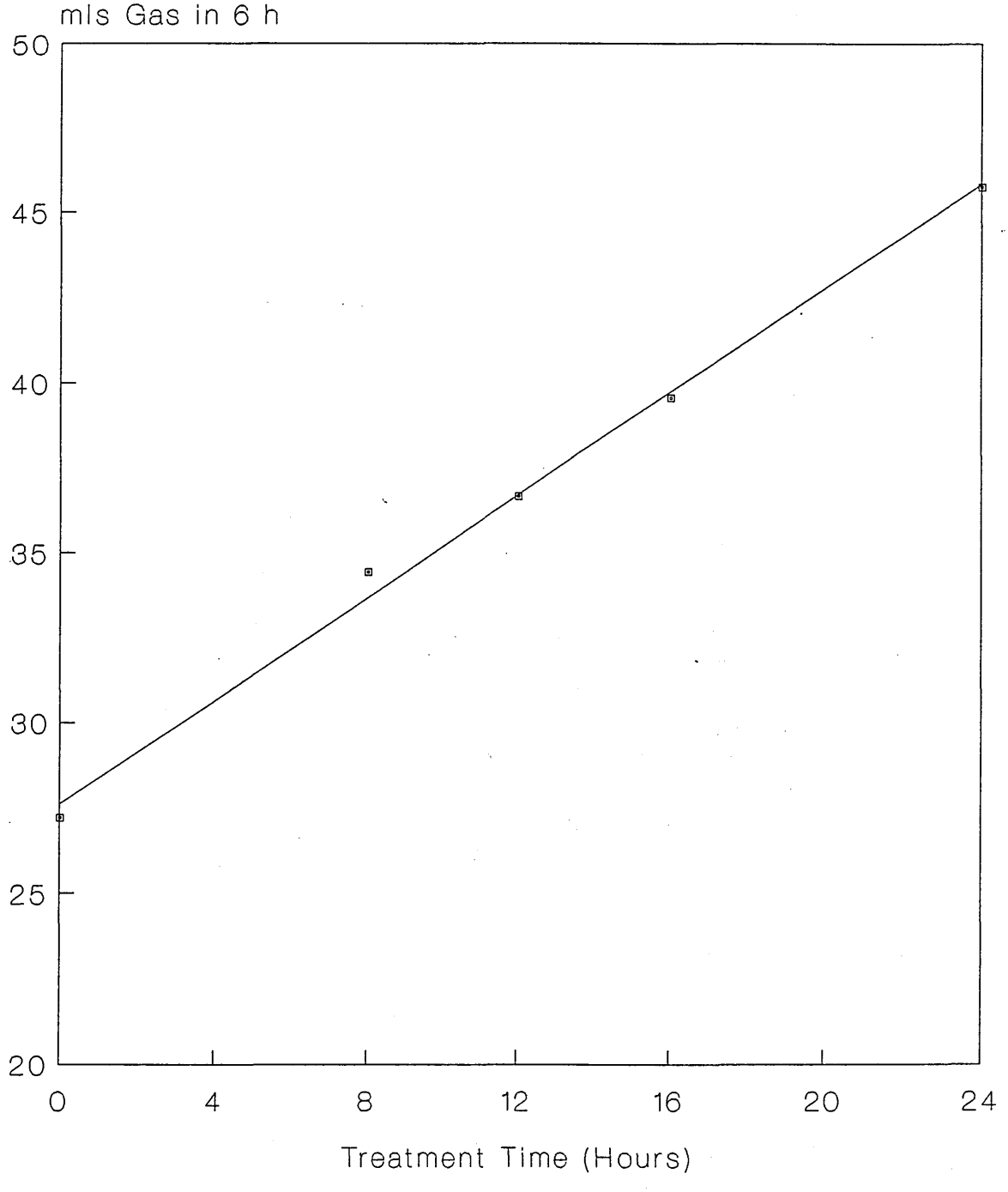
Treatment Time (h)	Mls Gas Produced in 6h
0	27.19
4	24.81
8	34.43
12	36.66
16	39.55
24	45.72

The results presented in Table 4.3 are shown graphically in Figure 4.1. If the value for the 4 hour treatment time is ignored, then the response to increasing treatment time is linear for gas production and can be defined by the following equation:

$$\text{Gas Production (mls)} = 28.1 + 0.732t \qquad R^2 = 99.7\%$$

Where t = treatment time in hours.

Figure 4.1 Effect of Treatment Time on In Vitro Gas Production from NID



The straight line nature of this response suggests that the mode of action of $Ca(OH)_2$ with increasing treatment time does not change, ie. more of the same material is solubilised in direct proportion to treatment time. This could be explained by the slow, progressive release of monosaccharide units from cell wall polysaccharides by the action of alkali described by Whistler and BeMiller (1958) in the so called 'peeling reactions'.

The rate of fermentation of plant material is also affected by the hydratability and the ion exchange capacity of the plant material. It is thought that these affect the attraction and attachment of micro-organisms to plant particles (Van Soest *et al.*, 1984) which themselves have considerable ion exchange and hydration capacities. The adsorption of other components of the diet such as lipids and minerals reflect the surface properties of the fibre. It has been suggested (Van Soest and Robertson, 1976) that constituents of the fibre such as uronic acid, the phenolic groups of lignin and the nitrogenous substances all contribute to its adsorptive properties. It has been shown in Chapter 6 that the absorptive properties of spent grains are increased by $Ca(OH)_2$-treatment. This may be a further factor responsible for the increased rate of gas production from spent grains with $Ca(OH)_2$-treatment.

The lack of response to NaOH treatment is unexplained but the effects of $Ca(OH)_2$ treatment were substantial. $Ca(OH)_2$ treatment increased the 6 hour gas production from the whole feed by 0.46. This large increase in the initial rate of fermentation was caused mainly by the increased content of rapidly fermented soluble material due to $Ca(OH)_2$ treatment (soluble content for untreated grains was measured at approximately 2% compared to 20% of the OM for the $Ca(OH)_2$ treated grains (see Chapter 8)). Also the soluble fraction of NID showed an increased initial fermentation rate compared to that of untreated draff (86 cf. 68). However the 0.46 increase in the *in vitro* gas production was less than the 3 fold increase in the 0 h *in sacco* loss. This suggests that not all the material lost from the bag by cold

water washing is rapidly fermented since the gas production data shows that only the truly soluble fraction is rapidly fermented. The discrepancy between the techniques is probably due to the loss of relatively slowly digested small particles from the bag during the washing procedure.

This finding suggests that the rumen fermentation characteristics of spent grains could be manipulated by varying the time that the grains are held at elevated temperatures during treatment. A feed that is rapidly fermented in the rumen could be produced by heating the grains for longer periods, or a feed which is degraded more slowly in the rumen could be made by drying the grains immediately after treatment. According to the 24 hour *in sacco* OMD values shown in Chapter 3 both of these treatments should result in grains of similar overall digestibility.

2. Rumen Degradation Characteristics *In Sacco*.

The raw data for the NID and MSBP *in sacco* digestibility at different incubation times and an example of the statistical analysis of variance are given in Appendix 3. The Orskov and McDonald (1979) model was fitted to the the measured values and the curves derived from this equation are shown in Figure 4.2. For the equation:-

$$\text{the percentage degraded at time t(hours)} = a + b\,(1 - e^{-ct})$$

where a = the instantaneous loss of soluble and small particles,
b = the potentially digestible fraction,
c = the rate constant of b.

Figure 4.2 In Sacco OM Degradation of NID and MSBP

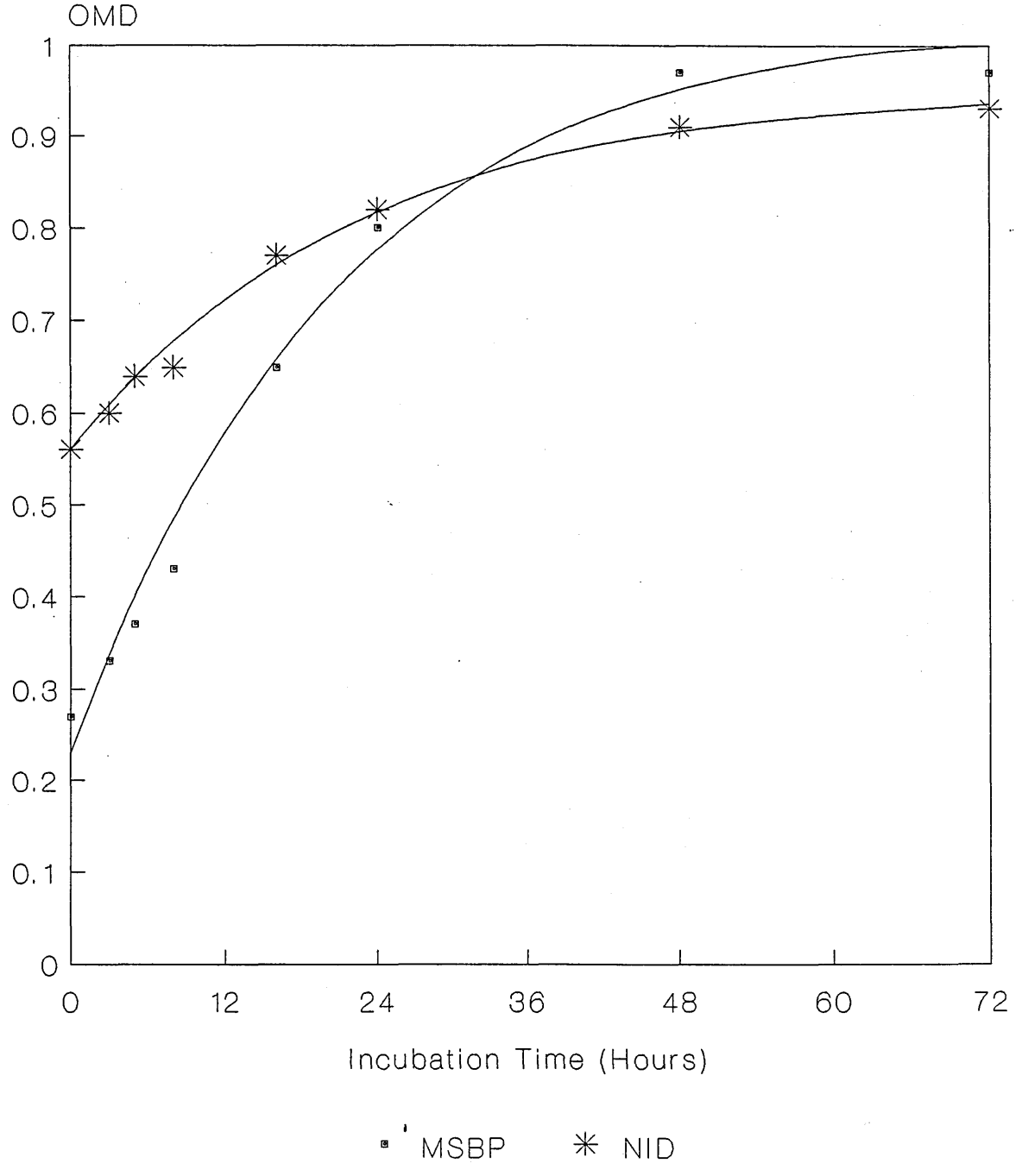


Table 4.4. a, b and c Constants for the *In Sacco* OMD of NID and Molassed Sugar Beet Pulp (MSBP).

	NID	MSBP	(SE)
a	54.94	22.95	(1.621)
b	41.17	84.62	(7.101)
c	0.05	0.05	(0.009)

The 'a', 'b' and 'c' terms for NID and MSBP are shown in Table 4.4. These values reflect the high instantaneous loss of soluble and particulate material from NID compared to SBP. For MSBP, most of the instantaneous loss is accounted for by WSC, however, for the NID, less than 50% of the instantaneous loss is truly soluble OM (levels of soluble OM were measured in Chapter 8). Since the a value for NID is high, the b value (potentially digestible residue) is much lower than for MSBP, however the rate of digestion of the b fraction (c value) is similar.

Comparison of the rates of gas production of NID and MSBP show that NID ferments more slowly over the 6 hour incubation time despite it having a higher 'a' value as shown in Table 4.4. The initial gas production rates of the soluble fractions from NID and MSBP are similar, it would therefore be expected that NID would be fermented at a faster rate than MSBP since it has a larger proportion of soluble material (0h loss). This suggests that the 0 hour losses from the bag do not consist of truly soluble material but contain a substantial proportion of particulate matter which is fermented more slowly. Without further study it is not possible to determine the fermentation characteristics of this particulate fraction.

The gas production and the *in sacco* findings allow the following comparisons of NID and MSBP in terms of their rumen degradation characteristics:

1. NID has a higher instantaneous loss of material from the nylon bag than MSBP. This loss consists of both truly soluble and particulate matter.

2. NID ferments more slowly over the first six hours than MSBP.

3. The particulate matter lost from the bags is fermented more slowly than the soluble material.

4. The insoluble NID residue remaining in the nylon bag is degraded at a similar rate to that of MSBP.

5. The truly soluble fractions of NID and MSBP ferment rapidly at similar rates.

These findings have important practical implications for feeding NID to ruminants. The fermentation characteristics of NID show that it could be fed as a supplement in the same way as MSBP to provide a source of short, fermentable fibre. MSBP is commonly used to reduce the content of sugar and starch to fibre ratios in rations by providing a source of highly digestible, readily fermentable fibre. This reduces the deleterious effects of large quantities of sugar or starch in the diet by stabilising rumen fermentations. It finds particular application in dairy cow diets as these rumen fermentation characteristics are beneficial for milk and butterfat production. The results from this experiment suggest that NID would act in the same way since it provides a similar source of fermentable fibre and a similar proportion of rapidly fermented soluble material.

4.2. The Effect of Free Draff Oil and Alkali on *In Vitro* OMD

The aim of the following experiments was to look in more detail at the depression of the IVOMD caused by the inhibitory effect of unsaturated fatty acids on rumen bacteria and the possible alleviation of this effect by addition of NaOH or $Ca(OH)_2$. To do this it was necessary to devise a method to measure the effects of treatment on the inhibition of digestion by the lipid fraction in a way unconfounded by the direct effects of alkali on the digestibility of the non-oil organic matter. This was achieved by extracting the oil from a quantity of untreated MDG in the Soxhlet apparatus using 40°C -60°C petroleum ether. The extracted oil and the oil-free residue were both retained. A range of levels of the oil and either NaOH or $Ca(OH)_2$ were then added to an *in vitro* tube and allowed to react at 60°C. The *in vitro* procedure was then carried out as described in section 2.4.1 using the ether extracted grain residue as the fermentation substrate. In this way, the fibrous (non-oil) component was never exposed to high concentrations of alkali and responses due to the action of alkali on fibre could be discounted.

Method

The extracted oil, weighed to 4 decimal places, was added to *in vitro* tubes in 0, 30, 60 or 90mg amounts. To these tubes was added 2 mls of a 2.5% aqueous solution of NaOH or $Ca(OH)_2$ (the $Ca(OH)_2$ was added as an aqueous suspension due to its low solubility). It was calculated that this amount of alkali should be sufficient to provide an alkaline pH for saponification to occur and sufficient cation concentration for maximum soap formation even at the highest level of oil. The tubes were thoroughly mixed, loosely stoppered and then placed in a waterbath for 4 hours at 60°C. The tubes were then mixed again so that the contents coated the inside of the *in vitro* tube and then dried in an oven at 60°C.

The *in vitro* analysis was then carried out as described in section 2.4.1 using 500 mg of ether-extracted spent grains as the fermentation substrate.

Results

The raw data for this experiment are given in Appendix 4.

Table 4.5 The Effect of Alkali on the Depression of IVOMD by Ether Extracted Oil from MDG.

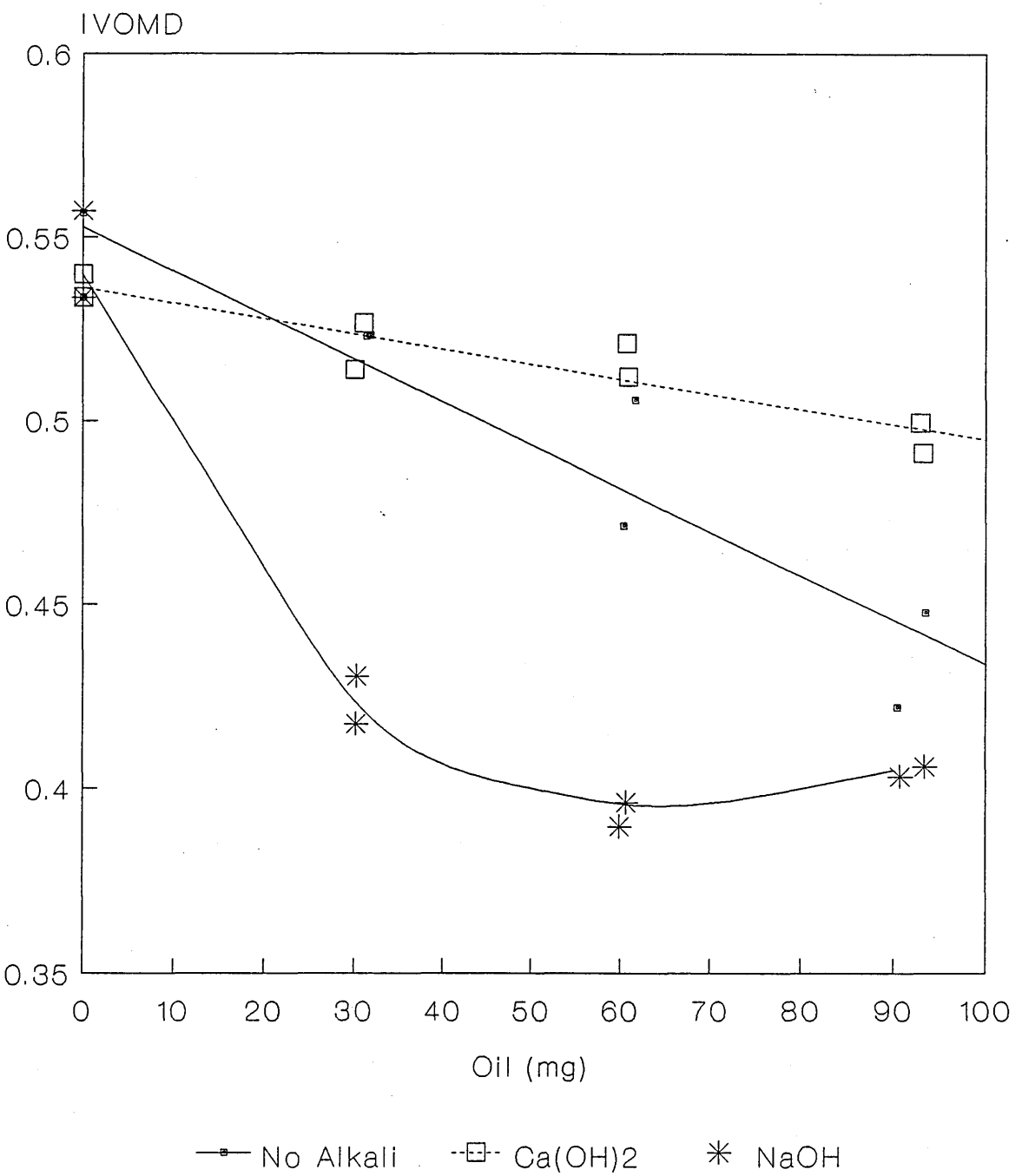
	Depression of IVOMD mg^{-1} oil	SD
Without Alkali	0.1189^a	0.01824
+ $Ca(OH)_2$	0.0417^b	0.00695
+ NaOH	0.1472^a	0.04166

Values with different superscripts differ significantly ($p<0.05$)

The results for experiment 1 are given in Table 4.5 and in Figure 4.3. The depression in IVOMD was linearly related to the quantity of added oil. This supports the findings of El Hag and Miller (1969) who reported that the digestibility coefficient was inversely related to the concentration of lipid and that this relationship was linear.

Treatment of the oil from MDG with $Ca(OH)_2$ reduces the inhibitory effect of oil on IVOMD although it does not negate it completely. The relationship between level of oil and IVOMD was still linear when oil was $Ca(OH)_2$-treated. There was a small but significant depression in IVOMD at the highest level of oil with the $Ca(OH)_2$ treatment, suggesting that either soap formation was not complete or that calcium soaps retain a degree of toxicity to the rumen micro-organisms. This finding is supported by studies on the effect of addition of linolenic acid to extracted MDG (El Hag and Miller, 1972). They reported that IVOMD was progressively decreased with increasing levels of fatty acid which occurred with and without the addition of calcium, even though the IVOMD was higher in the presence of calcium. Thus, calcium was not able completely to reverse the effects of linolenic acid. This

Figure 4.3 Effect of NaOH and $Ca(OH)_2$ on Reduction of IVOMD by Draff Oil



could have been due to the addition of $Ca(OH)_2$ as an aqueous suspension as observed by Jenkins and Palmquist (1980) who reported that calcium soap formation was incomplete in an aqueous medium.

In 1972 El Hag and Miller reported an experiment in which the effects of sodium salts and the corresponding acids on IVOMD were compared. They found that the effect of adding calcium was similar regardless of the form in which the fatty acid anions were added. In the present experiment addition of NaOH depressed the IVOMD below the level for the control treatment (without alkali). In the presence of NaOH the response to added oil was non-linear, the lowest level of oil giving a large depression in IVOMD. The greater depression in the IVOMD with NaOH addition suggests that the sodium soaps of fatty acids are more inhibitory than the untreated free oil. A likely explanation is that sodium hydroxide treatment hydrolyses the triglyceride in the MDG oil to the free acids which then form sodium salts of fatty acids which are soluble in aqueous medium. Since sodium salts are soluble in water the fatty acids would be more readily available to the micro-organisms and therefore cause greater inhibition of microbial function than the oil triglycerides which would yield free fatty acids at a relatively slow rate by the action of microbial lipases.

4.3. *In Vivo* Digestibility of $Ca(OH)_2$-Treated Spent Grains

The *in vivo* digestibility of the $Ca(OH)_2$-treated spent grains was measured in order to assess the digestible energy (DE) of the grains for ruminant animals. The digestibility of $Ca(OH)_2$-treated spent grains has been measured in different ways; *in sacco* and *in vitro* both of which give indications as to their digestion characteristics in the rumen. However, these measurements gave no indication as to the overall digestible energy value of the feed or its palatability or safety in the diet.

Method

The *in vivo* digestibility was measured using four wether sheep of approximately 70 kg liveweight, penned individually and bedded on sawdust. The animals were introduced to the diet by initially feeding 550 g DM/d of dried $Ca(OH)_2$-treated MDG (nutritionally improved draff or NID) and 750 g DM/d chopped hay. Over five days, the hay was phased out until by day six of the experiment the animals were offered 1000 g DM/d of NID. The experiment lasted for 14 days and animals were placed in metabolism crates and fitted with faecal collection bags for the final 6 days to facilitate complete faecal collection. All feed and faeces were subsampled and samples were analysed for DM, OM, NDF, nitrogen and GE as described in Chapter 2 and the digestibility for each of these parameters was calculated using these measurements.

Results

Digestibility measurements for each individual sheep are given in Appendix 5. The mean digestibilities and standard errors of the OM, CP, GE and NDF is shown in Table 4.6. The *in vivo* digestibility of the OM for NID was 0.64. Although, in this experiment there was no comparable measurement for untreated MDG, comparison with values measured by other workers shows that this is a significant increase in digestibility. The OMD of untreated MDG measured by Wainman and Dewey, (1982) and by Wainman *et al.*, (1984) was 0.54 and 0.50 respectively.

The apparent OM digestibility of NID was similar to that noted by El Hag and Miller (1969) for MDG in the presence of calcium (0.65).

Table 4.6. Analysis and Apparent *in vivo* Digestibility Coefficients of NID

Parameter (g/kg DM)	Content*	Mean (SE) Digestibility
Organic Matter	867	0.642 (0.0254)
Neutral Detergent Fibre	473	0.723 (0.0169)
Crude Protein	176	0.560 (0.0204)
Gross Energy (MJ/kg DM)	19.79	0.666 (0.0101)

* g/kg DM except for gross energy (MJ/kg DM)

The *in vivo* digestibility of MDG is markedly affected by level of feeding. Lewis and Lowman (1987) reported a depression in OMD of MDG at higher levels of feeding. They found that the OMD was reduced from 0.53 at maintenance level of feeding (M) to 0.46 when fed *ad libitum* (1.58 x M). This was thought to occur due to the increased rate of passage at higher levels of intake reducing the retention time of the short fibres of MDG in the rumen and thus reducing the digestibility of the grains. The level of feeding in the present experiment was approximately 1.3 x M on an ME basis so the digestibilities reported are likely to be an underestimate of the values that would be recorded at maintenance.

In 1970 Miller *et al.* studied the effect of additional calcium on the digestibility of other fractions of MDG. They reported digestibilities of 0.59, 0.78 and 0.63 for OM, CP and GE respectively in the absence of additional Ca. With Ca supplementation providing 4.72 g Ca/d these values increased to 0.65, 0.83 and 0.67. The values for OM and GE digestibility measured in the present experiment were of the same order as those observed previously by Miller *et al.* with supplementary Ca. The digestibility of NID crude protein, however, was markedly lower than the supplemented or unsupplemented values of

Miller *et al*.. Lewis and Lowman (1987) also commented on a low CP digestibility , although their value for *in vivo* CP digestibility of MDG was higher than that measured in the present experiment (0.68 compared to 0.56). They suggested that this arose due to the higher than average oil content of the grains in their study. The oil content of the grains used in this study was only average for the feed and therefore this does not explain this observation. They also observed that the CP digestibility was reduced to a value of 0.65 when the grains were fed *ad libitum*. Since the NID in this study was fed at above maintenance this may partly explain the low value obtained for CP digestibility.

The lower than expected protein digestibility for NID may also be related to increased hindgut fermentation. Palmquist (1985) suggested that high dietary levels of calcium, in particular limestone, enhance fermentation in the hindgut. Also, $Ca(OH)_2$ treatment has been shown to increase the proportion of soluble/small particle material in MDG which may increase ruminal by-pass and hence stimulate hindgut fermentation. Orskov *et al*. (1974) infused starch into the hindgut of sheep and observed an increase in metabolic faecal nitrogen loss. This was due to an increased production of microbial nitrogen which could not be absorbed and was lost in the faeces, depressing apparent nitrogen digestibility.

The NDF digestibility of NID was measured at 0.72 in this experiment. This value is much higher than NDF digestibility of untreated MDG measured by Hyslop (unpublished data) which ranged from 0.48 to 0.44. This reflects the effect of $Ca(OH)_2$ treatment on the fibre fraction and on the inhibitory effects of oil on digestion. The values (Chapter 3) for 24 h ISNDFD are of a similar magnitude; 0.45 for untreated grains and 0.78 for NID. Spent grains have a relatively small average particle size and this is particularly so after $Ca(OH)_2$ treatment, which increases the friability of the material. Consequently, its mean rumen retention time may be less than 24 hours. If this is the case then substantial hindgut digestion of NDF must occur to account for the similarity of the NDFD values measured *in sacco* and *in vivo*.

From measurements of the digestibility of the gross energy, the digestible energy (DE) was 13.18 (+/- 0.20) MJ/kg DM. When multiplied by a factor of 0.82 to allow for metabolic losses including methane as predicted by the Blaxter-Clapperton (1965) equation, this gives a metabolisable energy (ME) value of 10.81 MJ/kg DM. However, Wainman *et al.* (1982) noted that this equation does not accurately predict energy lost as methane for spent grains and that a value of 0.89DE gives a better prediction of ME. Using this figure, a value for the ME of NID of 11.73 MJ/kg DM may be calculated

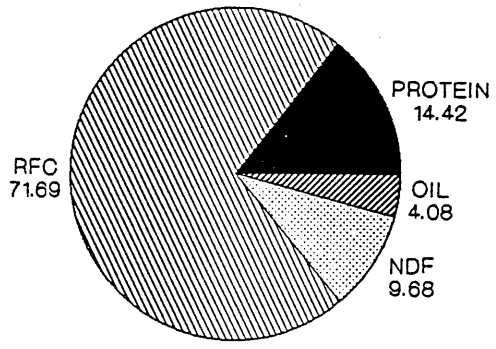
In the present experiment NID was fed at a level of 1 kg DM/d. At a ration M/D (MJ/kg DM) of 11.7 the maintenance requirement for energy for a 70 kg wether is approximately 8.93 MJ/d. Thus the sheep were fed at 1.31 times the maintenance requirement. Had the experiment been carried out at maintenance, the calculated ME of NID would have been higher. A value of 12.7 MJ/kg DM would be anticipated at maintenance on the basis of the effect of the level of feeding observed by Lewis and Lowman, (1987).

A calculation of the proportion of digestible energy from each fraction of the feed shows how this varies between barley, spent grains and NID and is represented in the pie charts shown in Figure 4.4. The source of digestible energy changes as barley is treated, firstly by malting and mashing, and subsequently by $Ca(OH)_2$-treatment. It shows that NID supplies a higher proportion of its DE from readily fermentable carbohydrate than spent grains and proportionally less from protein, NDF and oil. The proportions show how there is a shift from readily fermentable carbohydrate when the barley is malted towards protein, NDF and oil. $Ca(OH)_2$-treatment shifts the balance, at least partly, back to that for the whole grain by increasing the proportion of DE derived from the readily fermentable carbohydrate.

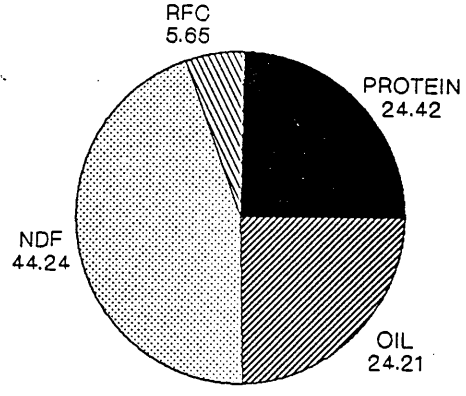
The benefits of $Ca(OH)_2$-treatment of spent grains are therefore threefold:

1. The effect on the fibre; the proportion of NDF in the grains is reduced and its digestibility is increased.

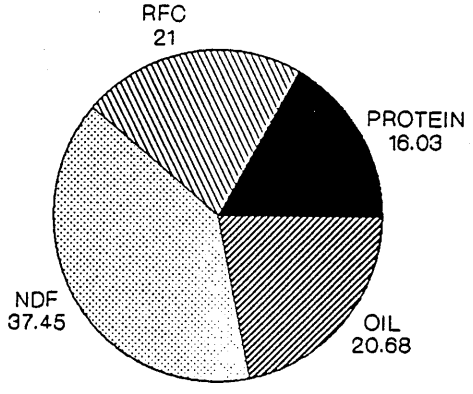
Figure 4.4 Source of DE
From Barley, MDG and NID



BARLEY



UNTREATED MALT DISTILLERS GRAINS



NID

2 The effect on the lipid; calcium soaps of fatty acids are formed which reduce their inhibitory effects on fibre digestibility.

3. The effect on the particle size and content of soluble material; solubilisation of the hemicellulose provides a source of rapidly fermentable carbohydrate which facilitates colonisation and digestion of the fibre by rumen micro-organisms.

CHAPTER FIVE

THE EFFECT OF $Ca(OH)_2$-TREATMENT OF MDG ON THE PERFORMANCE OF FINISHING LAMBS

5.1 Introduction

Chapters 3 and 4 described experiments designed to optimise conditions for the $Ca(OH)_2$ treatment of spent grains. The effects of treatment were assessed by chemical analysis and by digestibility measurements *in sacco*, *in vitro* and *in vivo*. All of these measurements gave an indirect indication that the nutritive value of the grains to ruminant animals and suggested that this was increased by treatment with $Ca(OH)_2$. The aim of the following experiment was to evaluate this increase directly by investigating the effects of $Ca(OH)_2$-treatment of spent grains on animal performance.

The main experiment reported in this chapter was a comparison of $Ca(OH)_2$-treated MDG (NID), untreated MDG mixed with minerals (min. draff) and a proprietary lamb finishing concentrate (Premium Sheep Fattening Pellets, Carrs Farm Foods Ltd, Solway Mills, Silloth) as a positive control as supplements to hay for finishing lambs. Two smaller experiments were carried out concurrently with this growth trial: the first to characterise the rumen degradation of the major nutrients (OM, N and NDF) of the three supplements, and the second to measure the *in vivo* digestibility of the three feeds in sheep when fed a similar amount of hay as fed to the finishing lambs. This permitted the the effects of $Ca(OH)_2$ treatment on animal performance to be related directly to the effects on rumen degradation and overall digestibility.

5.2 Methods

5.2.1 Preparation of Feeds

A 20 tonne load of malt distillers grains was compacted into two 10 tonne-capacity experimental silos and was stored anaerobically until required. For the mineralised draff diet, draff was removed from the clamps three times a week and weighed into polythene bags pending feeding. Immediately prior to feeding, the grains were thoroughly mixed with 10g/kg of the following mineral mix (g/kg);

NaCl	200
KCl	150
Calcined Magnesite	36
Dicalcium Phosphate	384
Limestone	180
TE + Vit. mix	50

The NID was prepared immediately prior to the begining of the experiments and stored until required in polythene dustbins. When the ensiled draff was taken from the clamps the pH was found to be very low (pH 3.15). To compensate for this, 8 mls/kg draff of 40% NaOH was added in addition to 20 g of industrial grade $Ca(OH)_2$ (Limbux, ICI) per kg of draff FW (approximately 80 g $Ca(OH)_2$/kg DM). These materials were thoroughly mixed in a cement mixer and then sealed in large polythene bags and placed in an oven at 60°C overnight (18 hours). After this the treated grains were returned to the cement mixer and mixed with 25 g molasses/kg and 13 g/kg of the following mineral mixture (g/kg) designed to redress the Ca:P imbalance caused by $Ca(OH)_2$ addition during treatment, and to supply the other mineral requirements of the lambs;

Magnesium Phosphate	77
Monodicalcium Phosphate	770
KCl	115
TE + Vit. mix	38

The resulting dough-like material was then passed through an industrial extruding machine and formed into long continuous pellets of 0.5'' diameter. The wet material was extruded directly onto large mesh-based drying trays and placed in a forced draught oven at 60°C until dry (>90% DM).

The analysis of these two feeds, the sheep finishing pellets (Carrs Nuts) and the hay is given in Table 5.1

Table 5.1 Analysis of Feeds used in Lamb Finishing Experiment.
(g/kg DM unless stated)

	Hay	Min. Draff	NID	Carrs Nuts
Dry Matter (g/kg)	958	250	984	985
Crude Protein	52	200	173	175
Organic Matter	952	930	851	877
NDF	686	774	451	409
Crude Fibre		199	162	126
Gross Energy (MJ/kg DM)		20.6	18.9	17.8
Acid Hydrolysed EE		73.0	61.5	36.5
IVOMD (%)	51.0	58.8	71.9	79.1
Ca	2.3	9.0	42.0	16.7
P	1.9	6.8	10.7	6.7
Mg	1.0	2.2	2.6	3.1
K	12.80	5.74	6.91	16.5

5.2.2 Lamb Finishing Experiment

Thirty Blackface cross lambs were purchased from Ayr Livestock Market on the 1st. September 1988. Lambs were weighed and penned in groups of 10 to give a mean liveweight of approximately 31kg for an initial acclimatisation period. Each pen was then randomly assigned to one of the three experimental diets which consisted of *ad libitum* hay with;

Carr's Premium lamb fattening nuts, NID or mineralised draff. All lambs were dosed with Panacur SC anthelmintic and an anticlostridial vaccine, Heptavac P. Lambs were weighed again 11 days later when all animals were seen to be eating the diet and were paired within each treatment group according to sex and weight.

During the experiment lambs were penned in pairs and bedded on sawdust. Animals were initially offered *ad libitum* hay with approximately 700g DM/pen/day of the supplements. This was increased as the animals were able to eat more to a maximum of 2400g DM/pen/day by day 50 of the trial. Refusals of both concentrate and hay from each pen were removed daily and the dry matter intake of each was determined. Animals were weighed weekly and condition scored 'blind' by the College Farm manager immediately after purchase and weekly approaching the time of slaughter.

After 60 days of the trial, 20 of the 30 lambs were judged ready for slaughter and were sent to the abattoir. The carcasses were graded and chilled overnight. The following day 50g fat samples were taken from the perirenal fat and from the subcutaneous fat surrounding the tailhead. The fatty tissue was chopped into small pieces and extracted as described in section 2.5.1. The fatty acid profile of each fat sample was analysed by derivatisation and GC analysis as described in sections 2.5.6. The melting point of the extracted fat was measured using an electrothermal melting point apparatus (Electrothermal Engineering Ltd., Neville Road, London. E.7.)

The ten remaining lambs were kept on their experimental diets for a further 32 days (until day 92). The weekly weighings were continued and the animals were condition scored weekly. The animals that were fed the mineralised draff continued to gain weight slowly but did not increase in condition score and it was therefore decided that these animals should be fed NID. The diet was introduced over a period of 11 days . Four more animals were slaughtered at this point (day 103) and the remaining 6 animals were fed entirely NID and hay for a further 11 days. On day 114 the remaining animals were slaughtered.

Samples of blood were taken from all animals on day 12 and day 53 of the experiment by venepuncture of the jugular vein and using heparinised vacutainer blood tubes. Blood samples were centrifuged for 15 minutes at 3000 rpm and the supernatant plasma was stored at -20°C awaiting analysis for calcium, magnesium and phosphorus.

The data for dry matter intakes of hay and supplements for the first 60 days were analysed using the Genstat V (1980) statistical package. Liveweight data was analysed first by using a regression analysis to give individual daily liveweight gains for each lamb, and then using analysis of variance of these values. Blood parameters, fatty acid data and killing out percentages were analysed using Edex (AFRC, Edinburgh). The date of slaughter of the lambs was analysed using the X^2 test.

5.2.3 Rumen *In sacco* Degradability of Feeds

The rumen *in sacco* degradability of the major components of the feeds used for the lamb finishing experiment were measured using four fistulated wether sheep fed a diet of 300 g/d of sheep concentrates and 800 g/d chopped hay. The experiment was carried out as described in Section 2.4.2. Nylon bags containing mineralised draff, NID, Carrs Nuts and chopped hay were incubated simultaneously in the four sheep for 0, 3, 5, 8, 17, 24 and 48 hours. The OM, NDF and nitrogen contents of the feeds and the residues remaining in the bags was measured as described in Sections 2.1.2, 2.1.5 and 2.1.3.1 respectively. The values obtained were used to calculate a, b and c terms for the Orskov McDonald equation (1979) defining the degradability curves for the three nutrients and allowing calculation of the effective degradability of nutrients at different outflow rates. These values were analysed for statistical significance by analysis of variance.

5.2.4 *In vivo* Digestibility Measurement

Measurement of the *in vivo* digestibility was carried out using four fistulated wether sheep of approximately 70kg liveweight. The experiment was of 4 x 4 latin square design. The animals were housed individually and bedded on sawdust for the first fortnight of each three week period. For the final week the animals were held in metabolism crates and were fitted with faecal collection bags to facilitate complete faecal collection. The four diets were:-

Mineralised Draff + Hay
NID + Hay
Carrs Nuts + Hay
Hay

Animals were fed approximately 1200g DM/d with a forage:supplement ratio of 33:67 (with the exception of the hay only diet). On day 13 of each period, a sample of rumen liquor was taken and the pH was measured, immediately before the morning feed and hourly thereafter for the following 7 hours. VFA concentrations of the rumen liquor samples were determined as described in section 2.7.1. On day 14, the *in sacco* dry matter digestibility of chopped hay was measured as described in section 2.4.2

The DM intake of the sheep was recorded daily, feeds and refusals were weighed and subsampled for analysis. Faecal samples from the 6 day collection period were weighed and subsampled. Feeds, refusals and faecal samples were analysed for DM, OM, N, ADF, NDF and gross energy as described in Chapter 2.

5.3 Results

5.3.1 Lamb Production Experiment

Only measurements made during the first 60 days of the experiment were analysed statistically and the analysis of variance of the dry matter intakes of supplements and hay are given in Appendices 6 and 7.

At day 60, all 10 of the lambs receiving Carrs Nuts and 8 of those fed NID were judged to be fit for slaughter. Only 2 of the lambs fed mineralised draff were finished at this time. Animals unfinished at day 60 remained on experimental diets for a further 32 days and were then reassessed for slaughter. Four animals were slaughtered on day 92 (the 2 remaining NID fed lambs and two of those fed mineralised draff). This left 6 lambs from the mineralised draff treatment which were assessed as unlikely to finish within an acceptable time on their diet. Consequently, they were transferred to NID and were judged to be finished three weeks later. The numbers of lambs finished at each date was analysed using a X^2 test and the differences due to treatment were found to be significant ($p<0.05$). The experiment proper was thus ended on day 60, however, the intakes of hay and concentrate of the animals remaining were recorded and are shown in Figure 5.4. Group mean liveweights, condition scores and intakes of hay and concentrates are given in Table 5.2 Mean lamb supplement and hay intakes are shown in Figures 5.1 and 5.2 respectively, mean liveweights are given in Figure 5.3.

The supplement dry matter intakes given in Figure 5.1 show that the intake of mineralised draff was consistently lower than that of Carrs nuts or of NID. Although the lambs were not fed the supplement *ad libitum*, animals offered NID and Carrs nuts ate significantly ($p<0.05$) more than those offered mineralised draff. The aim was to fix levels so that supplement DM intakes were near maximal but equal. Levels of supplement offered were limited to the amount of DM that lambs fed mineralised draff could consume but the intakes of this diet proved inconsistent so that less was actually consumed than for the other two supplements.

Figure 5.1 Lamb Finishing Experiment
Mean Daily Supplement Intake (DM)

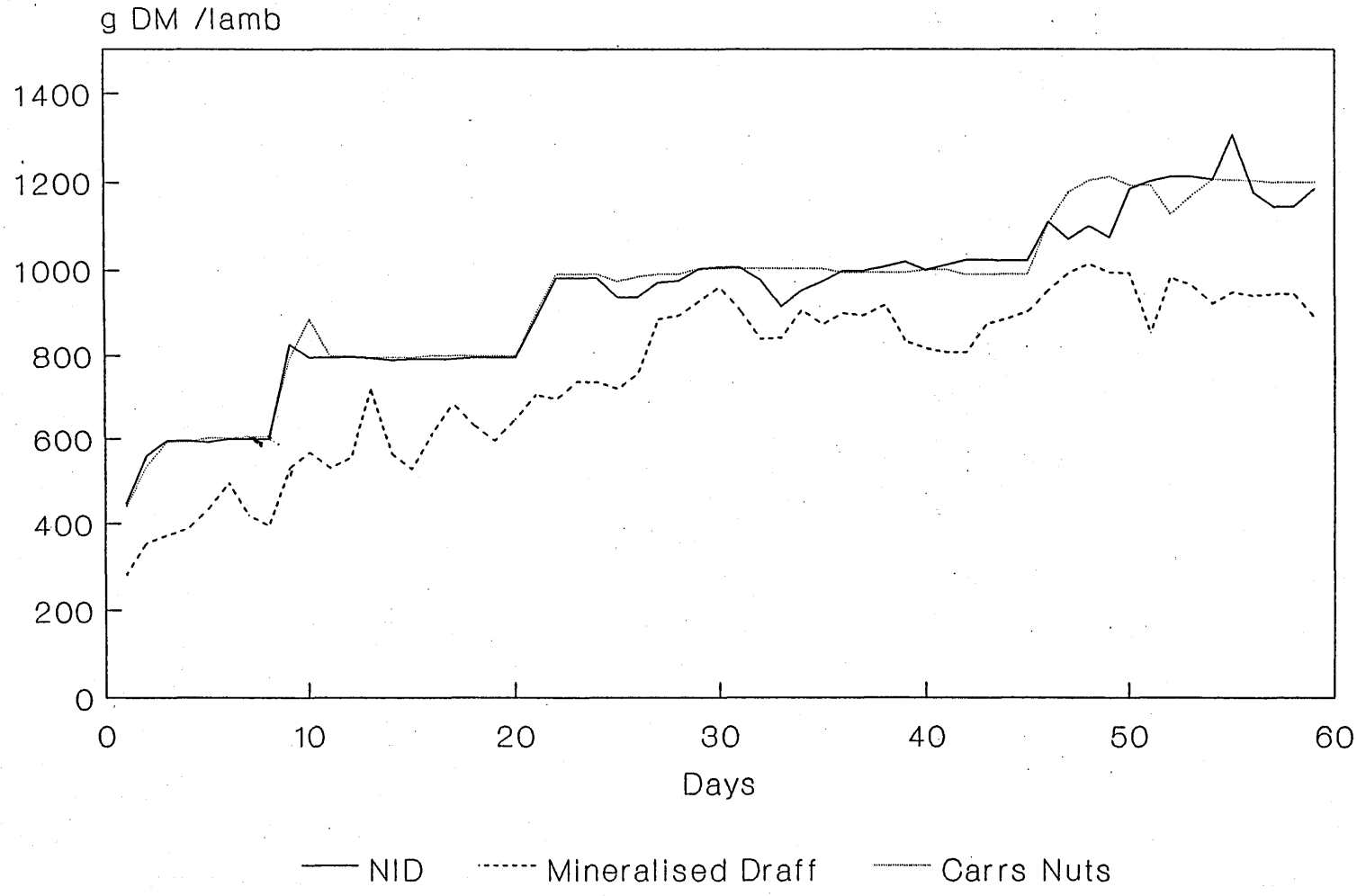


Figure 5.2 Lamb Finishing Experiment
Mean Daily Hay Intake (DM)

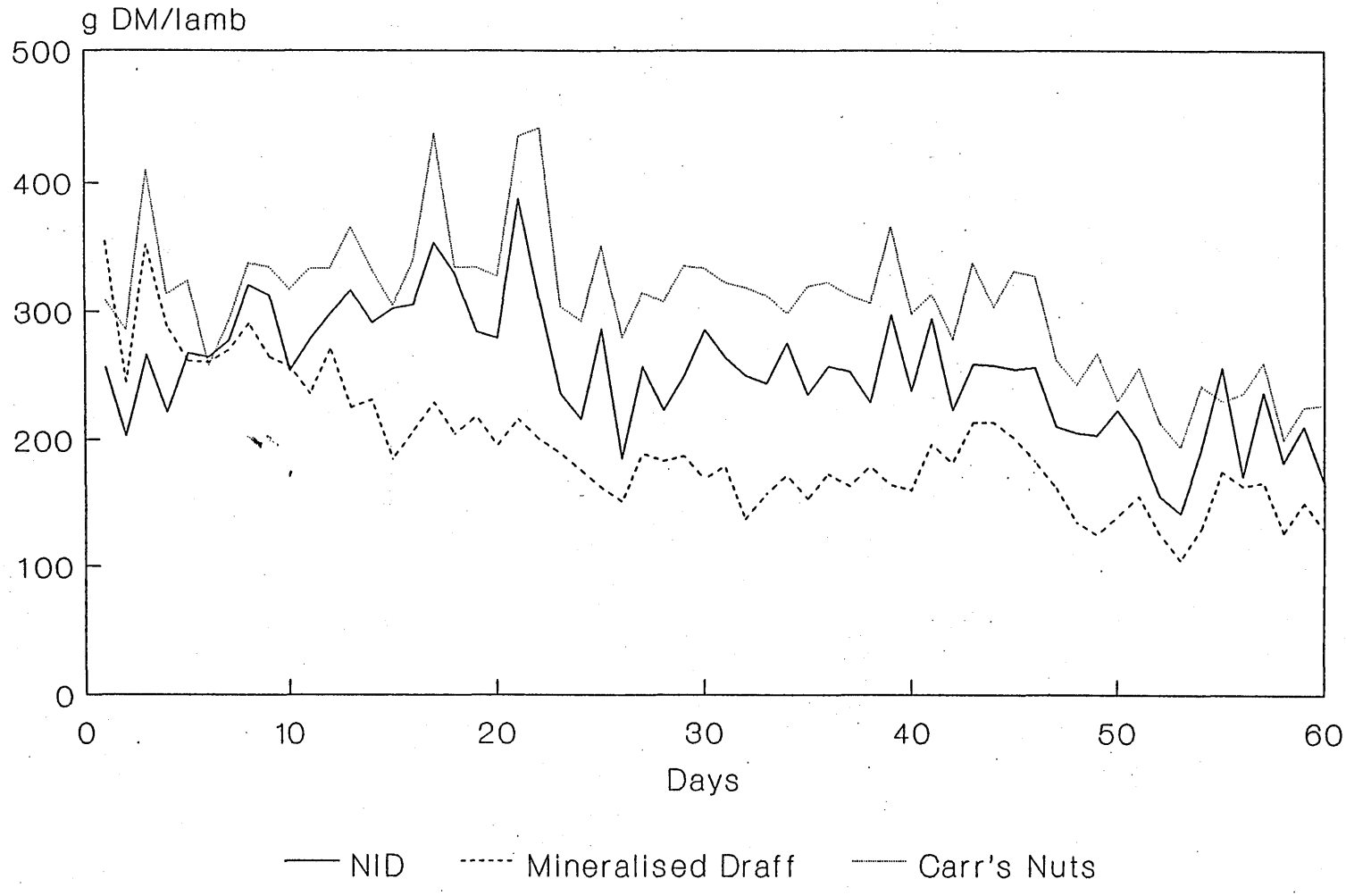


Figure 5.3 Lamb Finishing Experiment
Mean Lamb Liveweight (Weekly)

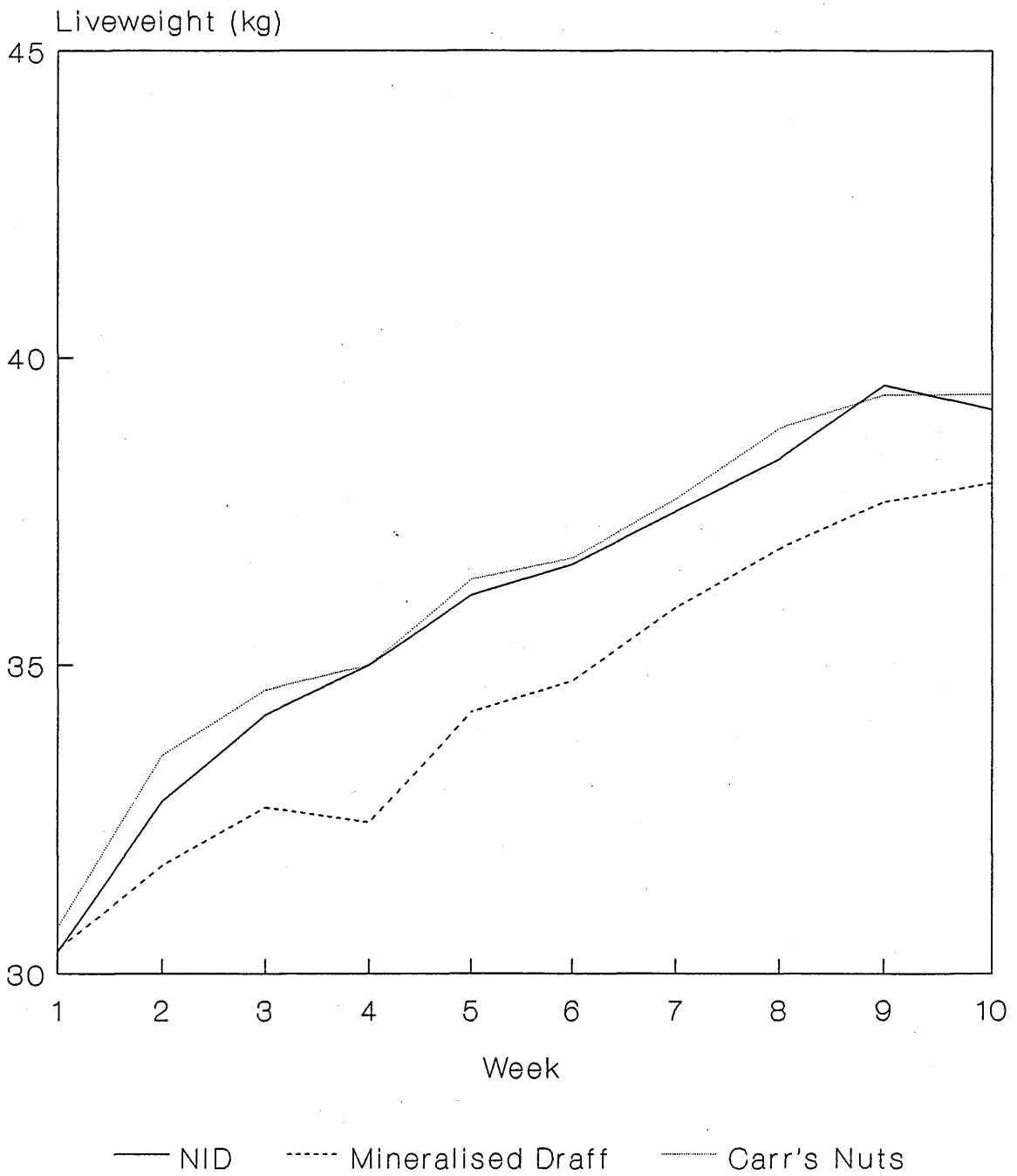


Figure 5.4 Lamb Finishing Experiment
Supplement and Hay DMI
Days 60-113

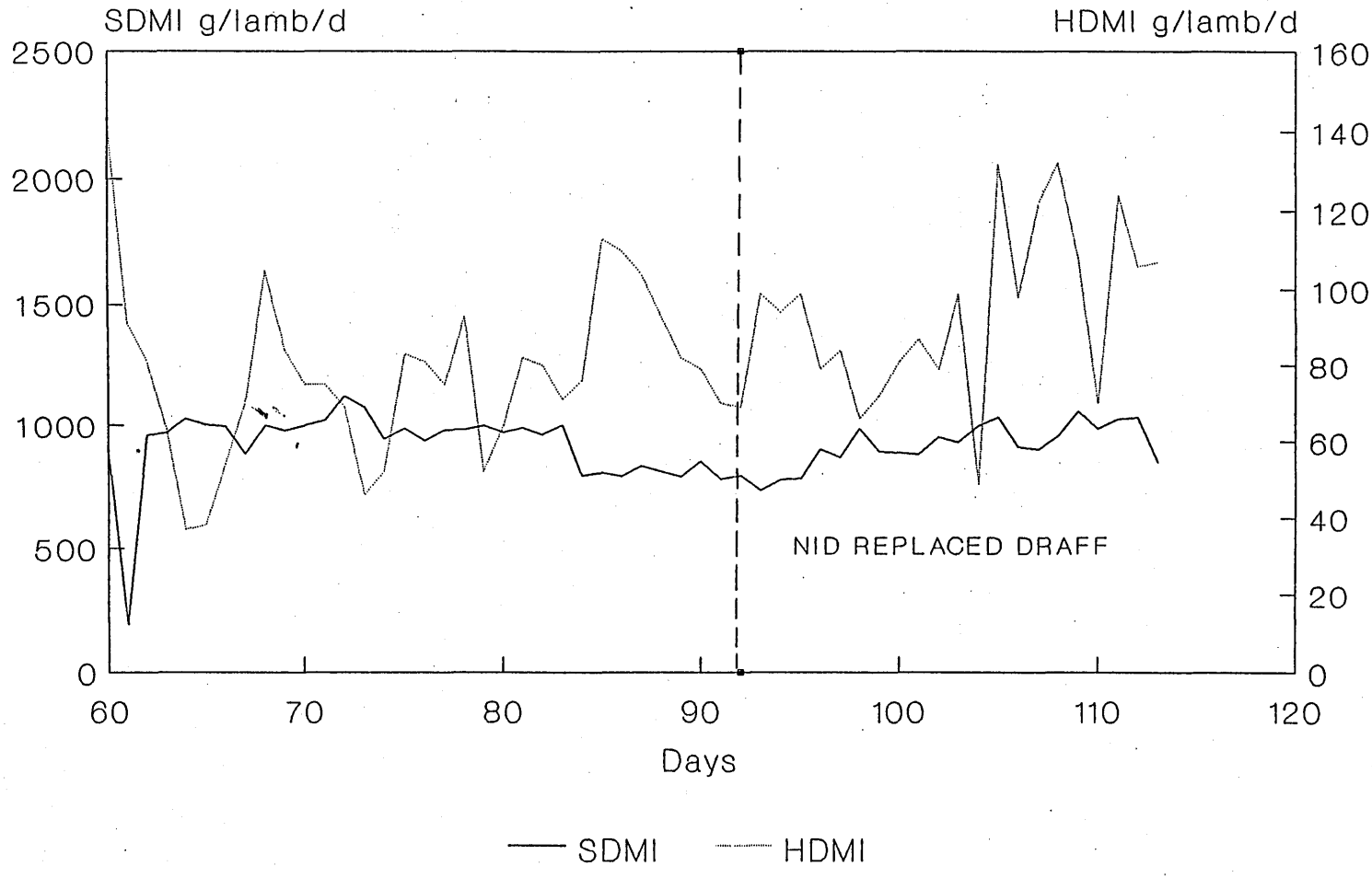


Table 5.2 Mean Liveweights, Condition Scores and Intakes of Lambs

	NID	Min. Draff	Carr's Nuts	SE
Initial LW (kg)	31.0	30.9	31.0	0.75
Day 60 LW (kg)	39.2	38.0	39.4	0.75
LWG g/day	136	123	130	10.9
No. Lambs Finished. Day 60	8	2	10	
Day 92	2	2	-	
Day 114	-	6	-	
Condition Score (day 1)	2.9	3.0	2.8	0.12
Condition Score (day 59)	3.9^{a}	3.6^{b}	4.0^{a}	0.06
Concentrate DMI (g/day)	925^{a}	750^{b}	934^{a}	19.2
Hay DM Intake (g/day)	258^{a}	200^{b}	311^{a}	17.4
Killing Out %	45.45	41.96	45.40	2.262

Means in the same row with different superscripts differ significantly ($p<0.05$)

Intakes of both draff diets showed considerably more variation than the intakes of the proprietary concentrate. This can be explained in part by the variations in the dry matter content of the feeds but, in the case of the mineralised draff, there were substantial refusals on occassions. Very few refusàls of the NID or the proprietary concentrate were recorded.

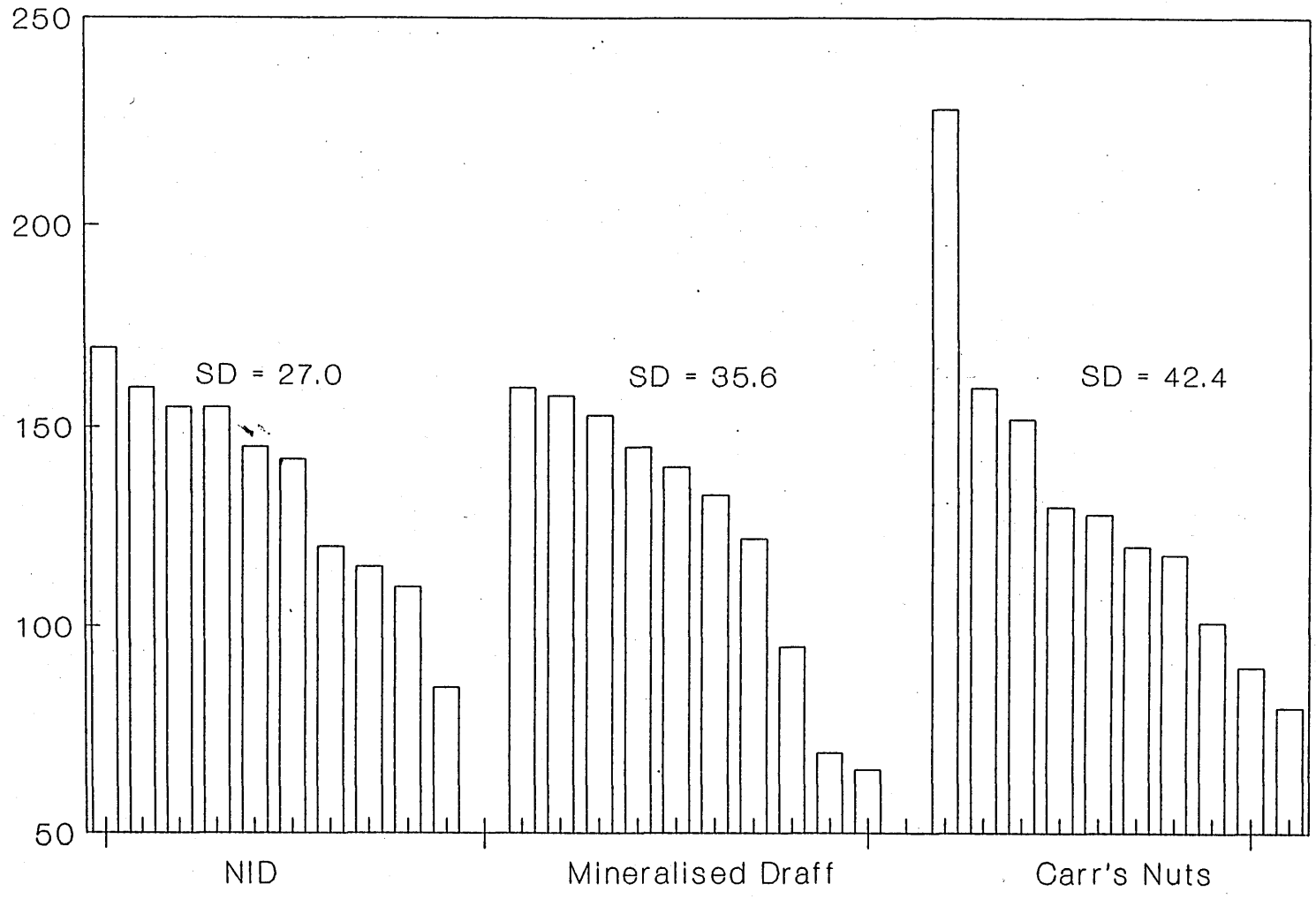
Hay dry matter intakes showed great daily variation. There was a trend towards lower hay intakes as the supplement level increased. Mean supplement:hay ratios were 78:22, 79:21 and 75:25 for the NID, mineralised draff and Carrs nuts diets respectively.

Although the differences in liveweight or liveweight gain were not significantly different ($p<0.05$) there was a clear trend for lambs fed Carrs nuts and NID to grow faster than animals fed mineralised draff. The lower growth rate observed for lambs fed mineralised draff and the lower intakes of both supplement and hay may have been related to the fact that this feed was much wetter than the other supplements (approx 250 vs. 950 g/kg thus limiting intake by a 'gut fill' effect. This is in accord with the findings of Porter *et al.* (1977) who suggested that bulk limitation effects may have been responsible for poor intakes at high levels of spent grain inclusion in ruminant diets. This is supported by the lower killing out percentages observed for the mineralised draff-fed lambs. However differences in digestibility and in the form and level of oil may also have been important.

Although there were differences in final liveweight on day 60 these were not significant ($p<0.05$). This was due to the large variation in liveweight gains between lambs and is illustrated in Figure 5.5 which shows that one lamb fed Carrs Nuts grew much faster than others fed the same diet. The variation in daily liveweight gain was least for animals fed NID. Analysis of variance of trimmed means however still did not prove the differences in LWG to be statistically significant at $p<0.05$.

Differences between the plasma levels of phosphorus and magnesium of lambs fed different diets were statistically significant on day 12 of the experiment as were differences between calcium levels on day 53. Mean levels of Ca, P and Mg measured in lamb plasma are given in Table 5.3. The levels of minerals measured fell well within the normal ovine range. This was an important finding in view of the Ca:P ratios in the supplements which were 1.32:1, 3.93:1 and 2.49:1 for mineralised draff, NID and Carrs Nuts respectively. The Ca:P ratio considered most suitable for ruminant livestock is within the range 1:1 - 2:1 (McDonald *et al.*, 1981). There is evidence (Wise *et al.*, 1963) that ruminants can tolerate higher but not lower ratios without adverse effect. The results of the present experiment would support this view. The observed plasma mineral levels suggest that the atypical Ca or P content of NID did not cause significant disturbance

Figure 5.5 Lamb Finishing Experiment
Individual Lamb Liveweight Gain (g/d)



of mineral metabolism although this finding would require further detailed investigation.

Table 5.3 Mean Levels of Ca, P and Mg in Lamb Plasma

		NID	Min. Draff	Carr's Nuts	SE
Day 12					
Calcium	(mg/100ml)	11.71	11.14	11.55	0.081
Phosphorus	(mmol/l)	2.39^{a}	1.78^{b}	2.00^{ab}	0.041
Magnesium	(mg/100ml)	2.65^{ab}	2.63^{a}	2.96^{b}	0.027
Day 53					
Calcium	(mg/100ml)	10.31^{ab}	10.35^{a}	9.58^{b}	0.064
Phosphorus	(mmol/l)	2.95	2.83	2.59	0.053
Magnesium	(mg/100ml)	2.45	2.57	2.53	0.018

Means in the same row with different superscripts differ significantly ($p<0.05$)

Mean fatty acid profiles for subcutaneous and perirenal fat are given in Table 5.4 and are shown in Figures 5.6a and 5.6b. In both sites of fat deposition, the NID-fed lambs had significantly ($p<0.05$) lower proportions of C18:0 and significantly more C18:2. Lambs fed the proprietary concentrate had proportionally less C18:3 than in the draff based diets, a difference which was significant ($p<0.05$) in the perirenal fat.

Figure 5.6a Lamb Subcutaneous Fat
Fatty Acid Content

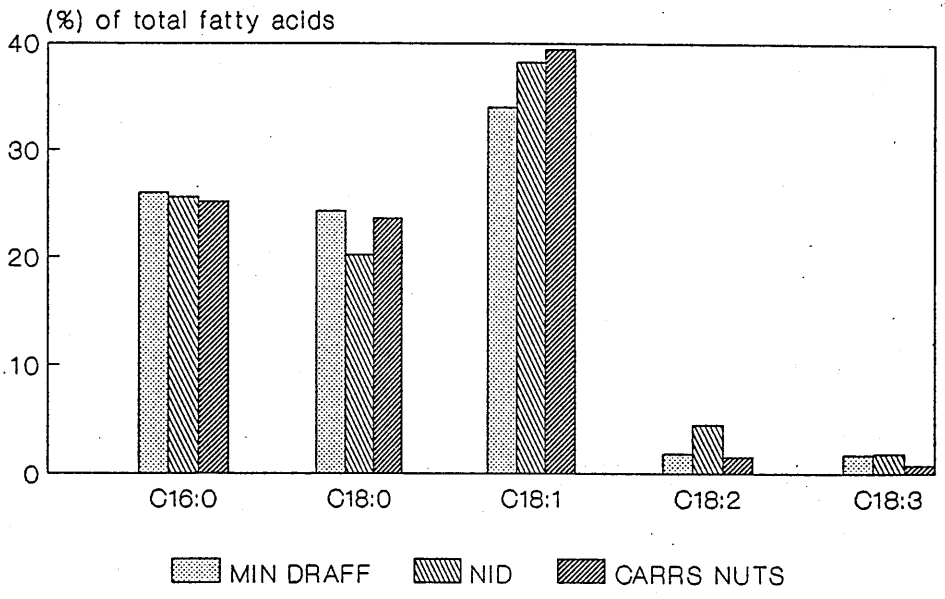


Figure 5.6b Lamb Perirenal Fat
Fatty Acid Content

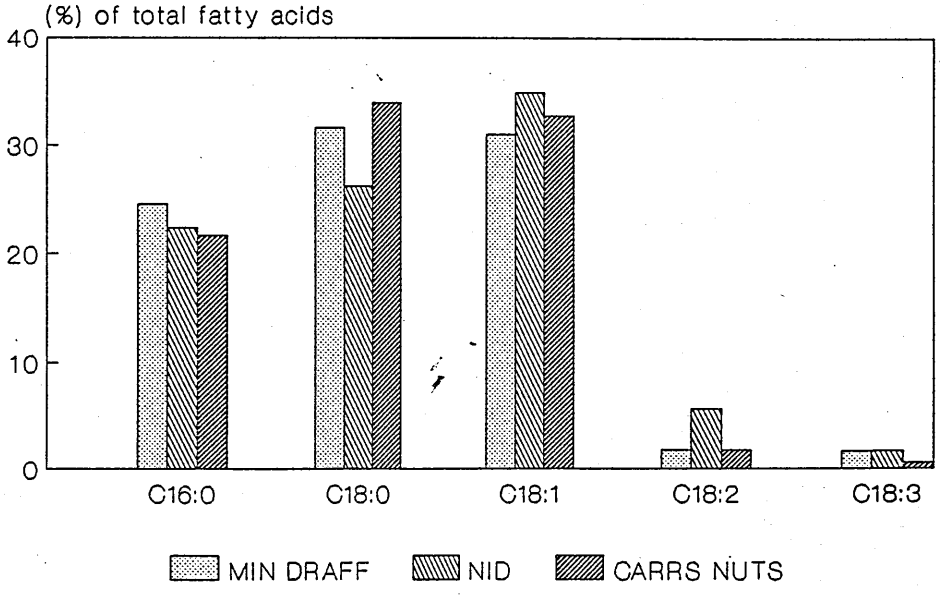


Table 5.4 Mean Lamb Fat Fatty Acid Profile.

% Total Fatty Acids	Subcutaneous Fat Min Draff	NID	Carrs Nuts	(SE)	Perirenal Fat Min Draff	NID	Carrs Nuts	(SE)
C16:0	25.96	25.57	25.12	(0.708)	24.63^{a}	22.38^{b}	21.67^{b}	(0.736)
C18:0	24.22^{a}	20.19^{b}	23.59^{a}	(1.077)	31.60^{a}	26.27^{b}	33.95^{a}	(1.055)
C18:1	34.05^{a}	38.25^{b}	39.42^{b}	(0.831)	31.02^{a}	34.85^{b}	32.76^{ab}	(0.744)
C18:2	1.84^{a}	4.55^{b}	1.57^{a}	(0.196)	1.79^{a}	5.73^{b}	1.80^{a}	(0.379)
C18:3	1.72	1.83	0.77	(0.354)	1.66^{a}	1.75^{a}	0.62^{b}	(0.244)

tentative stats only

Values in the same row, from the same site, with the same superscript do not differ significantly ($p<0.05$)

The largest effects of diet on fatty acid profile are found in the perirenal fat. This is in agreement with observations made by Duncan and Garton (1967) noted that there was preferential deposition of absorbed fatty acids in internal sites in ruminants. The differences in degrees of saturation between the two sites is also in accord with the findings of other researchers who have noted that in general, there is a progressive increase in saturation from peripheral through intermuscular and intramuscular fat, to deep body sites such as kidney fat. This trend applies to most types of carcass, including those from cattle sheep and pigs (Leat 1970). Such differences are the result of several factors such as age and dietary regime including fatty acid intake.

The change in fatty acid profile was reflected in the melting point of the depot fat. The mean melting points of subcutaneous fat from lambs fed mineralised draff, NID and Carrs nuts were 42.13, 41.69 and 42.85 respectively (SE 0.918). Mean perirenal fat melting points were 47.04, 44.84 and 46.60 respectively (SE 1.806). None of the differences due to diet were significant ($p<0.05$). L'Estrange and Mulvihill (1975) observed that the fatty acid most closely correlated

to melting point in lamb fat was C18:0. This observation explains the higher melting point of the perirenal fat, since levels of C18:0 from this site were higher than from the subcutaneous fat.

Although the absolute concentrations (g/kg tissue lipid) of the individual fatty acids were not measured in this experiment, the change in fatty acid profile of the fat tissue of lambs fed NID is of a similar proportion to that reported by Gibney and L'Estrange (1975). They showed that concentrations of linoleic acid in the kidney fat increased from 20 to 70 mg/g when lamb diets were supplemented with 58 g/kg of sunflower oil. They also reported a decline in the melting point of the fat from 46.7 to 43.3°C. Fat intake by lambs in the present experiment were 55.0, 57.3 and 34.6 g/d for mineralised draff, NID and Carrs nuts diets respectively).

Thus fat intakes for lambs on NID and mineralised draff diets were very similar yet the C18:2 levels were substantially greater in the NID-fed lambs. This suggests that the oil present in NID is to an extent escaping biohydrogenation in the rumen, in sufficient quantity to cause changes in the fatty acid profile of the depot fat. Cook *et al.* (1972) fed steers 200g/kg protected sunflower oil for 8 weeks and found linoleic acid in the kidney fat to have increased from 50 to 200mg/g after 2 weeks. Incorporation into internal depots was greater than into subcutaneous, supporting the view of preferential uptake into internal depots.

From reports in the literature, the degree of protection from biohydrogenation afforded by the formation of calcium soaps of fatty acids (CSFA) is variable, and is dependent on the calcium soaps remaining undissociated in the rumen. Ruminal biohydrogenation has been shown to require a free carboxyl group for one of the isomerisation reactions in the pathway to occur. Kepler *et al.* (1970 and 1971) tested the substrate specificity of a cis-12, trans 11 isomerase isolated from B. fibrisolvens against several positional and configurational isomers of octadecadienoic acid and showed an absolute requirement for the cis9,cis12 octadecanoic acid with a free carboxyl group on carbon 1 and the final double bond six carbon atoms

from the terminal methyl group. Although this is only one of many micro-organisms capable of biohydrogenation, Harfoot (1981) when reviewing lipid metabolism in the rumen suggested that it was this isomerase enzyme, or at least one very similar, that was present in most strains of biohydrogenating organisms.

Palmquist (1984b) noted that, despite feeding dairy cows large quantities of calcium soaps of fatty acids including 670 g/d of C18:2, there was little effect on the fatty acid content of the milk fat. He suggested that this was due to dissociation of the CSFA, particularly the soaps of the less saturated fatty acids which have a higher pKa value, at the low rumen pHs noted in the cows. Other workers, using more saturated CSFA (Sklan, 1989) have shown that they were not degraded in the rumen. Calcium soaps have been shown to form more readily with longer chain, less saturated fatty acids (Jenkins and Palmquist, 1982). Results from the present experiment suggest that at least some of the unsaturated fatty acids were escaping biohydrogenation and that this effect was increased by $Ca(OH)_2$ treatment.

5.3.2 *In Sacco* Feed Degradability

Organic Matter Degradation

Digestibility data and an example of the statistical analysis applied to the 'a', 'b' and 'c' constants for each fraction are given in Appendix 8. The OM degradability results given in Table 5.5 in Figure 5.7 show that NID had a significantly ($p<0.05$) higher 'a' value than mineralised draff. This reflected the increased instantaneous losses with $Ca(OH)_2$-treatment measured *in sacco* in this experiment and is supported by *in sacco* results given in Chapters 3 and 4. As discussed in these Chapters, this can be explained by the action of $Ca(OH)_2$ on the hemicellulose fraction, increasing solubility and small particle loss. With the exception of having a significantly lower 'a' term than Carrs Nuts, NID had similar rumen degradation characteristics to the proprietary concentrate.

Figure 5.7 In Sacco Digestibility
Organic Matter

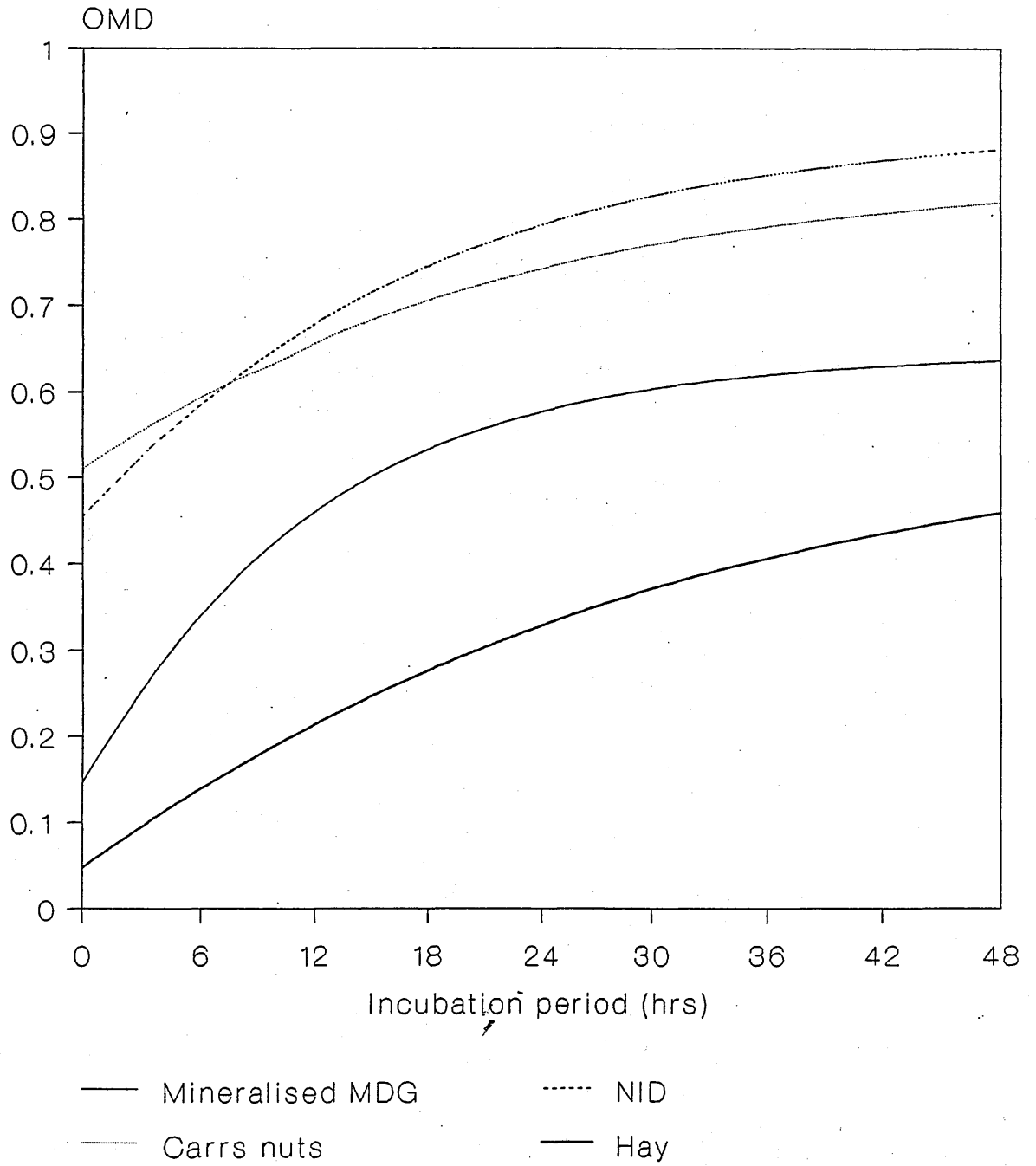


Table 5.5 *In Sacco* OM Degradability

	Min. Draff	NID	Carrs Nuts	Hay	SE
a	14.6^{a}	45.5^{b}	51.0^{c}	4.8^{d}	0.90
b	49.9	45.8	34.8	52.3	4.91
c	0.083^{a}	0.056^{ab}	0.046^{ab}	0.032^{b}	0.0103
*Dg (3%/h)	51.3^{a}	73.5^{b}	70.3^{b}	31.8^{c}	1.46
*Dg (6%/h)	43.3^{a}	66.3^{b}	64.3^{b}	23.0^{c}	0.85

Means in the same row not sharing common superscripts differ significantly ($p<0.05$).

* Effective rumen degradability at outflow rates of 3 and 6%/hour.

The results show that $Ca(OH)_2$-treatment increased the potentially digestible fraction (a + b) for MDG from 64.5 to 91.3%, increasing the amount of digestible OM in the supplement to a level greater than measured for the proprietary concentrate. Apart from a lower 'a' value (instantaneous loss) the rumen degradation of NID OM was not significantly different from Carrs Nuts.

The OM degradation curve constants for the mineralised draff differ from those reported by Hyslop and Roberts (1988). The 'a' terms for mineralised draff were much lower (14.6 vs 33.0) whilst the 'b' terms were higher (49.9 vs 37.5). The reasons for these differences are not apparent, but may in part, be explained by the variations in the feeds due to differences in processing conditions (for example, differences in milling, malting and mashing procedures for draff).

Mineralised draff had a similar potentially digestible residue (b) to NID. Figure 5.7 shows that the initial rate of OM degradation from this fraction is faster for mineralised draff than for NID. However

beyond 12-18 hours, NID OM is degraded more quickly. The curve for mineralised draff begins to reach a plateau at approximatley 60% OMD, whereas total OM loss from NID is still increasing at 48 hours giving digestibilities of >85% . Given the relatively small feed particle size of draff, it is unlikely that it would remain in the rumen beyond the 12 - 18 hours point identified from the graph. The increase in the instantaneous loss caused by $Ca(OH)_2$-treatment, therefore is likely to be of greater significance in the increase in draff rumen degradation than the faster rates of degradation observed at longer incubation times. These results suggest that $Ca(OH)_2$-treatment solubilises/particularises the more readily fermentable fraction of MDG and also increases the digestibility of the residual, more slowly degraded OM.

At outflow rates of 0.03 and 0.06, the OM degradability of the hay is low. In the *in vivo* situation outflow rates for long forages would be slower so degradability figures at rates of 0.03 and 0.06 for hay are therefore artificial but nonetheless serve as a useful comparison of the different types of feed.

Nitrogen Degradation

Table 5.6 *In sacco* Nitrogen Degradability

	Min. Draff	NID	Carrs Nuts	Hay	SE
a	47.8^a	57.6^b	54.3^c	14.9^d	0.68
b	46.4^a	34.0^b	34.6^b	50.7^a	1.91
c	0.117^a	0.087^b	0.081^b	0.047^c	0.0042
Dg (3%/h)	84.8^a	82.8^a	79.3^b	45.8^c	0.954
Dg (6%/h)	78.3^a	77.8^a	74.0^b	37.3^c	0.747

Means in the same row not sharing common superscripts differ significantly ($p<0.05$).

Figure 5.8 In Sacco Digestibility
Nitrogen

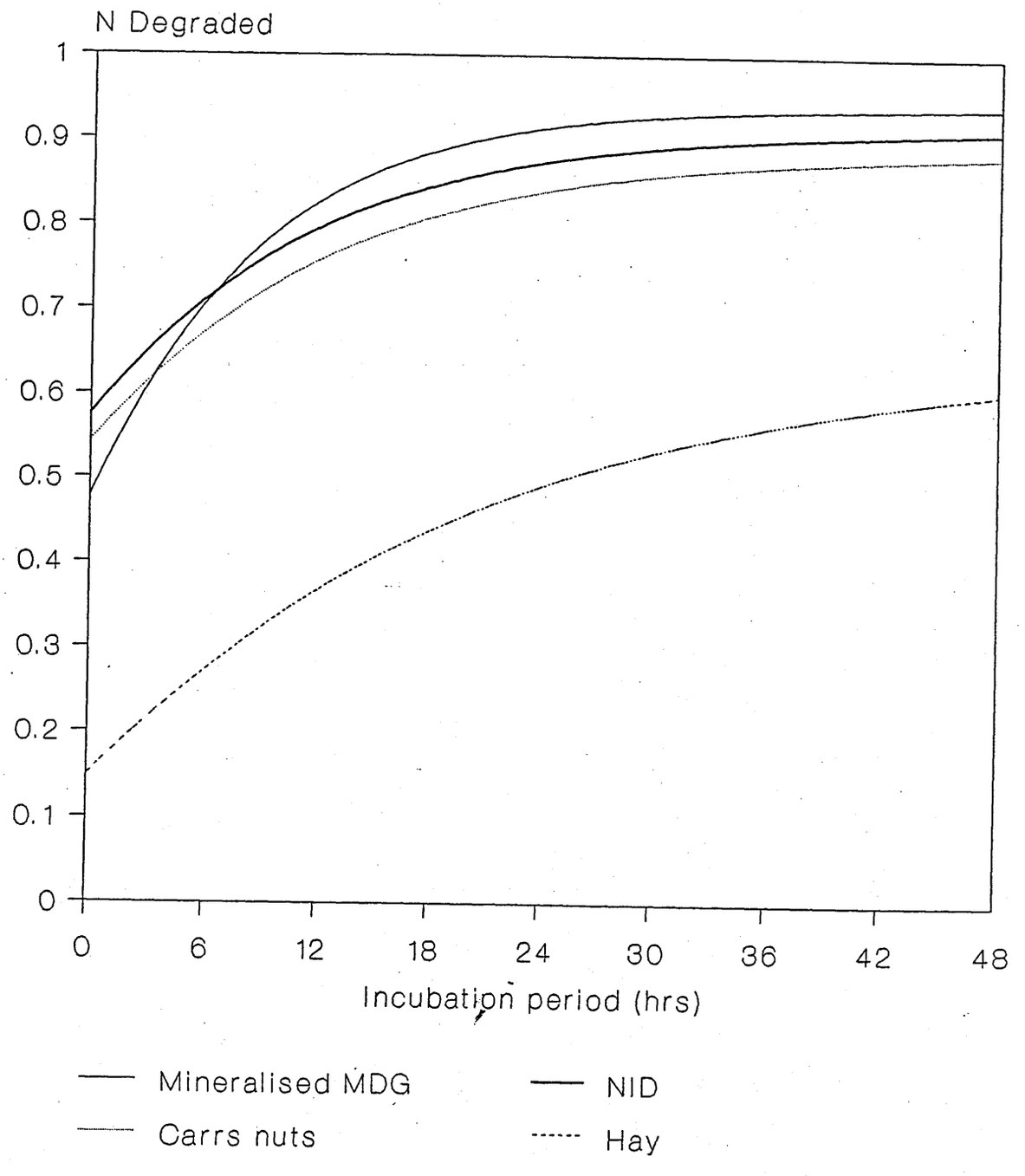


Table 5.6 shows the results for the protein degradability of the different feeds. As shown in Figure 5.8 the differences between protein degradability in the three supplementary feeds were small. However these differences were statistically significant ($p<0.05$).

The instantaneous loss of protein was significantly higher for NID than for the other feeds. This suggests that $Ca(OH)_2$ treatment was in some way acting to solubilise part of the protein in MDG. This is supported by measurements reported in Chapter 8 in which the solubilised material was shown to have a protein content of 120 g/kg DM. It is likely that such protein was associated with cell wall material, and was released as a result of the action of $Ca(OH)_2$ on cell wall polysaccharides. The 'b' and 'c' terms for NID do not differ significantly from Carrs nuts but are significantly lower than for mineralised draff. The higher 'a' value for NID and the lower 'b' and 'c' terms compared to mineralised draff result in the effective degradability of the protein of draff remaining unchanged due to $Ca(OH)_2$ treatment. Both mineralised draff and NID had significantly higher protein degradability at outflow rates of 0.03 and 0.06 than Carrs nuts.

Values for the *in sacco* protein disappearance were, as with the OMD, very different from those reported by Hyslop and Roberts (1988). They observed 'a', 'b' and 'c' terms for draff nitrogen degradation of 70.75, 24.83 and 0.098 respectively (compared to 47.8, 46.4, and 0.117 observed in this study). However, values from the present experiment agree well with comparable figures for draff nitrogen degradability observed by Lewis (personal communication) (41.25, 44.37 and 0.081). Similar measurements made by Topps (personal communication) showed different results (-24.1, 110.7 and 0.120 for the 'a', 'b' and 'c'terms respectively). The wide differences in the draff protein degradability may be partly explained by the grains originating from different distilleries and thus having been subject to different processing conditions. A further source of variation may be due to different dietary regimes of the animals incubating the bags. Neither of these explanations account totally for the wide variations observed.

NDF Degradation

The degradation curves for the NDF of the lamb feeds are shown in Figure 5.9 and the constants defining the curves are given in Table 5.7. Comparison of the values for mineralised draff and NID shows the effect that $Ca(OH)_2$ treatment had on the draff NDF. There was a significantly ($p<0.05$) higher instantaneous loss of fibre from NID, probably due to increased friability and reduction of particle size brought about by $Ca(OH)_2$ treatment. The increase in instantaneous loss of NDF from draff due to $Ca(OH)_2$ treatment cannot, by the definition of NDF, be due to an increase in soluble material (which would not be measured as NDF).

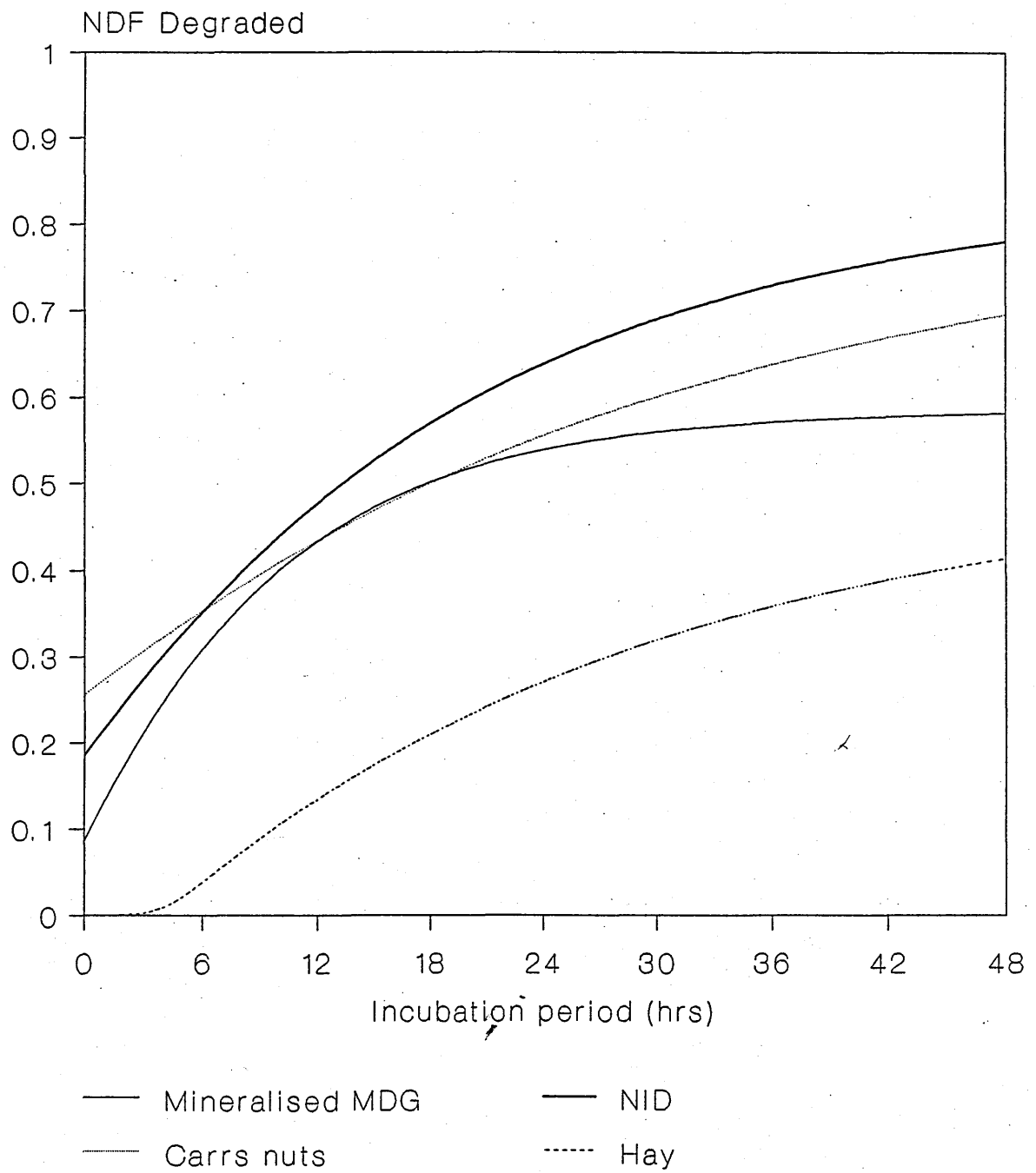
Table 5.7 *In sacco* NDF Degradability

	Min. Draff	NID	Carrs Nuts	Hay	SE
a	8.6^a	18.6^b	25.6^c	-8.1^d	1.35
b	49.8	65.5	55.8	58.9	6.26
c	0.100^a	0.049^b	0.032^b	0.038^b	0.0099
Dg (3%/h)	47.0^a	57.0^b	49.5^a	23.8^c	1.22
Dg (6%/h)	39.8^a	46.3^b	41.0^a	14.3^c	0.70

Means in the same row not sharing common superscripts differ significantly ($p<0.05$).

In addition to the increased instantaneous loss, $Ca(OH)_2$ treatment also results in an increase in the amount of potentially digestible fibre in draff. These observations suggest that $Ca(OH)_2$ treatment acts on the NDF in two ways; by liberating the more readily degradable material which is lost instantaneously both as truly soluble and as increased particulate loss, and by increasing the amount of potentially digestible fibre residue (the 'b' fraction). This results in a reduction in the amount of totally undigestible fibre in the

Figure 5.9 In Sacco Digestibility
Neutral Detergent Fibre



spent grains and significant increases in effective degradabilities at the given outflow rates.

The observations on the effect of $Ca(OH)_2$ treatment of draff on NDF degradation are reflected in *in sacco* OM losses where the curves for mineralised draff and NID converge slightly at approximately 12 hours and diverge thereafter. For OM, these effects are somewhat masked by the loss of other components of the OM, but nonetheless, they demonstrate the relative importance of the fibre fraction as a contributor to the total rumen OM degradation. The increase in the potentially digestible NDF fraction (a + b) due to $Ca(OH)_2$-treatment was 58.4 to 84.1%.

Comparison of the NDF degradation for NID with that for Carrs nuts shows that, apart from the proprietary concentrate having a higher instantaneous loss, degradation characteristics were not significantly different. Although the differences were not significant statistically, the increase in the amount and rate of degradation of the potentially digestible fraction was sufficient to give significantly higher effective degradabilities for NID compared to Carrs Nuts at the given outflow rates. However it must be remembered that the proprietary concentrate had a lower content of NDF than either of the draff supplements (409 vs 451 and 774 for NID and Min. Draff respectively) so fibre contributed a lower proportion of the total DE for this feed.

5.3.3 In Vivo Digestibility

Table 5.8. *In vivo* Whole Diet Digestibility.

	Hay	Min. Draff	NID	Carrs Nuts	SE
OM	0.550^{a}	0.534^{a}	0.626^{b}	0.651^{b}	0.0148
GE	0.536^{a}	0.555^{ab}	0.602^{bc}	0.647^{c}	0.0207
Nitrogen	0.374^{a}	0.713^{c}	0.539^{b}	0.720^{c}	0.0440
NDF	0.564^{ab}	0.551^{a}	0.687^{b}	0.572^{ab}	0.0371
ADF	0.521^{b}	0.356^{a}	0.520^{b}	0.360^{a}	0.0325

Values in the same row with differing superscripts differ significantly ($p<0.05$).

Table 5.8 gives the *in vivo* ration digestibilities for hay fed alone, and mineralised draff, NID and Carrs nuts fed with hay (67:33 on a DM basis). Results for the OMD show that $Ca(OH)_2$ treatment of spent grains significantly increased the diet digestibility. The OMD observed for the mineralised draff diet was not significantly different ($p<0.05$) from that found for the hay alone. The value recorded for the NID ration was not significantly different from that containing the proprietory concentrate.

The energy digestibilities observed follow a similar pattern to the OMDs, although in this case the increase in digestibility due to $Ca(OH)_2$ treatment was not significant ($p<0.05$). The observed protein digestibility for NID was significantly lower than for the mineralised draff and the proprietary concentrate. This is supported by the observations of Chapter 4 where possible reasons for this are discussed. $Ca(OH)_2$ treatment significantly increased apparent digestibilities of NDF and ADF in the total diet. For both measurements, the diet containing NID had the highest digestibility. This was supported by the *in sacco* findings.

The measurements of hay and diet digestibilities were used to calculate the individual feed digestibilities. This calculation assumed that the digestibility of the hay was not affected by the inclusion of other feeds in the ration, ie. that there were no associative effects. However, it is well known that inclusion of spent grains in rations depresses fibre digestibility by the inhibitory effect of the lipid fraction on microbial activity. This effect would be expected to be greatest for the untreated draff because of its higher content of oil in non-soap form. Consequently the values calculated for the individual apparent digestibilities may be under-estimates of the true value.

The significant ($p<0.05$) differences between digestibilities for the individual feeds shown in Table 5.9 are the same as for the whole diet digestibilities with the exception of the NDF digestibility, for which the digestibility of NID NDF is significantly higher than hay NDF.

Table 5.9. *In vivo* Feed Digestibility (Calculated by Difference)

	Hay	Min. Draff	NID	Carrs Nuts	SE
OM	0.550^{a}	0.531^{a}	0.669^{b}	0.704^{b}	0.0250
GE	0.536^{a}	0.575^{ab}	0.652^{bc}	0.702^{c}	0.0282
Nitrogen	0.374^{a}	0.754^{c}	0.568^{b}	0.773^{c}	0.0466
NDF	0.564^{a}	0.545^{a}	0.794^{b}	0.579^{ab}	0.0655
ADF	0.521^{a}	0.101^{b}	0.518^{a}	0.063^{b}	0.0324

Values in the same row with differing superscripts differ significantly ($p<0.05$).

The OMD for the mineralised draff is in good agreement with the findings of other studies. Lewis and Lowman (1987) reported an OMD for mineralised MDG of 0.53, whilst Wainman *et al.* (1984), measured 0.54. Hyslop (unpublished data) measured a slightly lower range of

0.48 - 0.52. The OMD for NID was 0.67, demonstrating the large increase in digestiblity due to $Ca(OH)_2$ treatment.

The digestibility of the gross energy for the mineralised grains (0.58) was the same as that noted by Lewis and Lowman (1987) for mineralised MDG. Other workers have reported similar values; 0.54 - 0.58 and 0.50, Hyslop (unpublished data) and Wainman *et al.* (1984) respectively. These values have been used to calculate ME values ranging from 10.80 - 11.67 MJ/kg DM. ME values for this study were calculated to be 10.50 for the mineralised draff and 10.97 MJ/kg DM for NID. These values were calculated using 0.89DE (Wainman and Dewey, 1982). The value for NID was low compared to that for mineralised draff given the improved digestibility of the OM and GE. This is due to the higher ash level in the NID. Expressed on an organic matter basis, the MEs become 11.29 and 12.89 MJ/kg OM respectively.

A further reason for the comparatively low ME values observed in this study is that the sheep in the *in vivo* digestibility experiment were fed at 1.31 and 1.35 times maintenance for the mineralised draff and NID diets respectively. Lewis and Lowman (1987) noted that the OMD of MDG was decreased from 0.53 when fed at maintentance to 0.46 when fed *ad libitum* (1.58 maintentance). Also as noted earlier, these digestibility measurements are likely to be under estimates of the true values due to the associative interactions between the hay and the concentrate feeds which were not taken into account when the feed digestibilities were calculated.

The results for the rumen pH and levels of volatile fatty acids and the *in sacco* hay digestibility of animals on the experimental diets are given in Table 5.10.

Table 5.10. Mean Rumen Parameters of Sheep Fed Experimental Diets

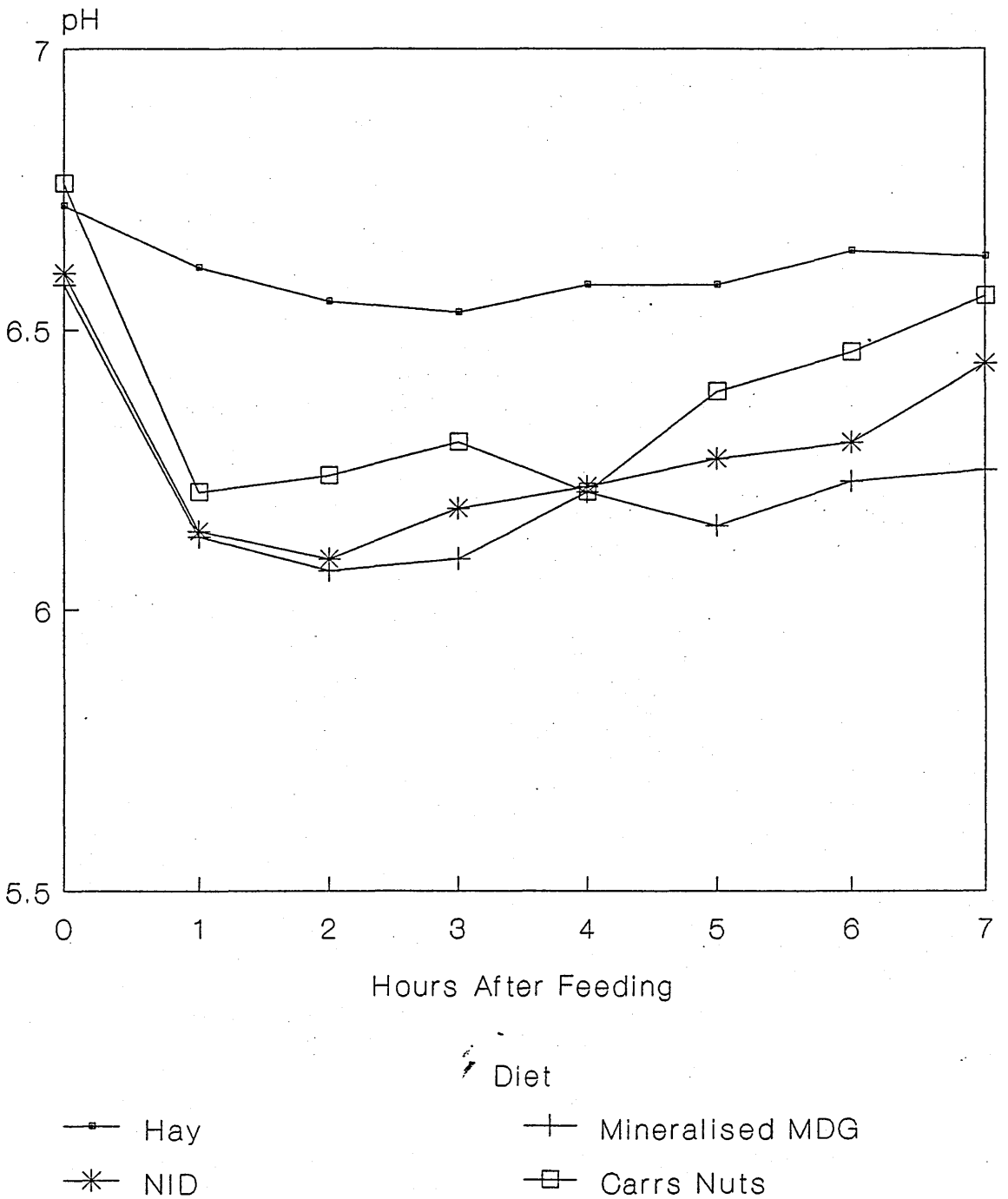
	Hay	Min. Draff	NID	Carrs Nuts	SE
pH	6.60[a]	6.21[b]	6.28[ab]	6.39[ab]	0.095
VFA Molar%					
Acetate	67.9[a]	66.1[b]	66.8[b]	66.4[b]	0.474
Propionate	21.6	23.3	22.0	22.7	0.558
Butyrate	8.1	8.2	8.4	8.2	0.113
Total VFA Concentration (g/l)					
	5.22	4.87	4.81	4.64	0.216
In Sacco Hay DM Digestibility					
	0.420	0.396	0.394	0.415	0.0381

Values in the same row with different superscripts differ significantly ($p<0.05$)

Samples of rumen liquor were taken and rumen pH was measured at hourly intervals, the results were analysed using analysis of variance. There was found to be no significant effect of sampling time and no significant interaction ($p<0.05$) between sampling time and diet for concentrations of VFAs. There were, however significant effects of sampling time and sampling time x diet interactions on rumen pH. The mean values for pH and rumen VFA levels are given in Table 5.10. and Figure 5.10 shows the variation in rumen pH after feeding for the four diets.

Figure 5.10 shows that the pH for sheep fed hay remained higher and more constant than those given concentrates reflecting the more continuous intake and the slower rates of fermentation of the hay. The mineralised draff gave the lowest rumen pH which probably resulted

Figure 5.10 Effect of Diet on Rumen pH



from the intake of relatively large quantities of low pH draff (pH approximately 3.3).

The rumen VFA levels did not vary significantly ($p<0.05$) between diets including the supplementary feeds, but acetate levels in sheep fed hay as the sole diet were significantly higher than when hay was fed with mineralised draff, NID or Carrs nuts. Hay *in sacco* DM digestibility was also not significantly affected by diet, although it was slightly lower for the draff based diets. These findings suggest that the rumen fermentation was not adversely affected by any of the supplementary feeds. It should be noted that the level of supplementation in this experiment was lower than in the lamb production experiment, so care should be taken in extrapolation of the results between experiments.

5.4 Discussion

The lamb finishing experiment showed that the $Ca(OH)_2$ treatment of MDG significantly increased lamb condition score and reduced the time taken to finish animals fed high levels of draff. With the measurements of *in vivo* and *in sacco* digestibility of the feeds carried out concurrently it is possible to explain these observations.

The reasons for the increased lamb growth are two fold:

1. The increase in OM and energy digestibility due to $Ca(OH)_2$ treatment. *In sacco* data shows that the improvement in NDF digestibility is one of the main factors causing this increase. This results in an increase in the ME of the diet.

2. The animals were able to eat significantly more $Ca(OH)_2$ treated draff than mineralised draff. This was thought to have been due to the reduction in microbial inhibition by the formation of calcium soaps, and to the increased rate of fibre digestion. A further limitation on DM intake may have been the 'gut fill' effect of consuming 3 kg/d of FW wet draff. For similar reasons the lambs fed mineralised draff also ate significantly less hay, further limiting energy intake. This is

supported by the killing out percentage data.

A slight increase was observed in the daily liveweight gains of lambs fed NID and Carrs nuts compared to those fed mineralised draff. The similarity of LWG between the mineralised draff treatment and the others in spite of the substantial difference in ME intake may be related to different weights of gut contents of the lambs. It is possible that the lambs consuming large quantities of wet grains, of lower digestibility than NID, had heavier gut contents than other lambs and thus gut fill accounted for a larger proportion of body weight. Empty body weight for lambs fed mineralised draff may have been considerably lighter than for other lambs. This is supported by the significant differences in condition score and numbers of animals finishing by day 60.

Lamb tissue fatty acid analyses suggested that feeding NID is a potential method for manipulating ruminant fat compostition. Reduction in the saturated fat intake in the human diet is now widely recommended by bodies such as COMA (1984) to reduce the incidence of cardiovascular disease. Ruminant fat is one of the most saturated forms of fat eaten, and manipulation of the degree of saturation has, in the past been limited by ruminal biohydrogenation. Calcium soaps of fatty acids are thought to escape biohydrogenation and may provide the means to reduce the saturation of ruminant fat.

A further benefit from manipulation of the fatty acid profile of lamb fat may be an improvement in the cooking and eating quality of the meat. The firmness and melting point of ruminant fat is most closely correlated to the concentration of stearic acid (L'Estrange and Mulvihill, 1975). The high concentration of C18:0 causes the ruminant fat to be hard when cold and gives the sticky taste to cold lamb fat which many people dislike (Wood, 1984). Feeding NID in this experiment significantly reduced the levels of C18:0 and could be a method of producing lambs with softer, less saturated fat which is more acceptable to the consumer.

CHAPTER SIX

THE USE OF SPENT GRAINS AS EFFLUENT ABSORBENTS IN GRASS SILAGE

6.1 Introduction

The use of effluent absorbents on farms has increased dramatically in the last five years. Most commercially available absorbents are based on dried sugar beet pulp which has proved efficacious in reducing effluent volume and improving the fermentation characteristics of the silage (Done, 1988). Offer *et al.*, 1989 review strategies for the management of silage effluent including the use of absorbents. The properties of an ideal absorbent are listed as:-

High moisture holding capacity under load.

Resistance to microbial degradation in the silo.

High digestibility and feeding value.

High density.

Low content of soluble material.

Availability during the silage season.

Many substances have been tested as effluent absorbents including sodium bentonite (Everson *et al.*, 1971 and Woolford and Camp, 1977); newspaper (Salo and Sormunen, 1974); chopped straw (Pederson, 1979) and rolled barley and dried sugar beet shreds (Jones and Jones, 1987 and Done, 1988) but none of these fulfil all of the above characteristics. It was concluded (Offer and Al-Rwidah, 1987) that the more fibrous materials had higher absorbency than cereal grains.

In 1987, Offer and Al-Rwidah reported an experiment in which the relative absorbency of a range of materials was compared using 200

litre drum silos and 10 tonne experimental silos. The materials tested included chopped barley straw, dried distillers grains, molassed beet shreds, alkali treated straw cubes, molassed beet nuts and rolled barley. They noted that after chopped barley straw, the most absorbent material tested was dried distillers grains. Conversely, O'Keily (1990) observed that dried distillers grains were less absorbent than dried sugar beet pulp. He reported effluent retention rates (kg effluent/kg absorbent) for barley, corn gluten, dried distillers grains and soyabean meal of less than 1, malt culms and dried sugar beet pulp of 1 -2 and greater than 2.5 for straw when these materials were ensiled with grass in 2m high silos.

The aim of the work described in this chapter was to measure the relative absorbencies of dried distillery spent grains using both laboratory and large-scale techniques and to investigate the effects of $Ca(OH)_2$-treatment on absorbency. The Chapter is divided into two parts with the following aims:-

1. Measurement of the absorptive capacity of untreated and alkali treated spent grains and an assessment by laboratory techniques of their efficacy as effluent absorbents.

2. An investigation of the use of spent grains as absorbents for ensilage with grass in bunker silos and the measurement of the feeding value of the silages when fed to growing bulls.

6.2 Part 1. Measurement of Absorptive Capacity and Laboratory-Scale Ensilage Experiments

6.2.1 Feed Absorbency Comparison

6.2.1.1 Methods

The comparative feed absorbencies were measured by soaking 500g subsamples of oven dried material in either 1 or 2 litres of water for 20 hours and then squeezing the wet material in a test rig. Details of the apparatus are described by Al-Rwidah (1987). The test rig consisted of a home-made wine press with a load cell attached so that the pressures exerted by the press could be controlled and recorded. The feeds tested included:-

Dried Molassed Sugar Beet Pulp
Untreated Maize Draff (From a Grain Distillery)
Untreated MDG (From a Malt Distillery)
$Ca(OH)_2$-Treated MDG
NaOH-Treated MDG

Initially, the wet material was allowed to drain over the collection vessel for 3 minutes without being subjected to any load. The load was then increased sequentially through the following pressures; 28, 57, 114, 170 and 227 kg/dm^2. Each pressure was maintained for three minutes and then the material was allowed to drain for a further three minutes before being taken up to the next pressure. The volume of liquid collected at each pressure was measured.

6.2.1.2 Results

The results from Table 6.1 are illustrated in Figures 6.1a and 6.1b. They show that untreated maize draff (UMD) was similar in absorptive capacity to MSBP under these conditions, but untreated MDG was superior. Alkali treatment, with either 80 g $Ca(OH)_2$/kg DM or 80 g NaOH/kg DM, of barley draff greatly increased its water-holding

Figure 6.1a Relative Absorbency of MSBP and Treated and Untreated Spent Grains Feed:Water 1:2

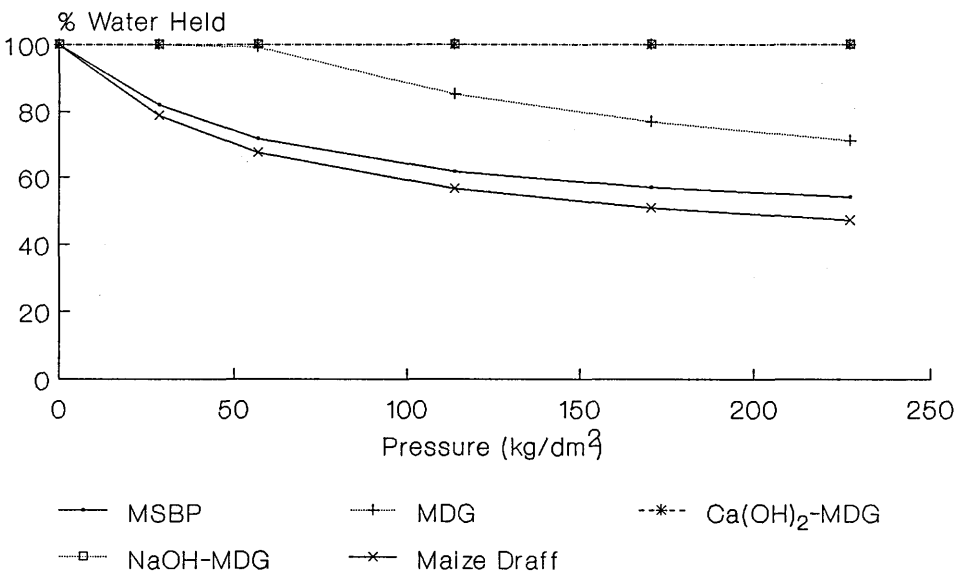
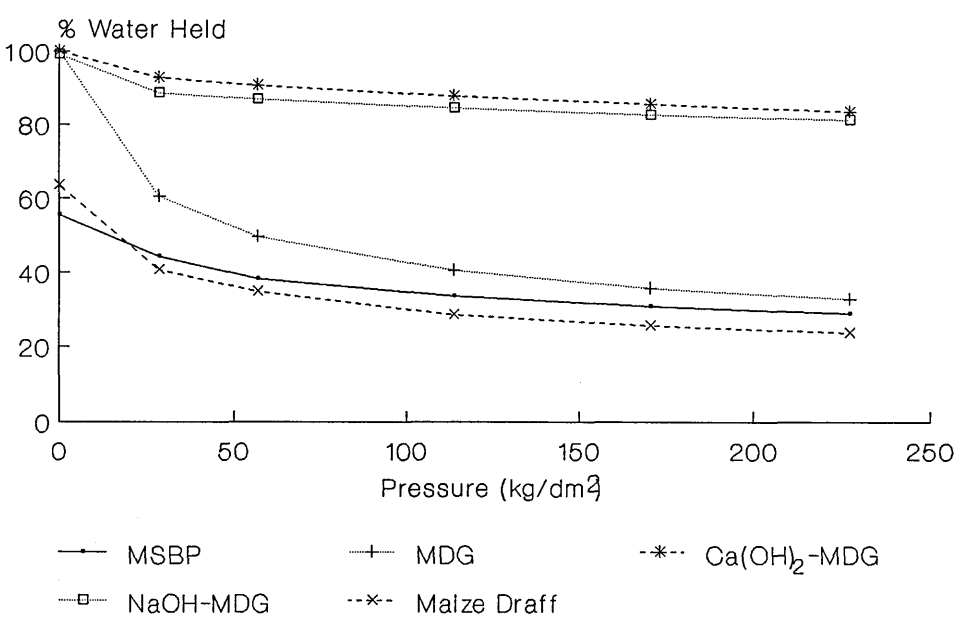


Figure 6.1b
Feed:Water 1:4



capacity. When these feeds had been soaked in twice the weight of water, no water could be squeezed out over the range of pressures applied in this experiment. The reasons for this increase in water-holding capacity with alkali treatment are not known, however it seems likely that it was due to the changes in the draff fibre fraction described in Chapters 4 and 8. This is supported by Van Soest and Robertson (1976) who suggested that the absorption of substances onto fibre reflect its surface properties, which are determined by constituents such as phenolic groups of lignin.

Table 6.1 Cumulative Volumes of Water Squeezed From Soaked Feedstuffs

Pressure (kg/dm^2)	MSBP	Maize Draff	Barley Draff	$Ca(OH)_2$ treated Draff	NaOH treated Draff
1 litre		% Held			
0	100	100	100	100	100
28	82	79	100	100	100
57	72	68	99	100	100
114	62	57	85	100	100
170	57	51	77	100	100
227	54	47	71	100	100
2 litres					
0	56	64	100	100	99
28	44	41	61	93	89
57	38	35	50	91	87
114	34	29	41	88	85
170	31	26	36	86	83
227	29	24	33	84	81

This initial finding suggested that NID would perform well as a silage effluent absorbent, particularly as the pressures used in this test were much greater than those measured at the bottom of a silo (an

average figure for this would be approximately 50 kg/dm^2). However O'Keily (1990) noted that, whereas many potential absorbents show high water holding capacities in absorptive tests, their capacity to retain effluent under the physical and chemical conditions of the silo is often much less. Similarly, Offer *et al.* (1989) observed that there was little relationship between the absorptive capacity of materials measured using "beaker and water" techniques and the volumes of effluent retained under ensilage conditions. Therefore the absorptive capacity of NID was tested under more practical conditions as described below.

6.2.2 Effect of Inclusion of Absorbent Materials on Effluent Production and Fermentation Characteristics of Grass Ensiled in Laboratory Silos

Having observed the relatively high absorbency under load of alkali treated draff compared to MSBP or dried draff, these materials were then tested under more practical conditions to investigate how absorbency was affected by the fermentative conditions of the ensilage process.

6.2.2.1 Method and Materials

The experiment was of a 4 x 5 factorial design using duplicate samples subjected to four different absorbent treatments: no additive (control), MSBP, NID or dried untreated MDG. Absorbents were added at 80 g/kg grass FW. Five ensilage times (2, 24, 48, 96 or 144 hours) were used.

A 50 kg load of chopped grass of 110 g/kg dry matter was obtained from the College farm. The grass was thoroughly mixed and 40 x 1 kg amounts were taken by random sampling. The remaining grass was subsampled for DM and OM analysis.

The grass was ensiled so as to reproduce, as closely as possible, conditions in larger scale silos. This was done by compressing the grass and vacuum packing it in a double layer of polythene. The

tightly sealed packs were then packed under 10kg of granite chippings. The 'silos' were then opened after 2, 24, 48, 96 or 144 hours and the silages were squeezed in a test-rig at a pressure of 34 kg/dm^2. Effluent volume, pH, DM, and OM were measured as described in sections 2.1.1 and 2.1.2. The concentrations of various fermentation products were measured as described in section 2.7.4.

6.2.2.2 **Results**

Table 6.2 The Effect of Addition of MSBP, NID and untreated MDG to Grass Silage on pH and Effluent Production.

		Control	+MSBP	+NID	+Untreated MDG
Effluent Volume (mls/kg Silage FW)					
	2	186	104	47	90
Ensilage	24	446	268	231	294
Time	48	514	291	280	343
(Hours)	96	639	298	300	327
	144	620	328	327	352
Effluent OM Loss (g OM/kg Silage DM)					
	2	1.26	1.95	1.63	1.16
Ensilage	24	5.49	4.20	5.54	3.90
Time	48	7.49	6.70	7.05	6.14
(Hours)	96	9.34	7.26	7.08	5.71
	144	10.27	8.03	7.51	6.22
Effluent pH					
	2	5.51	5.18	7.22	4.85
Ensilage	24	4.73	4.32	5.78	4.46
Time	48	4.53	4.08	5.40	4.20
(Hours)	96	4.61	3.96	5.15	4.15
	144	4.55	3.80	5.03	4.06

pH

Figure 6.2 shows how the silage pH changed with time after ensilage. The results in Table 6.2 show the greatest effect on pH was due to the inclusion of NID as an absorbent. The pH after 2 hours with this treatment was above pH 7, presumably due directly to the presence of NID (pH 8.5). The initial pH of the other silages were very similar, the lowest pH being observed with the dried MDG treatment. This too can be explained by the pH of the absorbent material itself which was acidic (pH 4.3) and helped to reduce the pH of the silage.

Over the first 24 hours of ensilage, the pH in all silages fell to below pH 5 for the control, MDG and MSBP treatments, but only as far as pH 6 for the silage containing NID. After this time, there was little change in silage pH apart from a slow drift downwards in the NID silage to pH 5.5 over the following 5 days. The MDG and MSBP silages had pH values at 5 days (4.1 and 3.8 respectively) which indicate that stable preservation had been achieved. The pH for the control silage (4.5) was at the upper limit for good preservation while the value for the NID silage (5.2) indicated poor preservation quality.

Effluent Volume

Figure 6.3a shows the volumes of liquid that were squeezed from the samples at the different times that the 'silos' were opened. The volume of effluent that could be squeezed from the silages was initially lower for the NID treatment and highest for the control silage. This is in accordance with absorptive capacity findings from the previous experiment. However, the benefit of ensiling with NID over the other absorbents tested was lost after 48 hours. At this point, although all absorbent treatments were lower than the control, there is little difference between treatments. This pattern persisted for the longer ensilage times. This observation is in accord with the views expressed by O'Keily 1990 regarding the use of potential absorbents in more practical situations. Reduced efficacy of NID as and absorbent after 48 hours suggests that the materials formed during

Figure 6.2 Changes in Silage pH

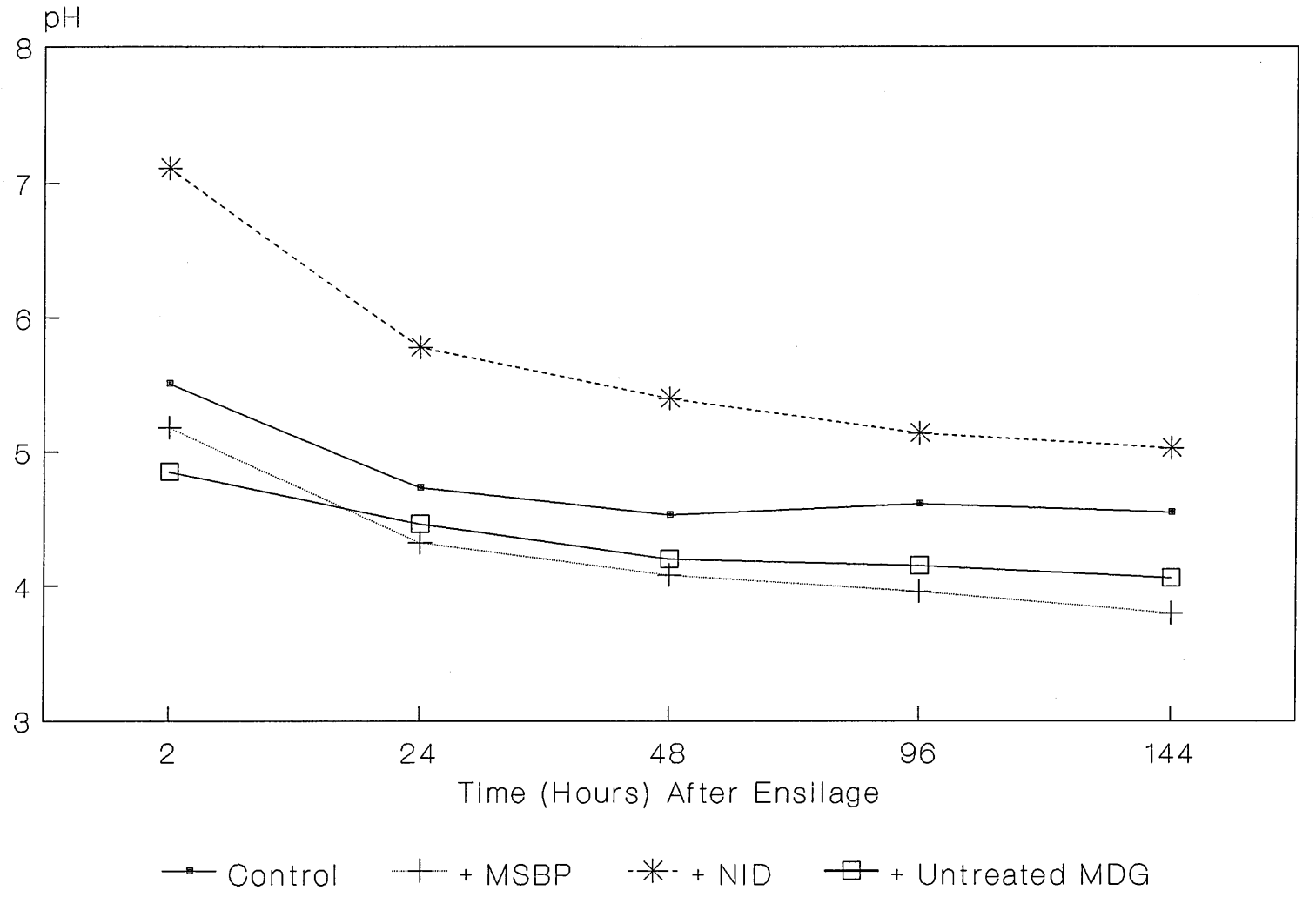


Figure 6.3a Effluent Volume

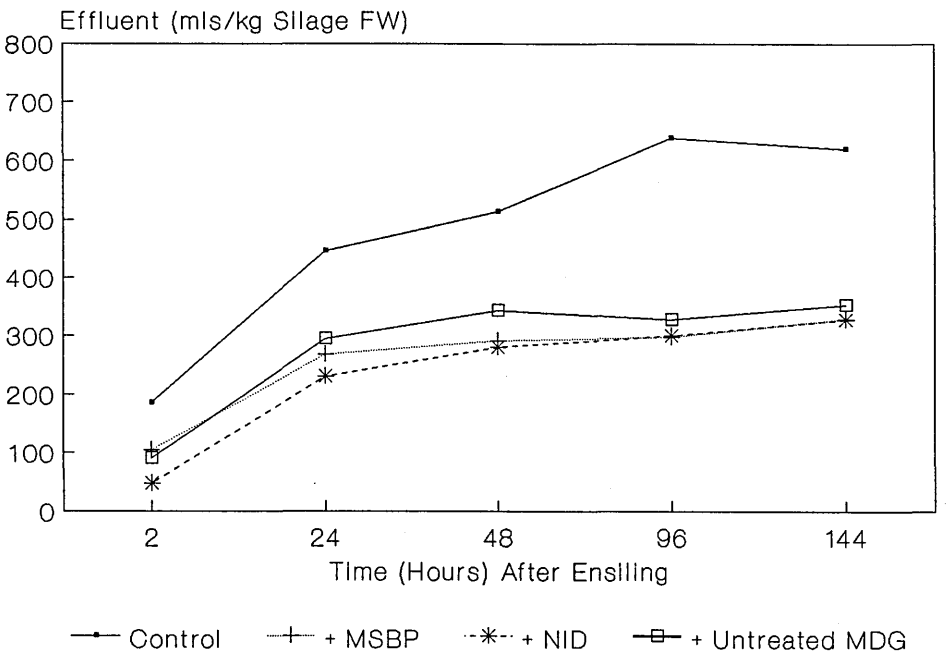
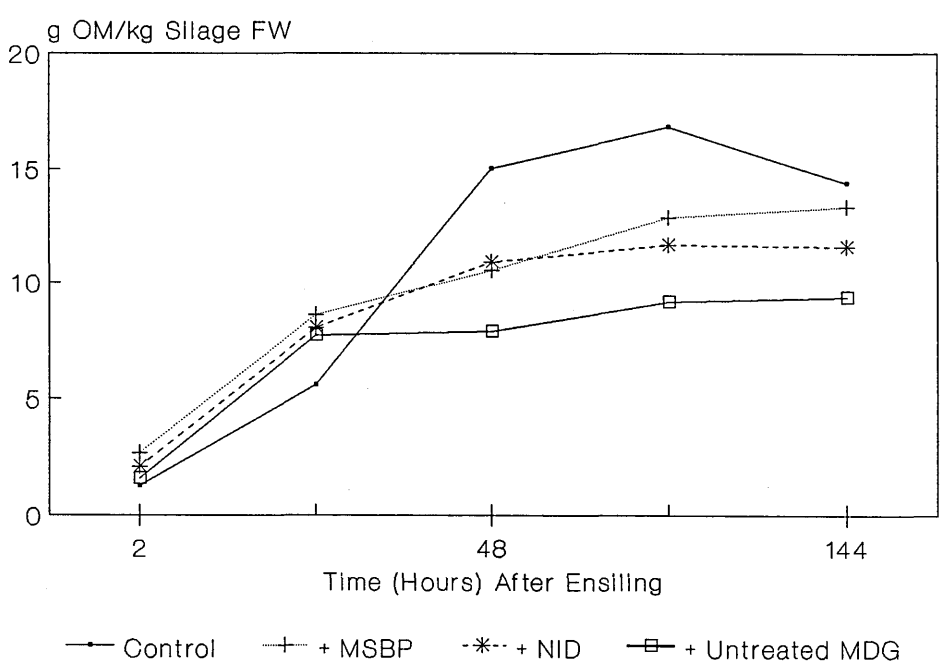


Figure 6.3b Effluent OM Loss



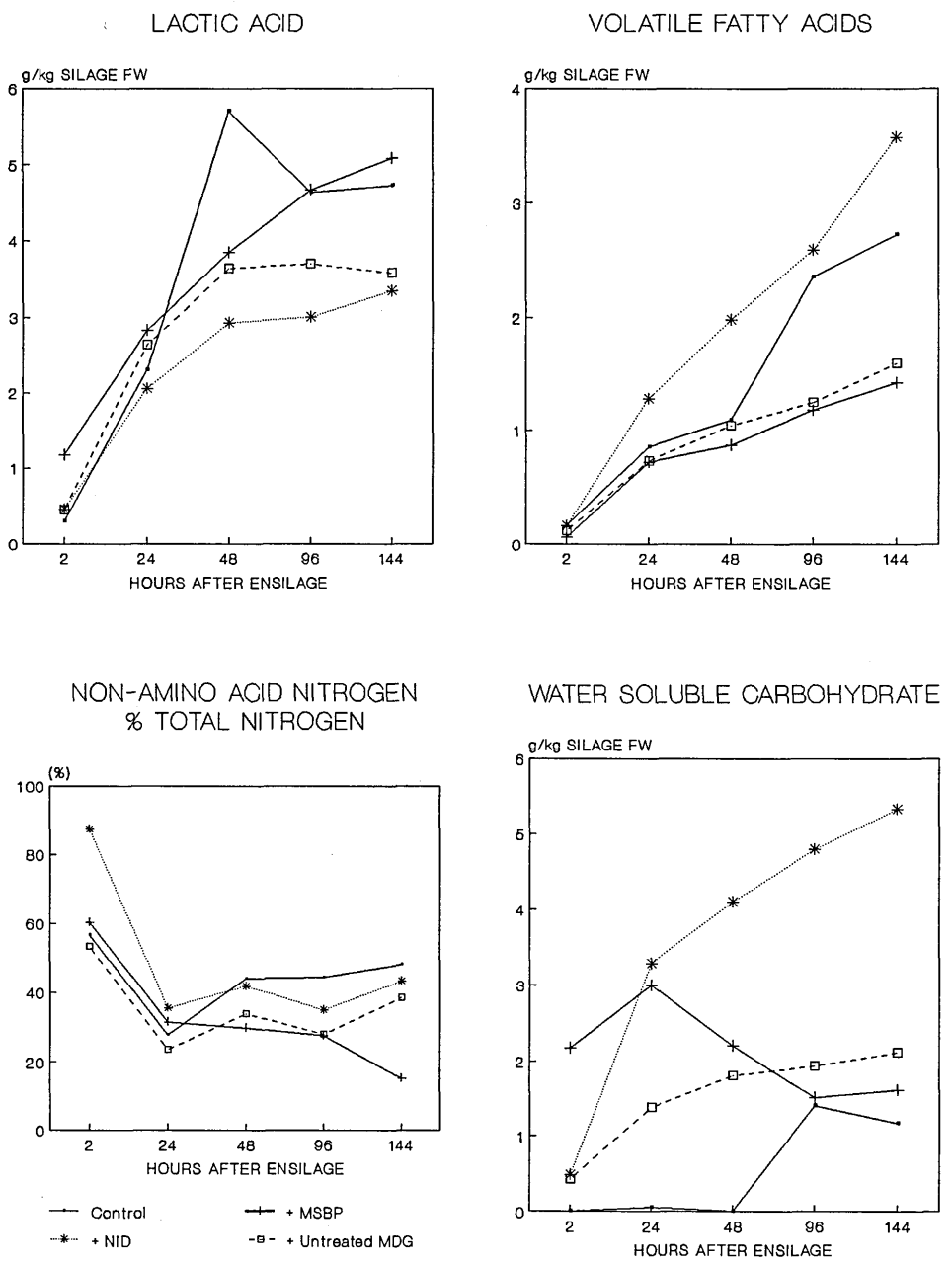
$Ca(OH)_2$-treatment which were responsible for its increased water-holding capacity, were lost in the fermentative environment of the grass silage.

The organic matter lost in the effluent from each treatment is shown in Figure 6.3b. It shows that initially the OM loss was least from the control silage, indicating that although the volume of effluent was greatest from the control, initially this contained a low concentration of OM. The higher initial loss of OM for the absorbent silages reflects the loss of soluble OM from the absorbents themselves and is similar for all treatments. After 24 hours, when fermentation processes have reduced the pH, the OM loss from the control silage was increased. This reflects the release of soluble cell contents from the grass which were subsequently lost in the large volumes of effluent produced at that time. OM losses from absorbent silages were less than for the control after 24 hours due to the reduction in effluent volume. OM losses were particularly low for the silage including dried MDG reflecting the low content of soluble OM in MDG. $Ca(OH)_2$ treatment of MDG increased the effluent OM loss due to the solubilisation of the OM. At longer ensilage times (<48 hours) the loss of OM from MSBP-treated silage was greater than for either of the draff treatments, however it was still lower than for the control silage.

Fermentation Products

The results for the GC analysis of the fermentation products are given in Appendix 9 and are shown in Figure 6.4. The levels of fermentation products measured show the reasons for the high pH observed with the NID silage. Concentrations of VFAs in all silages increased with ensilage time but were always highest for the NID silages. Concentrations of lactic acid also increased with ensilage time but were at all times lowest for NID silages. These findings suggest that, for the NID silage, heterofermentative organisms predominate yielding high levels of VFA and low levels of lactic acid. The high levels of VFAs in the NID silages could have resulted from fermentation of the MDG cell wall material solubilised by $Ca(OH)_2$-

Figure 6.4 Fermentation Characteristics of Silages



treatment. In Chapter 4 it was shown that these substances were rapidly fermented.

The water soluble carbohydrate levels followed a different pattern. Levels initially were, not surprisingly, highest for the MSBP silage reflecting the molasses content of the absorbent. WSC levels in these silages decreased with ensilage time as the sugars were fermented to VFAs and lactic acid causing the observed reduction in pH. WSC levels for the draff-based absorbents increased with ensilage time although the increase was much greater for the $Ca(OH)_2$ treated draff. This suggests that WSC was being progressively released from the absorbent material as fermentation proceeded and that this effect was dramatically enhanced by $Ca(OH)_2$ treatment.

The breakdown of protein during ensilage followed a similar pattern for all silages. The proportion of the nitrogen present in the silage juice as non-amino acid nitrogen was reduced in the first 24 hours of ensilage. The proportions were reduced still further after this time for the MSBP silage but for the control silage and silages containing draff or NID the proportions of non-amino acid nitrogen tended to rise slightly.

The silages produced with dried MDG as an absorbent were comparable to those produced with MSBP although there were differences in fermentation characteristics during the initial phase of ensilage. Addition of dried MDG, which is acidic in nature, to the grass helped to reduce the silage pH thereby promoting lactic acid production and further pH reductions. Addition of MSBP, which contains high levels of soluble sugars, promoted rapid fermentation and production of lactic acid and caused the pH to fall rapidly. This also resulted in the MSBP silages having higher levels of lactate and a lower final pH than the draff silages. Final pHs for MSBP and draff silages were 3.80 and 4.06 respectively. The results suggest that both of these absorbents would promote the production of well-preserved silages.

The high pH, low levels of lactic acid and the high levels of VFAs measured in the NID silages suggest that clostridial fermentation had

taken place. However clostridial type fermentations are usually characterised by low WSC levels (McDonald, 1981) which was not found in the NID silages. The high levels of WSC measured arise from the release of sugars from the material solubilised by $Ca(OH)_2$ treatment although why these sugars were not fermented is unexplained.

The cause of the clostridial fermentation may be to be linked to the high pH of the NID when it was added to the grass. However, this factor alone may not have caused the secondary fermentation to occur since alkali treated straw (pH > 9) has been used as an absorbent, giving a well preserved silage of pH 4.0 (Al-Rwidah, 1987), despite the absorbent having a pH of greater than 9.

A further factor determining the preservation quality of a silage is the ability of the herbage to resist change in pH (buffering capacity). Since the herbage used in each treatment was randomly sampled from a well mixed heap of grass, it was assumed that differences in the buffering capacity of silages was due to differences in the buffering capacity of the absorbents mixed in with the grass. Most of the buffering capacity properties of plant material can be attributed to the presence of anions, including organic acids, orthophosphates, sulphates, nitrates and chlorides (McDonald, 1981) and it is possible that $Ca(OH)_2$ treatment of spent grains could affect these properties in draff. In order to test this theory buffering capacity measurements were carried out on samples of all absorbent material used.

6.2.3 **Buffering Capacity Measurements**

The buffering capacity of the absorbents over the pH range of 4 - 6 is of most interest for this experiment since most plants, after maceration, have pH values of approximately 6 and the pH of a well-preserved silage is about ph 4. Buffering capacity was measured by titrating a slurry consisting of 1 g of dried, milled absorbent in 20 mls of distilled water, firstly with molar hydrochloric acid down to pH 4 and then up to pH 6 with molar sodium hydroxide. The titration was carried out using an autotitrater (Radiometer, Copenhagen) which

recorded the pH whilst adding small aliquots of acid and then alkali to the slurry which was constantly stirred. The buffering capacity was expressed as mEq of alkali required to change the pH from 4 to 6 and the results are given in Table 6.3.

Table 6.3 The Buffering Capacity of Absorbent Materials

Sample	Initial pH	Acid to pH 4 (mequivalents/kg DM)	Alkali from pH 4 to pH 6 (mequivalents/kg DM)
Barley Draff	4.32	60	300
MSBP	5.68	200	230
NID	8.77	1440	840
Maize Draff	3.92	0	210
Alkali Treated Straw	10.44	1290	630

Table 6.3 shows the differences between the buffering capacities of various absorbent materials. It demonstrates the high buffering capacity of $Ca(OH)_2$-treated spent grains compared to other potential absorbents. Even compared to alkali treated straw cubes which have a higher pH to begin with, NID requires more acid to reduce the pH to pH 4 due to its higher buffering capacity. This provides an explanation of the high silage pH and poor fermentation characteristics achieved when NID was used in this role. It may explain why the poor fermentation characteristics observed when the alkali treated spent grains were included as an absorbent where not noted when alkali treated straw cubes were used. It confirms the unsuitability of NID for use as an absorbent in grass silage.

6.3 The use of Maize Draff as an Effluent Absorbent and the Effect of Inclusion in Grass Silage on Intake and Performance of Growing Bulls

6.3.1 Introduction

In view of the findings from part 1 of this Chapter it was decided that $Ca(OH)_2$-treated spent grains were unsuitable for use as an absorbent but that untreated spent grains have substantial potential for this use. Due to availability and the commercial funding of much of this work, dried maize draff from Port Dundas Distillery, Glasgow, was used as the untreated spent grain after a small laboratory scale ensilage experiment was carried out to confirmed its suitability for this purpose as described in Section 6.3.2.1.

Recent experiments (Jones and Jones (1987), (1988); Offer and Al-Rwidah (1987), Done (1988) and Kennedy (1988)) have shown that inclusion of dried sugar beet pulp as an effluent absorbent in grass silages has resulted in improved intakes and liveweight gains in cattle and sheep fed the silage. Additionally, synergistic effects of MSBP inclusion in silage have been noted on dry matter intakes (Dulphy and Demarquilly, 1976; Dulphy and Andrieu, 1978). Jones and Jones (1987), (1988) observed an increase in DMI for young steers from 0.87 to 0.96 kg/d when MSBP was ensiled with grass rather than fed with grass silage as a separate feed but at a similar rate. Conversely, later work (O'Keily, 1990(b)) showed no synergism due to ensilage. He observed no significant differences in DMI or animal performance between feeding MSBP as a separate feed with grass silage or ensiling it with the grass.

Reports in the literature of experiments in which spent grains have been used to supplement diets for growing animals generally focus on the value of draff protein when fed with poor quality forages such as straw (Miller *et al.*, 1970; Alawa *et al.*, 1988). Studies which have been carried out in the USA have investigated the supplementation of corn silage-based diets with spent grains, again with the emphasis on draff protein utilisation (Crickenberger and Johnson, 1979; Abrams *et*

al., 1983; Rogers *et al.*, 1988).

No reports of the effect of supplementation of grass silage-based diets for growing cattle have been found, although, several studies have been reported in which draff has been used to replace either silage or concentrates in dairy cow diets (Verbeek Oosthuizen and Marais, 1958; Maoli and Mazziotti, 1960; Castle and Watson, 1982; Aston *et al.*, 1987; Hyslop, in preparation). Since the number of such experiments is small and they are of diverse nature and type, there are no recognisable trends due to the effect of feeding spent grains on milk yield and composition. Differences in the nutritional characteristics of the spent grains would also add to the lack of consistency in the results between experiments.

Previous studies given in Chapter 3 indicated that $Ca(OH)_2$ treatment was effective in increasing the digestibility *in sacco* and *in vitro* of different types of spent grain whether produced on an industrial scale or in the laboratory. In Chapter 5 the effects of $Ca(OH)_2$ treatment of MDG on digestibility characteristics and lamb growth were demonstrated. However no comparative data for different types of spent grain exists. The following experiment aimed to:-

1. Investigate the nutritional implications of $Ca(OH)_2$ treatment of maize draff when fed to growing bulls.

2. To investigate the use of dried maize draff (UMD) as an absorbent and to identify any synergistic effects associated with ensiling draff with grass.

6.3.2 Preliminary Experiments

Two small preliminary experiments were conducted to confirm the suitability of maize draff as an effluent absorbent and to optimise the level of $Ca(OH)_2$ application.

6.3.2.1 Measurement of the Absorptive Capacity of Maize Draff

In this experiment the absorptive capacity of dried untreated maize draff (UMD) was compared to that of molassed sugar beet pulp (MSBP) by ensiling 80 g of the dried material with 1 kg of chopped grass of 117 g/kg DM. Grass and absorbent samples were ensiled in triplicate, with triplicate grass samples ensiled without absorbent serving as the control. The grass was ensiled as described in Section 6.2.2. All silos were opened after 5 days of ensilage the silages were squeezed in the test rig at a pressure of 34 kg/dm^2 as described previously in Section 6.2.2. The volume of silage juice squeezed out, its pH, OM and levels of fermentation products were measured as described in Section 6.2.2.

Results

The results given in Table 6.4 show that UMD and MSBP gave significant ($p<0.05$) reductions in the volume of effluent that could be squeezed from the silages. Although UMD was slightly less effective in this respect than MSBP, inclusion of UMD resulted in lower losses of OM in the juice than either MSBP-silages or control silages. Although levels of fermentation products were generally lower for UMD than for other silages, UMD silages did not differ significantly from the control. It was concluded therefore, that since UMD reduced effluent losses and did not adversely affect fermentation characteristics, that it was suitable for inclusion as an absorbent in grass silages.

Table 6.4 Results from Preliminary Ensilage Experiment

	Control	MSBP	UMD
pH	4.27^{a}	4.16^{b}	4.16^{b}
Effluent			
Vol(mls/kg Silage FW	491.33^{a}	352.67^{b}	373.67^{b}
OM (g OM/kg Silage FW)	10.21^{a}	13.61^{b}	10.20^{a}
Fermentation Products			
Lactic	4.44	4.76	3.98
VFA	1.15^{a}	1.57^{b}	1.04^{a}
AN % TN	67.90^{a}	77.13^{b}	64.80^{a}
WSC	3.00^{a}	5.49^{b}	4.38^{ab}

Values in the same row with similar superscripts do not differ significantly ($P<0.05$).

6.3.2.2 **In Sacco Digestibility Experiment**

Table 6.5 Effect of Level of $Ca(OH)_2$ on Maize Draff *In Sacco* DM Digestibility.

Level of $Ca(OH)_2$	% DM Loss 0h	24h
0	11.88	60.96
60	42.30	73.34
80	57.73	77.38
100	64.36	81.20

SE = 2.037

A preliminary *in sacco* digestibility experiment was carried out on the maize draff to be used for the subsequent feeding experiment to establish the optimal $Ca(OH)_2$ treatment conditions. A sample of maize draff was obtained from Port Dundas Distillery and was mixed with $Ca(OH)_2$ at rates of 60, 80 or 100 g $Ca(OH)_2$/kg DM. The grains were then sealed in polythene bags and held at 60 °C for 18 hours. The treated grains were then dried at 60 °C to a DM of at least 90 %. The 0h and 24h digestibility was measured as described in Section 2.4.2. The 0h and 24h *in sacco* DM losses are given in Table 6.5.

It was decided from these results to use the 80 g $Ca(OH)_2$/kg since treatment levels above this gave a diminished increase in digestibility and higher levels of ash in the feed which would depress the ME of $Ca(OH)_2$-treated grains (TMD).

6.3.3 Ensilage and Feeding Experiment

6.3.3.1 Materials and Methods

Ensilage

Silage was made on the 14th of September 1989 from third cut perennial rye grass which had been cut two days previously as silage making was delayed by wet weather. Grass was precision chopped and Add Safe (BP Nutrition) was added to all the grass used at a rate of 4 litres per tonne of grass. Approximately 36 tonnes of grass were compacted into five experimental silos each of approximately 11 m^3 volume. The silos were constructed of railway sleepers on concrete bases which drained into subfloor collection vessels to allow collection of effluent from each pit. The walls of each pit were lined with heavy duty black polythene sheets which were large enough to be folded across the surface of the grass once the pits were filled. Grass was loaded into the pits using a fore-end loader tractor and was compacted by tramping with four people per silo. Dried maize draff (UMD) was added at a rate of 87.5 kg/tonne grass FW to one pit by layering draff between each load of grass placed in the silo. The pits were sealed by folding the polythene sheets across the surface and weighting them down with sandbags. Once sealed, a roof was built over the pits to prevent rain water running into the collection vessels.

The exact weight of silage in each pit was weighed as it was removed for feeding. Silage which was discoloured or contained mould was discarded after a subsample had been taken for DM analysis and the weight of waste material had been recorded. A record was also kept of the amount of silage from each pit that was fed to the bulls. These measurements were used to calculate the percentage DM wasted from each pit.

The effluent produced from each pit during the first six weeks after ensilage was measured and sub-sampled for measurement of DM, OM and biochemical oxygen demand (BOD_5) as described in Sections 2.1.1, 2.1.2 and 2.7.2.

Grab samples of grass were taken from each bucketful of grass loaded into the pits and were analysed for DM, OM, CP, WSC and IVOMD. Core samples from the silages were taken when the pits were opened on the 22nd of January 1990 for the feeding experiment. These were analysed for pH, DM, OM, CP, IVOMD, NH_4N. as described in Chapter 2. Water extracts were made and analysed for ethanol, lactic acid and volatile fatty acids as described in Section 2.7.4.

Feeding Experiment

The feeding experiment was carried out using 20 young bulls of a wide range of initial liveweights (115 to 228kg). The bulls were allocated to 4 groups by liveweight to give a similar mean and SD of liveweight for each group. They were penned individually and bedded on sawdust. Bulls were weighed at the same time each week to minimise differences due to variable gut fill.

Animals were offered either the control silage or one containing UMD *ad libitum* and the supplements as shown in Table 6.7. Supplements were fed initially at 2.5 kg/day but from day 45 of the experiment onwards this was increased to 3 kg/day. Where UMD or TMD were included as the concentrate, minerals were added to satisfy the animals requirements according to ARC (1980) as modified by SAC nutritionists, and to provide sufficient calcium to form soaps with the dietary oil. Mineral mixtures for each supplement are given in Table 6.6. The TMD mineral mix was included at 15 g/kg FW and the UMD was mineralised using 44 g UMD mineral mix/kg FW.

Intakes of concentrates and silage were recorded daily. The bulls offered UMD and TMD concentrates took several days to adapt to the experimental diets with some animals still refusing large proportions of the concentrate after 10 days on the diet. At this point the UMD and TMD draff were pelleted in an attempt to improve the concentrate intakes on these diets. The feeds were coarsely milled through a hammer mill fitted with a 5mm screen and were then pelleted through a 5/16th inch die. The mineral supplements for each concentrate was

mixed with the milled material and 7% molasses was added to facilitate pelleting. The dietary treatment groups are shown in Table 6.7.

Table 6.6 Mineral Supplements For UMD and TMD Supplements.

(g/kg)	TMD	UMD
Limestone		180
Calcined Magnesite		60
Dicalcium Phosphate		525
Magnesium Phosphate	130	
Monodicalcium Phosphate	635	
Sodium Chloride	75	75
Potassium Chloride	75	75
TE + Vits	85	85

Table 6.7. Treatment Groups.

Treatment	Silage	Concentrate
1	+ UMD	Beef Nuts
2	Control Silage	TMD
3	Control Silage	Beef Nuts
4	Control Silage	UMD

The animals were weighed weekly. Blood samples were taken at the beginning and end of the experiment by venepuncture of the jugular vein into evacuated heparinised 10 ml blood collection tubes to check the mineral status of the animals. The tubes were centrifuged at 3,000 rpm for 15 minutes and the plasma was analysed for Ca, Mg and P as described in Section 2.3. The experiment ended on the 4th of April (day 70) when all the silage had been eaten.

6.3.3.2 **Results**

Ensilage Experiment

1. Analysis of Grass and Maize Draff

A sample of the grass, taken by repetitive grab sampling during filling the silos, was analysed and the results are given below in Table 6.8. The analysis shows that the grass used for this experiment had relatively low DM and OM contents. These values reflect the wet weather conditions at the time the grass cut was picked up off the field and the consequent soil contamination. The value (82.3 g/kg DM) for the WSC is low compared to the range of values 105 - 211 measured by Henderson (1973) for grass cut between the 1st of August and the 24th of September. Again this reflects the weather conditions prevailing at the time and also the time of year the grass was cut. ADAS (1979) suggest that a WSC concentration of between 25 - 30

Table 6.8. Analysis of Grass Ensiled.

Parameter (g/kg DM unless stated)	Mean of 5 values	(Std Dev)
Dry Matter (g/kg)	158	(7.75)
Organic Matter	881	(7.13)
D Value	68	(1.93)
WSC (g/kg)	13	(2.73)
Crude Protein	228	(7.44)
IVOMD (%)	72.5	(1.64)
M/D	10.9	(0.30)

g/kg FW as the minimum requirement for successful ensilage without the use of preservatives. Since the grass had been treated with 4 l/t of Add Safe, the low WSC should not have affected fermentation or storage characteristics.

Table 6.9. Analysis of Maize Draff Ensiled with Grass.

Parameter (g/kg DM unless otherwise stated)	
Dry Matter (g/kg)	873
Organic Matter	971
Crude Protein	330
AHEE	99.8
NDF	637
ADF	391
Lignin	13.4
IVOMD	64.1
Cu (mg/kg DM)	91.0
Gross Energy (MJ/kg DM)	22.0

The dried maize draff collected from Port Dundas Distillery and used as an effluent absorbent was also analysed and the results are shown in Table 6.9. The analysis of the maize draff shows it to have an IVOMD lower than that of the grass although this may have resulted from inhibition *in vitro* caused by its high oil content.

2. Effluent

The effluent produced from each pit was collected separately in tanks at the bottom of the silos. Six weeks after ensilage the total weight of effluent produced from each silo and its content of DM, OM and BOD_5 were measured. The results from individual silos are given in Appendix 10, mean results for the control silages compared to the silage containing absorbent are shown in Table 6.10.

Table 6.10. Weight and Composition of Effluent Produced

Parameter	Mean Control	(SE)	UMD
Weight (kg/t grass)	134	(14.87)	29
Effluent DM (g/kg)	92.18	(1.33)	111.93
Effluent OM (g/kg)	68.75	(1.44)	84.04
% Grass DM Lost *	7.72	(0.83)	2.02
% Grass OM Lost *	6.49	(0.64)	1.78
BOD_5 (mg/l)	24,160		33,280

* Figures normalised for small differences in grass DM between clamps

There was a large variation in the weight of effluent from the control pits (97 - 165 kg/t, mean 134 kg/t). The mean was lower, but of the same order as the value of 157 l/t calculated using the regression equation of Bastiman (1976) relating effluent production to grass DM. Although the volume of effluent produced is influenced mainly by the DM of the grass, other factors such as type of silo, crop pretreatment and degree of consolidation will also affect flow rate and final volume (McDonald, 1981). Bastiman's equation was derived from effluent measurements from 50 different silages made in bunker-type silos. Grass in the present experiment was treated with Add Safe which tends to increase effluent although the main effect is to increase initial rates of effluent loss (O'Keily, 1990a). Formic acid is known to induce rapid release of the cell sap from fresh plant material (Perdersen *et al.*, 1973). The degree of consolidation may have been less than that achieved by common agricultural practice, particularly since the height of the silage pit is lower than commonly used and may have resulted in the lower effluent volume.

Earlier experiments Al-Rwidah (1987) investigating the effects of inclusion of different types of absorbents using the same pits as in this experiment showed that use of MSBP reduced effluent volumes from 137 to 71 l/t and effluent OM loss from 4.0 to 3.0 kg/t grass. The results from the present experiment using UMD compared favourably with this and suggest that UMD is more effective than MSBP in reducing effluent volume and organic matter loss. UMD incorporated into the grass at ensilage reduced both the quantity of effluent and the amount of nutrients lost in the effluent. Effluent losses of dry matter or organic matter from the control silage were approximately 4 times greater than for the silage made with UMD.

The effluent produced from the silage made with UMD had a higher biochemical oxygen demand than that from the control silage. This has important practical implications because, unless effluent loss can be prevented completely, the benefit for pollution and nutrient loss from the use of draff as an absorbent may be less than indicated by the reduction in volume or organic matter loss. The level of absorbent required to prevent effluent loss is dependent on many factors such as compaction or the use of additives but the major one is the grass dry matter content.

Analysis of the silages produced with and without UMD as an absorbent show that dried maize draff is suitable for use as an effluent absorbent. It reduces the losses associated with ensiling dramatically, largely by reducing the nutrients lost with the effluent. The effluent produced from silage containing UMD was much lower in volume than that from the control pits, but in common with other observations of absorbent inclusion, the OM concentration in the effluent was more concentrated. In the present experiment this represented a 25% increase in OM (g/l effluent) over the value for the the control pits. However, this was considerably less in comparison than the increase in effluent OM concentration observed for other more commonly used absorbents. MSBP gave 46% increase in OM g/l, Viton Straw cubes gave 41% increase in silage effluent OM g/l (Al-Rwidah, 1987). Although the use of absorbents ideally prevents all effluent loss, these results would suggest that, if effluent loss did occur

when an absorbent had been used, then the effluent would be less concentrated, and therefore less environmentally damaging, if the absorbent was UMD than if it was alkali treated straw or MSBP.

3. Silage Analysis

The silos were cored at the time they were opened for feeding approximately 4 months after ensilage. The samples obtained were analysed and the results are given in Table 6.11.

The analysis shows that the silage produced was of moderate to low quality. Silage produced using UMD as an absorbent had a much higher dry matter, organic matter and crude protein content and a lower IVOMD and IVD Value. These changes in the silage composition can be attributed directly to the presence of the UMD in the grass rather than to changes in the fermentation characteristics. The UMD added was drier, contained less ash and had a higher protein content than the grass which was ensiled.

Table 6.11. Silage Analysis.

Parameter	Mean Control	(SE)	+UMD
Dry Matter (g/kg)	172.7	(5.40)	215.5
OM (g/kg DM)	881.5	(2.10)	899.0
CP (g/kg DM)	202.5	(6.78)	275.0
IVOMD (%)	71.3	(0.88)	66.3
M/D	10.0	(0.13)	9.5
pH	4.0	(0)	4.0
D Value	62.3	(0.88)	59.5
NH_4/N (g/kg TN)	80.75	(12.82)	95.0

The M/D of the silage containing absorbent was lower than expected, in view of the increased DM, OM and CP and reduced effluent losses.

Since effluent contains highly digestible components of the grass such as soluble carbohydrates, organic acids and soluble nitrogenous compounds, it follows that the production of large volumes of effluent tends to increase the concentration of the less digestible components such as the cell wall material in the silage.

One explanation for the lower M/D of the absorbent-silage may have been the reduced IVOMD and D values for the UMD compared to the grass. This resulted in lower IVOMD and D values for the silage made with absorbent than for control silage. This may be due to the unsaturated oil content of the UMD depressing microbial activity and therefore reducing the digestibility *in vitro*.

The proportions of DM (%) that were lost as effluent or due to moulding in the pits are given in Table 6.12. A further source of loss would be the invisible losses from the silage which arise from respiration and fermentation losses during the ensilage process. Table 6.12 shows that for the control silage by far the greatest losses were incurred as a result of effluent loss. For the absorbent silage, losses due to moulding were greatest.

Table 6.12. Sources of DM Loss From Silage (% DM Ensiled)

	Control Silage		+UMD
	Mean	(SE)	
Effluent	7.72	(0.83)	2.02
Moulding	2.75	(0.79)	3.70

In order to characterise the fermentation of each silage, water extracts were made from the core samples, these were then analysed by GC. The results are given in Table 6.13.

Table 6.13. Analysis of Silage Fermentation Products

Silage	Control Mean (SE)	+ UMD
	Concentrations g/kg Silage FW	
Ethanol	1.126 (0.291)	0.884
Acetic	4.771 (0.336)	7.030
Propionic	1.471 (0.164)	0.825
Lactic	19.102 (0.439)	22.074

The results obtained from the water extracts suggest that adding dried maize draff as an absorbent did not adversely affect fermentation. They show that both the control silages and that including UMD were high in lactic acid and low in acetic acid. No butyric acid was detected in any of the samples. With these fermentation characteristics, both silages could be classified as lactic (McDonald, 1981). The fermentation of the silages and the lack of effect of incorporation of the UMD is probably attributable to the Add Safe which has achieved a low pH and good preservation in all the silages.

Figure 6.5 Mean Concentrate Intake

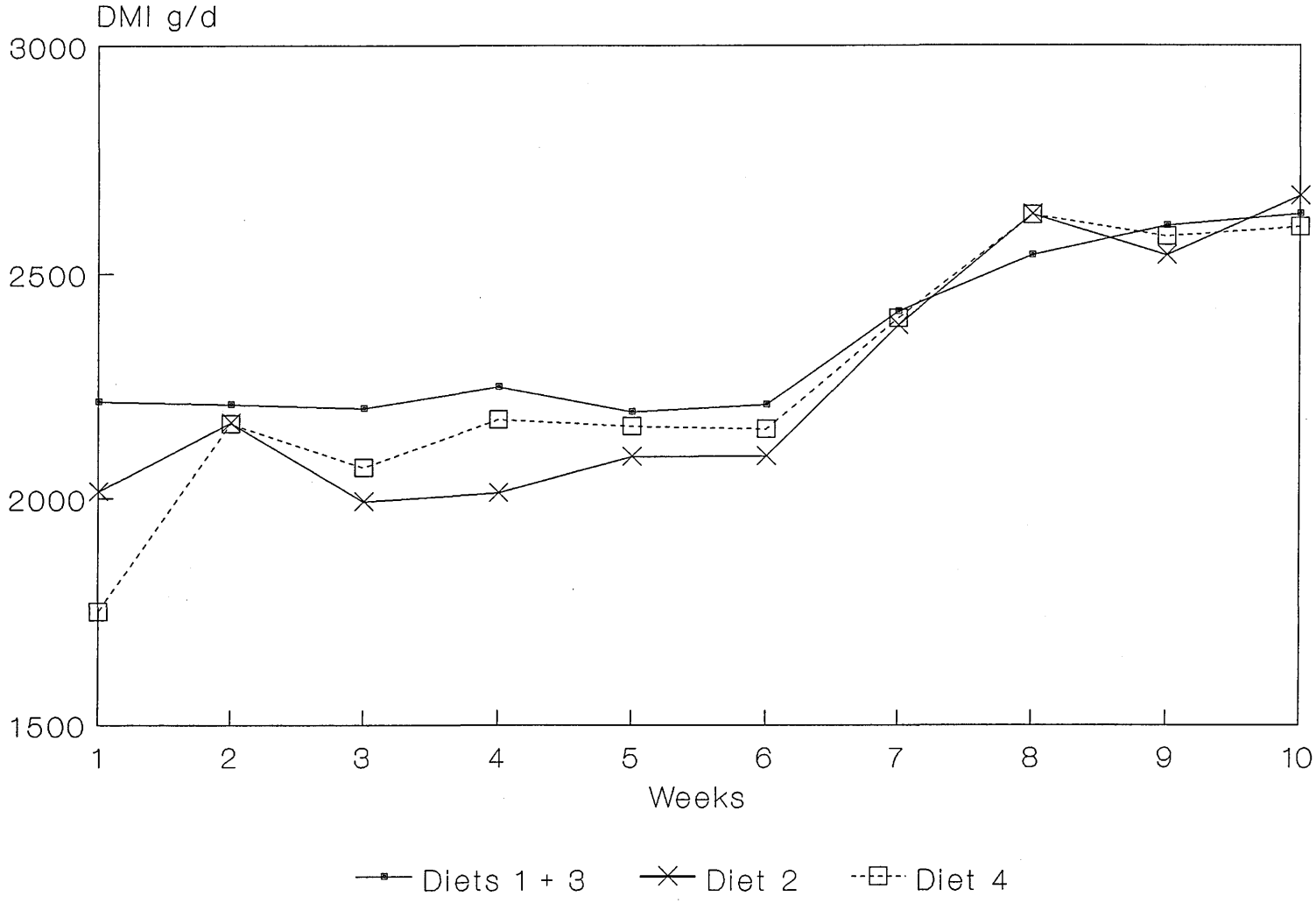
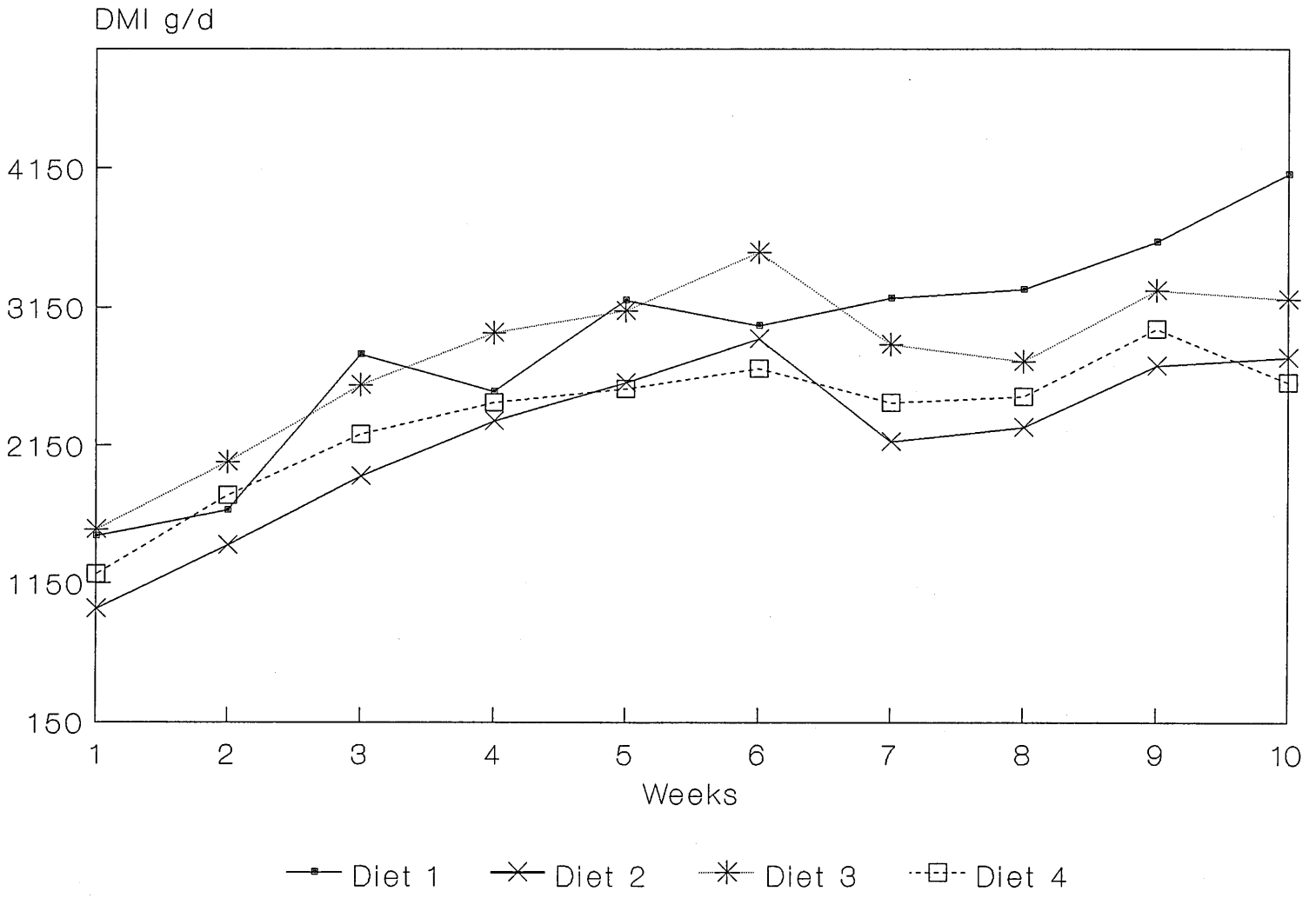


Figure 6.6 Mean Silage Intake



Feeding Experiment

1. Feed Intake.

The concentrates fed to the bulls were analysed (see Appendix 11) and the results are shown in Table 6.14. Analysis of the silages is given in Table 6.11.

Table 6.14. Analysis of Concentrates.

Parameter (g/kg DM unless stated)	TMD	UMD	Beef Nuts
Dry Matter (g/kg)	887	853	867
Organic Matter	855	928	868
Crude Protein	325	355	180
Ether Extract	57.0	71.3	60.8
AHEE	76.5	89.5	78.0
IVOMD (%)	67.9	71.9	70.2
M/D (MJ/kg DM)	11.8	12.1	11.1
Gross Energy (MJ/kg DM)	19.6	20.1	18.2
NCD	706.0	705.0	650.0
Ca	40.3	11.4	16.8
P	9.6	6.4	5.4
Mg	2.5	2.2	2.7

The M/D was calculated using the E3 equation (Thomas *et al.*, 1988)

Weekly means of daily concentrate dry matter intakes are shown in Figure 6.5. Intakes of concentrates for the first few days of the experiment were low for the draff-based feeds, however once these feeds had been pelleted, concentrate intakes for most bulls were determined by the amount offered. Two animals offered the TMD still refused significant quantities of their concentrate and this continued throughout the trial. Group mean concentrate dry matter intakes (CDMI) for the experiment were 2347, 2260, 2348 and 2269 g/day for

diets 1 - 4 respectively (see Table 6.8 for identification of diets). Although groups fed the UMD and more especially the TMD had concentrate dry matter intakes which were lower than for groups offered the beef nuts, these differences were not significant ($p<0.05$).

The NCD values for the untreated and $Ca(OH)_2$-treated maize draff products were very similar (706 and 705 g/kg DM respectively). This result was not expected as in previous work with barley draff the NCD value was increased from 560 to 720 g/kg when draff was $Ca(OH)_2$-treated and large increases in draff digestibility *in vivo* were observed.

Statistical analysis of the silage intake data (Appendix 12) showed that weekly group mean intakes were significantly higher for animals fed diets 1 and 3 than for those fed 2 and 4 (Table 6.15). However when silage intakes were analysed using weekly mean figures for individual animals, differences between diets were no longer significant although the interaction between diet and the number of weeks on the trial was highly significant ($p<0.001$). A graph of the weekly mean silage intakes is shown in Figure 6.6.

Table 6.15. Weekly Group Mean Silage Intakes (g DM/d)

Diet	Silage	Supplement	Intake
1	+ UMD	Beef Nuts	2896^a
2	Control	TMD	2204^b
3	Control	Beef Nuts	2804^a
4	Control	UMD	2349^b

Values with different superscripts differ significantly ($p<0.05$)

Silage intakes for bulls fed the control silage diets dropped considerably, particularly for diets 2 and 3, on week six of the

experiment. This coincided with increasing the level of concentrate offered from 2.5 to 3.0 kg/d. Silage intakes of bulls fed the silage + absorbent continued to increase at this time and remained higher than for the control silage for the rest of the experiment. This difference with respect to the days on trial was significant as indicated by the significant diet.days on trial interaction referred to above. However the reasons for the observations are unknown.

Feeding pelleted maize draff, either limed or unlimed reduced the *ad libitum* silage intakes compared to the diet 3 where the animals were offered the same silage but with beef nuts as the supplement. The reasons for this were not apparent. Analysis of the concentrates showed that they all had similar levels of oil although the draff oil would be expected to be highly unsaturated. The NCD figures for the draff feeds were 50 g/kg DM higher than for the beef nuts suggesting that there is more readily digestible OM in the draff than in the beef nuts. It seems unlikely therefore that it was the poor digestibility of draff fibre that was responsible for the reduced forage intakes.

2. Liveweight Gains

The bulls were weighed weekly and these values were used to calculate the daily liveweight gains (DLWG) by regression. Group mean liveweights are shown in Figure 6.7. The initial and final liveweights and DLWG are given in Table 6.16.

Bull DLWG were statistically analysed by analysis of variance using EDEX 6H.2 statistical package (AFRC Unit of Statistics, Edinburgh) with initial liveweight (V2) taken as the covariant. The analysis was carried out on data recorded for the whole experiment and also on the LWGs up to day 35 and from day 36 to day 70 so that the effects of the initial feed refusals did not influence the analysis. The analyses are given in Appendix 13. The analyses showed that there were significant differences in LWG between treatments and although these were slightly greater during the latter half of the experiment, these were similar to those shown in Table 6.16 for the whole experiment.

Figure 6.7 Mean Bull Liveweight.

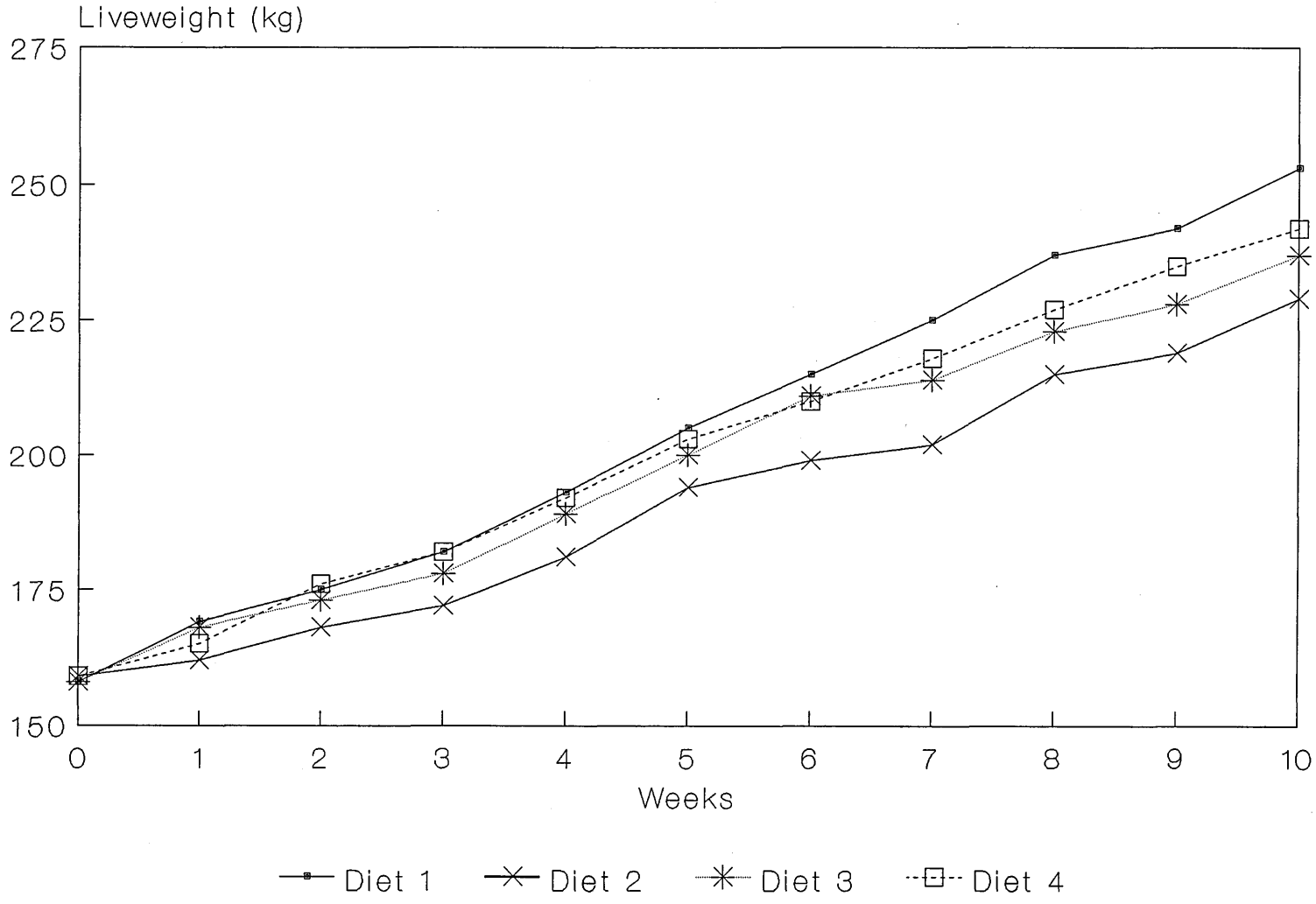


Table 6.16. Mean Bull Liveweights

Diet	Silage	Supplements	Liveweight Initial (kg)	Liveweight Final (kg)	DLWG (kg/d)	FCE (kgDM/ kgLWG)
1	+ UMD	Beef Nuts	158	253	1.38^{a}	3.85^{a}
2	Control	TMD	159	229	1.07^{b}	4.22^{ab}
3	Control	Beef Nuts	158	237	1.12^{b}	4.68^{b}
4	Control	UMD	159	242	1.25^{ab}	3.80^{a}

Values in the same column with different superscripts differ significantly ($p<0.05$).

The results showed that bull daily liveweight gain was statistically higher ($p<0.01$) for animals on diet 1 than for diets 2 and 3. Bulls offered UMD either as a concentrate supplement or in the forage performed better than those fed the other diets. Correspondingly, the efficiency with which they utilised the total dry matter eaten for liveweight gain was also significantly greater ($p<0.05$). 1kg LWG required 3.85, 4.21, 4.68 and 3.80 kg of diet dry matter for animals on diets 1, 2, 3 and 4 respectively.

The increase in feed conversion efficiency when draff is included in the ration is supported by many other studies in which draff has been fed to similar effects (Farlin, 1981; Abrams *et al.*, 1983; Firkins *et al.*, 1985;). Since no attempt was made to equalise the protein intake of the bulls in this experiment, it is possible that this effect was due to the higher levels of protein in the draff diets compared to the beef nuts diet.

Similar calculations were carried out on the ME intakes to indicate the energetic efficiencies of each diet for liveweight gain. Values for k_f (the efficiency with which the dietary ME was utilised for gain) were calculated using the program given in Appendix 14 using

equations from ARC 1980. Values were also calculated to give the 'goodness-of-fit' of the bull data to the ARC 1980 model, given by :-

$$\frac{\text{Actual NE Stored}}{\text{Predicted NE Stored}}$$

The actual NE stored was calculated by multiplying the observed LWG by the energy value of that gain as given by derived regression equations (ARC, 1980). Values of k_f and goodness-of-fit were analysed by analysis of variance (Appendix 15) using the EDEX 6H.2 statistical package (AFRC Unit of Statistics, Edinburgh). Means and statistical significances are given in Table 6.17.

Table 6.17. Efficiency of Utilisation of Energy of Bull Diets

Diet		k_f	Goodness of Fit
1	UMD Silage + Beef Nuts	0.68^{a}	1.45^{a}
2	Control Silage + TMD	0.54^{bc}	1.10^{bc}
3	Control Silage + Beef Nuts	0.49^{c}	1.03^{bc}
4	Control Silage + UMD	0.62^{ab}	1.22^{ab}
	SE	0.039	0.085

Values in the same column with different superscripts differ significantly ($p<0.05$).

Table 6.17 shows that the diet containing the Beef Nuts and UMD silage gave a significantly higher k_f value while diet 3 (Beef Nuts and control silage) gave significantly lower ($p<0.05$) k_f values than the other diets. It also shows that the more conventional diet of proprietary concentrates and control silage gave performance which could be predicted by the ARC 1980 model more accurately than diets

including draff. The predictions underestimate the k_f of diets including draff. The use of this model therefore for such diets may be arguable.

The validity of these results may be questionable since the calculations were based on estimations rather than observations. For example, the ME intakes were calculated from predicted and not measured ME values for the feeds and no actual measurements of the composition of liveweight gain were made. The estimation of feed MEs were based on equation E3 which is derived from the regression of a wide range of compound feeds which generally contain more readily fermentable carbohydrate than draff and therefore give larger losses of DE as methane. This would result in an underestimation of the ME of draff and an overestimation of efficiency.

Differences in LWG may have been due to differences in gut-fill between diets but since all diets were of a similar physical form these are unlikely to have significantly influenced the calculations of k_f. Differences between diets are large, particularly those between diets 1 and 3 (UMD and control silages fed with Beef Nuts), and suggest that the observations of the effects of diet on efficiency of utilisation of ME for gain are real. Given that this is so, there are several possible explanations as to why this effect may have occurred:

1. The diet containing the UMD silage contained more lipid and therefore less rumen-fermentable material. Baldwin (1980) found that the energy cost of ATP generation from acetate was 10% more than from long chain fatty acids (LCFA) and Palmquist (1984) suggested that LCFA increased the synthetic as well as oxidative energetic efficiency. Thus less heat of metabolism would be lost from animals fed the draff based diets and particularly the UMD silage diet where total intake of lipid was greatest.

2. Higher levels of unsaturated oil in the diet may have altered the pattern of rumen fermentation, increasing the proportions of propionate produced. It has been shown that propionate is more

efficiently utilised for growth than the other VFAs (Armstrong and Blaxter 1957) and that utilisation of DE was highly correlated to the relative proportions of propionic acid in VFAs (Elliot and Loosli, 1959). Orskov and Allen (1966) noted a similar but smaller effect.

3. The silage containing UMD contained less long fibre than the control silage and would therefore require less chewing and rumination and less of the ME would be lost as heat of digestion. Since particle size is less for UMD silage, the retention time in the rumen would be reduced and lower energy losses via heat of fermentation could also be expected.

Increases in energetic efficiency when spent grains have been included in the diets of growing ruminants have been noted by many researchers (Abrams *et al.*, 1983; Firkins *et al.*, 1985). Increases in the efficiency with which ME is used for milk production (kl) when spent grains are included in the diet have also been noted (Castle and Watson, 1982; Johnson and Huber, 1987; Hyslop, 1990)

Increases in the k_f have also been observed in studies in which other absorbent materials have been included in silages. Offer and Al-Rwidah (1987) noted an increase in k_f in growing calves from 0.45 for the control silage, to 0.58 and 0.49 for silage made with chopped straw and MSBP respectively. Very few studies have carried out definitive carcass composition analyses to confirm the increases in k_f thought to occur when such absorbent materials are included in silages. However in a study by Reveron *et al.* (1970) comparative slaughter techniques were used on lambs fed either dried pelleted grass or a mixture (20:80) of grass:malt distillers grains. Lambs fed diets including spent grains finished more quickly and had slightly less fat and more protein in their carcasses. The efficiency with which ME was used for growth was increased from 51% to 63% when spent grains were included in the diet.

3. Plasma Mineral Levels

Mean plasma mineral levels of samples taken on day 3 and day 70 of the experiment are shown in Table 6.18. Although there were significant differences between treatment groups in the levels of the three parameters measured, all fall within the normal bovine ranges. The low value for Mg for animals on diet 2 and differences in the phosphorus levels were not apparent by the end of the trial. The differences in the calcium values were reversed by day 70 with those groups of animals showing the highest Ca levels initially showing the lowest at the end.

Table 6.18. Plasma Mineral Levels of Bulls at the Begining and End of the Experiment.

		Diet 1	Diet 2	Diet 3	Diet 4	SE
Ca mg/100ml	Day 3	11.440^{b}	12.260^{a}	11.720^{ab}	11.180^{b}	0.2230
	Day 70	11.600^{a}	10.800^{c}	10.960^{bc}	11.200^{b}	0.1329
Mg mg/100ml	Day 3	2.200^{a}	1.774^{b}	2.098^{a}	2.170^{a}	0.0598
	Day 70	2.290	2.274	2.268	2.244	0.0694
P mmol/l	Day 3	2.506^{a}	2.208^{b}	2.408^{ab}	2.586^{a}	0.0738
	Day 70	2.666	2.764	2.566	2.728	0.0760

Values in the same row with different superscripts differ significantly ($p<0.05$)

These results confirm that the rations were adequately supplemented and that the high levels of calcium in diet 2 did not adversely affect plasma mineral status.

6.3.4 *In Sacco* Evaluation of Diets

A small scale preliminary trial demonstrated that the 24 hour *in sacco* OM digestibility was increased from approximately 61% to 77% when maize draff was treated with 80 g$Ca(OH)_2$/kg DM.

The lack of benefit for animal performance due to $Ca(OH)_2$-treatment of maize draff was unexpected in view of the response to treatment observed when MDG was fed to lambs (Chapter 5). Further, more detailed *in sacco* measurements were therefore made to investigate the possible causes for the differences in the responses between barley and maize. Since increases in NCD were lower than expected and the animal responses to $Ca(OH)_2$ treatment of maize draff were lower than expected, an *in sacco* time course digestibility experiment was carried out to compare the rates of rumen degradation of the three concentrate feeds fed in the bull experiment.

Methods

The feeds were incubated in the rumens of two sheep as described in section 2.4.2. for 0, 4, 8, 16, 24 and 48 hours. The OM, N and NDF *in sacco* degradation curves were determined as described in the same section and the 'a', 'b' and 'c' terms for the Orskov and McDonald (1979) equation were calculated.

Results

Raw data for the *in sacco* digestibility of each fraction is given in Appendix 16. The results for OM, N and NDF degradation *in sacco* are given in Table 6.19 and Figures 6.8, 6.9 and 6.10 respectively.

The degradation curves for $Ca(OH)_2$-treated and untreated barley and maize draff OM *in sacco* are compared in Figure 6.11. The main effect of treatment for both types of grain was an increase in the instantaneous loss of material from the bag. The increase in digestibility due to $Ca(OH)_2$-treatment diminished with increasing incubation times, until at 48 hours there was no effect of treatment

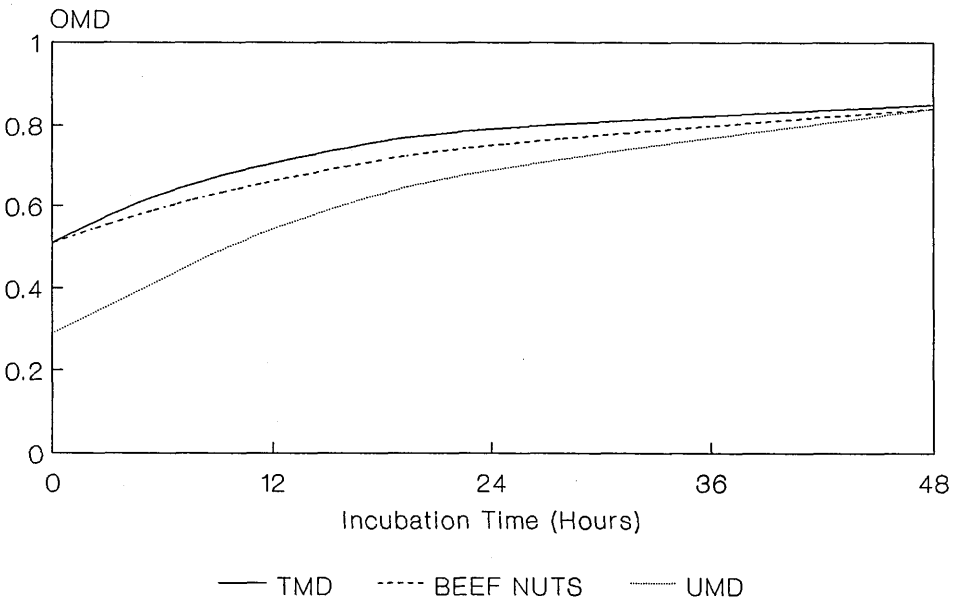
on OM loss. The curves confirm the increase in digestibility at 24 hours demonstrated in the preliminary *in sacco* experiment to determine the level of $Ca(OH)_2$ (Section 6.2).

Table 6.19. Mean *In Sacco* Degradation Curve Constants.

Constant	TMD	UMD	Beef Nuts	Control Silage	UMD Silage
Organic Matter					
a	51.03	29.12	51.10	47.27	57.01
b	35.38	61.59	37.80	56.38	42.97
c	0.074	0.046	0.044	0.036	0.043
Effective Rumen Digestibility					
k=0.03/h	0.76	0.66	0.74	0.78	0.82
k=0.06/h	0.71	0.56	0.67	0.69	0.75
Nitrogen					
a	55.40	32.65	65.99	69.34	84.96
b	21.01	64.16	31.15	24.68	14.94
c	0.084	0.027	0.040	0.148	0.047
Effective Rumen Digestibility					
k=0.03/h	0.71	0.63	0.84	0.90	0.94
k=0.06/h	0.68	0.52	0.78	0.86	0.91
NDF					
a	28.21	10.66	23.99	31.16	30.39
b	53.87	75.48	79.95	94.32	74.25
c	0.059	0.051	0.020	0.021	0.036
Effective Rumen Digestibility					
k=0.03/h	0.64	0.58	0.56	0.70	0.71
k=0.06/h	0.55	0.45	0.44	0.56	0.58

Figure 6.8 In Sacco OM Degradation

Concentrate Feeds



Silages

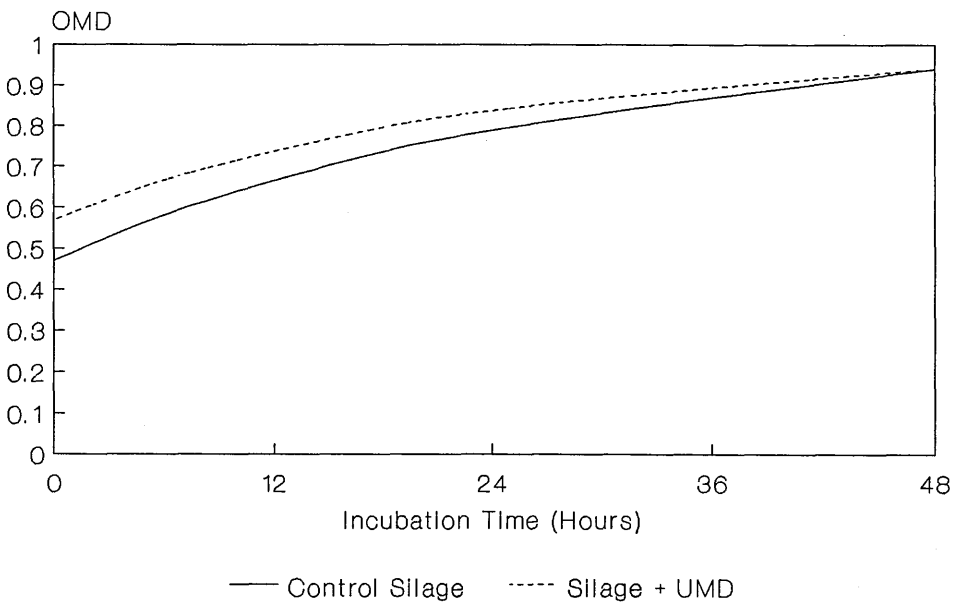
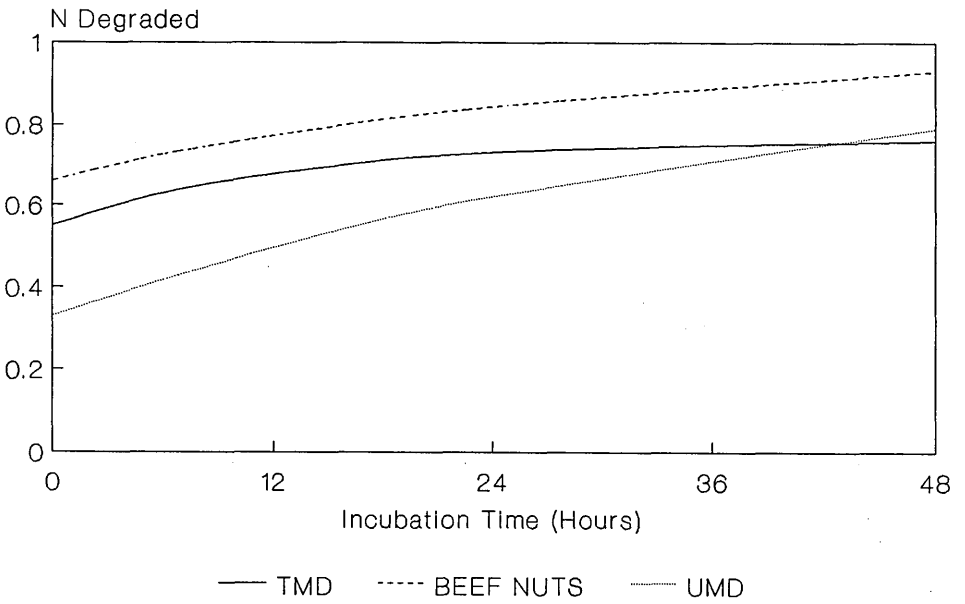


Figure 6.9 In Sacco Nitrogen Degradation

Concentrate Feeds



Silages

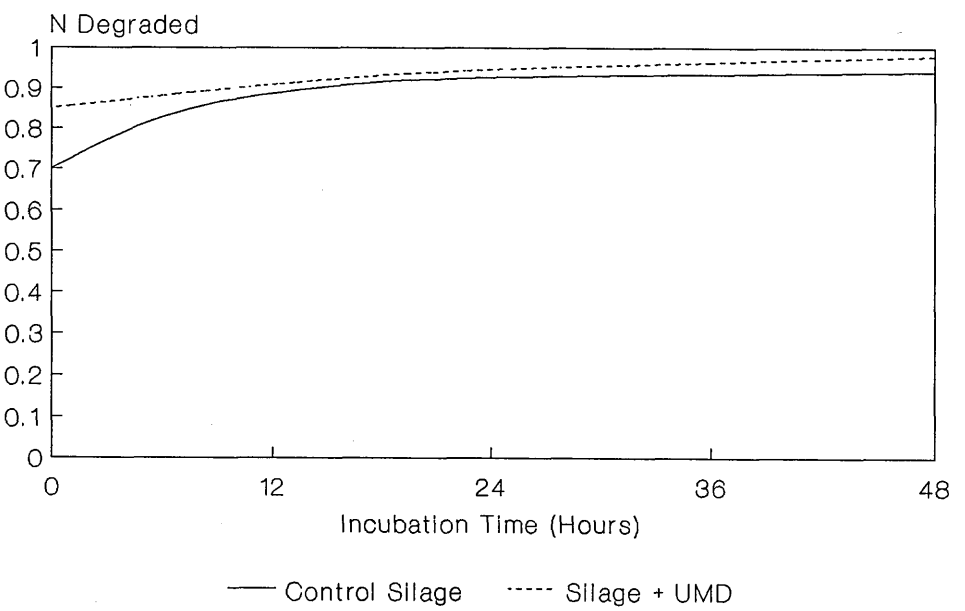
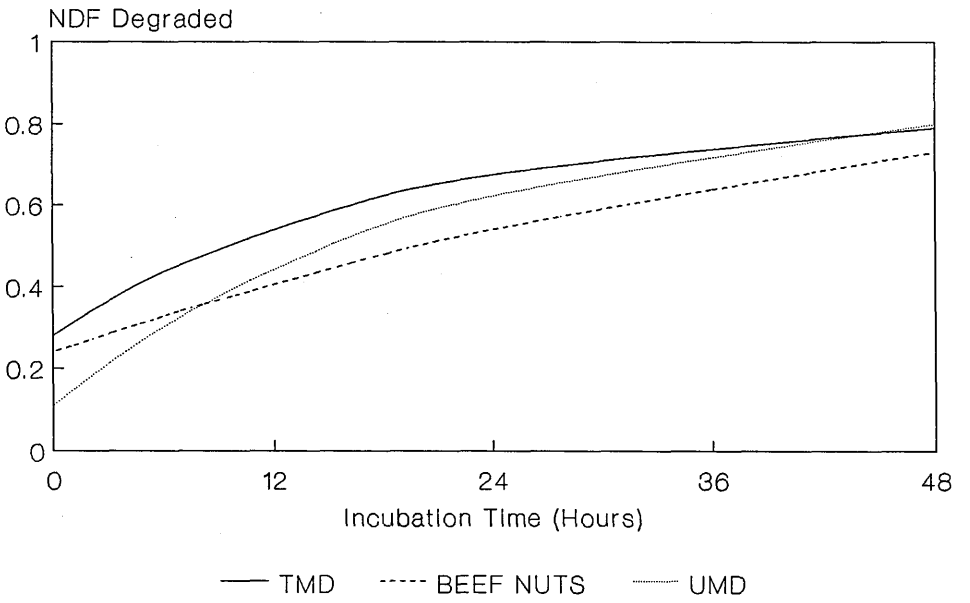


Figure 6.10 In Sacco NDF Degradation

Concentrate Feeds



Silages

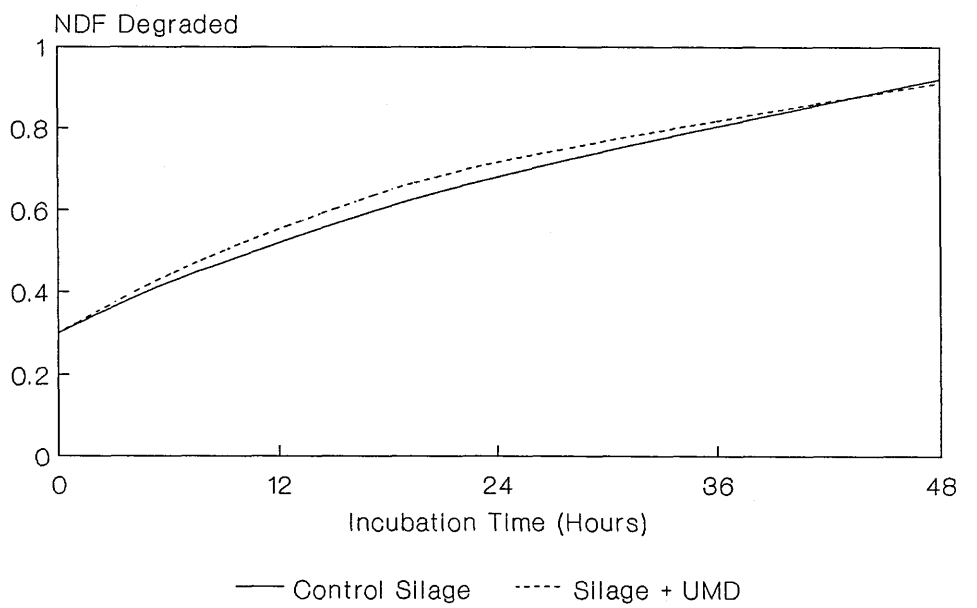
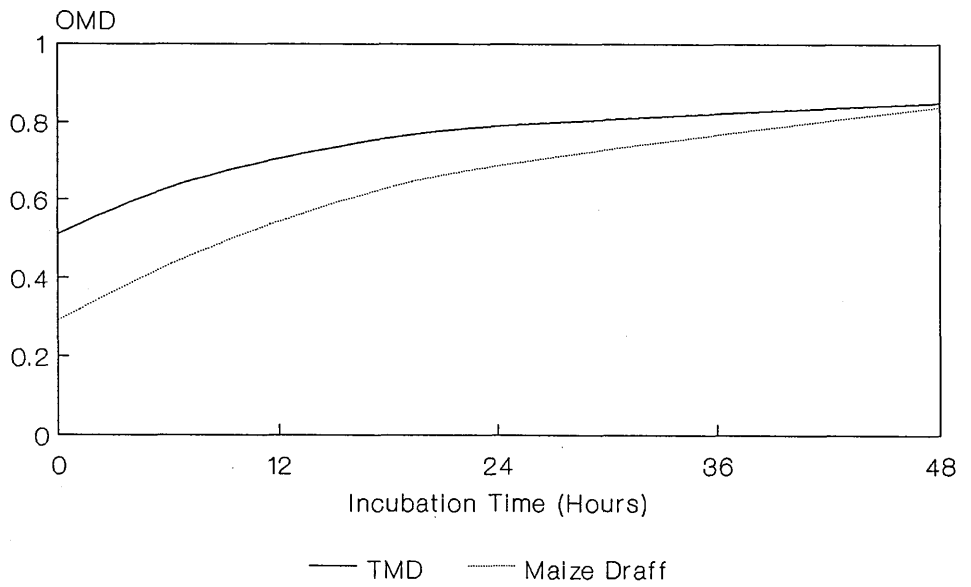
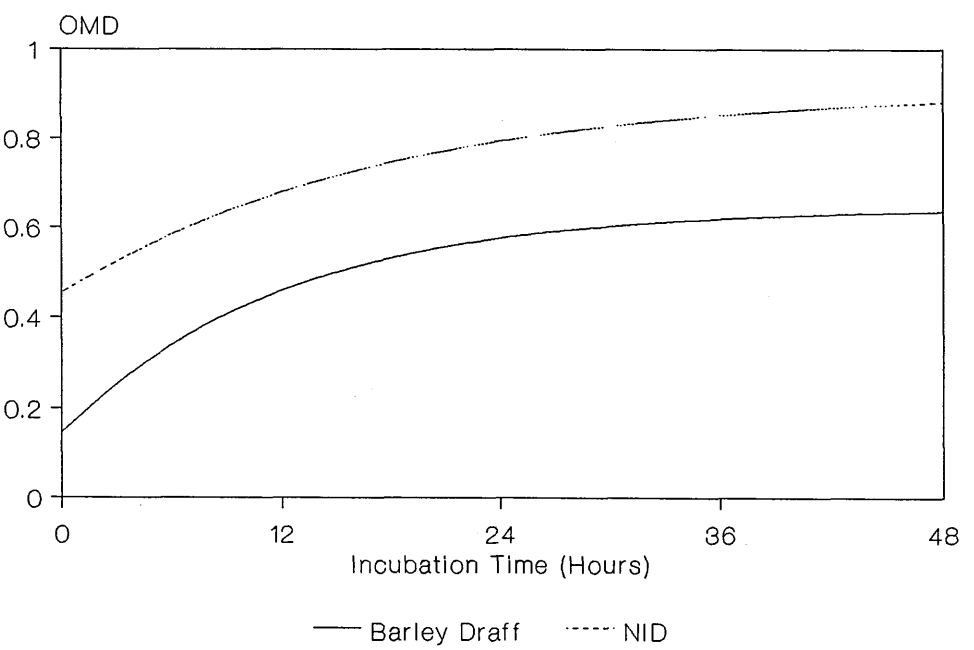


Figure 6.11 Comparison of Lime Treatment of Maize and Barley Draff



Maize Draff



Barley Draff

Comparison of the silage OMD curves shows that inclusion of UMD as an absorbent increases the instantaneous loss of OM from the bags. The curves converge at 48 hours, suggesting that there was no benefit in terms of OMD at longer incubation times, although at high outflow rates silage containing UMD absorbent would have a higher digestibility.

The nitrogen degradation curves for maize draff are also different from those for MDG. Whereas there was little effect of $Ca(OH)_2$ treatment on the nitrogen degradation of MDG, apart from a small increase in the instantaneous loss, treatment of maize draff causes a much larger increase in instantaneous loss. The shape of the curve was also altered by $Ca(OH)_2$ treatment of maize draff due to the decrease in the 'b' fraction and the increase in the 'c' term. $Ca(OH)_2$-treatment of maize draff solubilises the protein but does not increase the total proportion of the nitrogen which can be degraded. For MDG, the small increase in N degradability at 0 hours with $Ca(OH)_2$-treatment is maintained at all incubation times.

The nitrogen digestibility curves of the silages show a large increase in instantaneous loss of nitrogen when UMD is used as an absorbent and an increased nitrogen loss at all incubation times.

The curves for the NDF degradation for TMD and UMD are similar to those for nitrogen in that there is a large increase due to $Ca(OH)_2$-treatment in degradability at zero time which diminishes with increasing incubation times. For MDG, the reverse is true, and the curves for treated and untreated draff NDF diverge at longer incubation times.

The effect of treatment on the rumen digestibility of the spent grains would be bigger at faster outflow rates. The improvement in rumen OM degradation due to $Ca(OH)_2$-treatment of maize draff is therefore dependent on residence time in the rumen. At low outflow rates, the response would be smaller than when the feed particles are retained for longer periods. Thus as outflow rates of $k = 0.03/h$ (Table 6.20) the effective digestibility in the rumen increases from 0.66 to 0.76

due to $Ca(OH)_2$-treatment (a 17% increase). However, at a faster outflow rate (k = 0.06/h) the response is greater; 0.56 to 0.71 (39% increase). The spent grains are made up of small particles and the outflow rate in practice is likely to be high and the effect of $Ca(OH)_2$-treatment on rumen OM degradation is therefore also likely to be maximised.

The total potential digestible OM in the feed is given by the sum of 'a' and 'b' constants. For TMD this is 86% and for UMD 91%. Thus $Ca(OH)_2$-treatment does not increase the total proportion of maize draff that is potentially degradable, although, as shown above, it would be expected to cause an increase in the rumen degradability of the OM. It is likely therefore that the effect of treatment is to reduce the proportion of draff OM that is digested in the hindgut but the effect on the whole tract digestibility may be small.

Comparable figures for the *in sacco* OMD of MDG illustrate the differences between responses of barley and maize draff to $Ca(OH)_2$-treatment. Whereas $Ca(OH)_2$-treatment did not increase the proportion of potentially digestible material for maize draff, for MDG it was increased from 58% to 84%. The differences in response to treatment can be seen by comparing Figure 6.11a showing the effect of $Ca(OH)_2$-treatment on the ISOMD of maize draff, with Figure 6.11b showing ISOMD of MDG. For the MDG the curve for the $Ca(OH)_2$-treated material remains approximately parallel to that for the untreated. For the maize draff however, the degradation curves converge after 48 hours.

These results suggest that the differences in responses to $Ca(OH)_2$ treatment observed for the two different draffs are due to differences in the cell wall structures of maize and barley. In the maize draff, the curves for N and NDF degradation were similar, suggesting that the two fractions are in some way associated in the cell wall material. The same cannot be said for MDG. $Ca(OH)_2$-treatment of MDG gives greater increases in *in sacco* NDF digestibility at longer incubation times whereas the reverse is true for maize draff. It is likely that this phenomenon is due to differences in the polysaccharide content of the cell walls of maize and barley draff leading to differences in

their responses to alkali.

Conclusions

Inclusion of UMD as an effluent absorbent reduced effluent loss from grass silage and the amount of DM and OM lost per kg of grass ensiled. The silages produced were of a similar chemical analysis, suggesting that fermentation was not adversely affected by the inclusion of dried draff.

Bulls offered silages (either control or UMD silage) supplemented with beef nuts consumed significantly more silage DM ($p<0.05$) than those offered the control silages supplemented with maize draff (UMD or TMD).

Bulls offered silage made with UMD had a significantly higher ($p<0.05$) daily liveweight gain than any other treatment group. Those offered maize draff either incorporated into the silage or fed as a separate supplement, had superior feed conversion efficiencies to those for other treatments.

$Ca(OH)_2$-treatment of maize draff was not effective in increasing the proportion of potentially digestible OM whereas with MDG it gave a 48% increase in potentially digestible OM.

CHAPTER SEVEN

INVESTIGATIONS INTO THE USE OF NID AS A CARRIER FOR DIETARY FAT

7.1 Introduction

In Chapter 6 the absorbtive nature of $Ca(OH)_2$-treated spent grains was described and it was speculated that this property may extend to the absorption of fats. The aim of the work described in this chapter was to investigate the use of NID as a carrier for dietary fat.

Several studies on the absorption of fatty acids onto food particles have been carried out in experiments investigating the inhibitory effects of long chain fatty acid on rumen bacteria (Harfoot *et al.*, 1974; Maczulak *et al.*, 1981). Harfoot *et al.* (1973) showed that biohydrogenation of the long chain fatty acids of linoleic acid was incomplete when it was included at high levels *in vitro*. It was suggested that this was because at concentrations in excess of 1 mg/ml, the levels of linoleic acid was greater than could be absorbed onto the surface area of the food particles. Henderson (1973) noted that long chain fatty acids became inhibitory to the growth of bacteria when their concentrations became greater than could be absorbed onto a bacterial cell. In 1974 Harfoot *et al.* showed that there was competition between rumen bacteria and food particles for the absorption of long chain fatty acids and that inclusion of inert particles of silica or food particles reduced the amount of fat which was absorbed onto bacterial cells. This would suggest that absorption of long chain fatty acids onto food particles could be a method of reducing their inhibitory effects on rumen function. Apparently, no studies have been carried out to investigate the degree of binding shown by different long chain fatty acids onto different feedstuffs.

As observed in Chapter 3, $Ca(OH)_2$-treatment of spent grains results in the formation of insoluble calcium soaps. This is thought to occur firstly by the hydrolysis of the triglycerides which are the major part of the draff lipid, and subsequently by the saponification of the

free fatty acids with the free calcium ions. Supplementary oil added to draff would undergo a similar reaction on addition of $Ca(OH)_2$ and may give increased levels of preformed soaps in the feed.

Thus there are potentially two ways that the rumen-inhibitory effects of supplementary oil added to spent grains would be reduced by treatment with $Ca(OH)_2$;

1. By adsorption of the free fatty acids or triglycerides on to the surface of the $Ca(OH)_2$-treated spent grains.

2. By the formation of insoluble calcium soaps.

In the following experiment MDG were supplemented with different levels of maize oil, both with and without $Ca(OH)_2$ treatment, and the effects on *in vitro* digestibility were compared. Maize oil was chosen for this purpose since it was readily available and had a similar fatty acid profile to MDG oil. The fatty acid profiles of each oil are given in Table 7.1 for comparison.

Table 7.1. Fatty acid Profiles of Maize and MDG Oil
(% Total Fat)

Fatty Acid	Draff Oil	Maize Oil
C16:0	22.5	10.0
C18:0	1.7	2.0
C16:1	1.4	
C18:1	21.5	22.9
C18:2	48.6	64.4
C18:3	4.8	0.8

7.2 *In Vitro* Measurements of the Inhibitory Effects of Maize Oil Absorbed onto MDG

7.2.1 Preparation of Oil Supplemented MDG

Oil-supplemented MDG (OSD) and oil-supplemented NID (OSNID) were prepared to give MDG-based feeds with a wide range of lipid contents with and without $Ca(OH)_2$-treatment. Since supplementary oil was added it was assumed that additional $Ca(OH)_2$ would be required to maximise saponification. It was calculated stoichiometrically that the $Ca(OH)_2$ requirement g/kg of fresh MDG would be:

$$16 + 0.14(\text{g of total oil})$$

$$(Ca{:}oil = 1{:}13,\ Ca(OH)_2{:}oil = 1{:}7)$$

Table 7.2 Ingredients Added to 1 kg (FW) of MDG.

Code	$Ca(OH)_2$ (g)	Oil (g)	Emulsion (mls)	Total Oil (g)	% Oil Final DM
OSNID					
1T	20	0	0	25	10
2T	28	31	39	56	20
3T	37	125	156	150	40
4T	48	200	250	225	50
5T	64	313	391	338	60
OSD					
1C	0	0	0	25	10
2C	0	31	39	56	20
3C	0	125	156	150	40
4C	0	200	250	225	50
5C	0	313	391	338	60

Oil was added to give final concentrations of 10, 20, 40, 50 and 60 % of the final DM assuming the MDG had a lipid content of 100 g/kg DM as shown in Table 7.2.

Oil was added as an emulsion consisting of 4 parts oil: 1 part water + a few drops of an emulsifier (Tween 20). After adding the emulsion, the draff and emulsion were thoroughly mixed in a large food mixer (Hobart Manufacturing Co. Ltd., 73, Dykehead St. Queenslie Industrial Estate, Glasgow G33 4DE). For $Ca(OH)_2$ treated samples, industrial grade lime (Limbux, ICI) was added according to the above calculation and the mixture was again thoroughly mixed. All mixtures were sealed in polythene bags and were held at 60°C for 48 hours. The oil-supplemented grains were then dried at 60°C and, once dry (>90% DM) were stored in polythene containers until required.

7.2.2 **Experimental Design**

Table 7.3 Additions to *In Vitro* Tubes.

Code	(mg)	Hay (mg)	Final Oil Conc.(g/kg)
1C	500	-	100
1T	500	-	100
2C	500	-	200
2T	500	-	200
3C	312.5	187.5	250
3T	312.5	187.5	250
4C	250.0	250.0	250
4T	250.0	250.0	250
5C	208.3	291.7	250
5T	208.3	291.7	250
Hay		500.0	10

Duplicate samples of each treatment and of untreated MDG and hay were incubated *in vitro* as described in Section 2.4.1. For treatments with oil concentrations of > 250 g/kg DM, hay was added to the *in vitro* tube to dilute the oil so that fermentation would not be completely inhibited. This also provided sufficient fermentable substrate to maintain the system over 48 hours and allowed the degree of inhibition for each treatment to be measured. Additions of substrate to each tube is shown in Table 7.3..

7.2.3 Results and Discussion

The mean IVOMD values are shown in Table 7.4. Figure 7.1 shows the effect of $Ca(OH)_2$-treatment on the IVOMD of the oil-supplemented products. At all levels of oil supplementation, $Ca(OH)_2$ treatment increased the IVOMD. The differences between $Ca(OH)_2$-treated and untreated samples decreased at higher levels of oil supplementation, this was due, in part, to higher levels of hay inclusion with these treatments.

These values were used to calculate the oil-free IVOMD (OFIVOMD) for each sample. The digestibility of the oil-free OM was calculated assuming that all the oil originally put in the tube was recovered in the OM at the end of the incubation. These values are referred to as OFIVOMD values.

Oil-free IVOMD values for hay incubated separately (0.468) and for the $Ca(OH)_2$-treated and untreated MDG without supplementary oil (1T and 1C respectively) were used to predict the oil-free IVOMD for the oil supplemented feeds incubated with hay, assuming no associative effects of the oil on digestibility. These are referred to as the predicted OFIVOMD values.

Comparison of the calculated and predicted oil-free IVOMD as shown in Figure 7.2, which shows, for each sample, the extent of the inhibitory effect of oil supplementation on that sample. These calculations assume that the oil-free IVOMD for the OSNID was the same as for the

Figure 7.1 Effect of Supplementary Oil on IVOMD of MDG

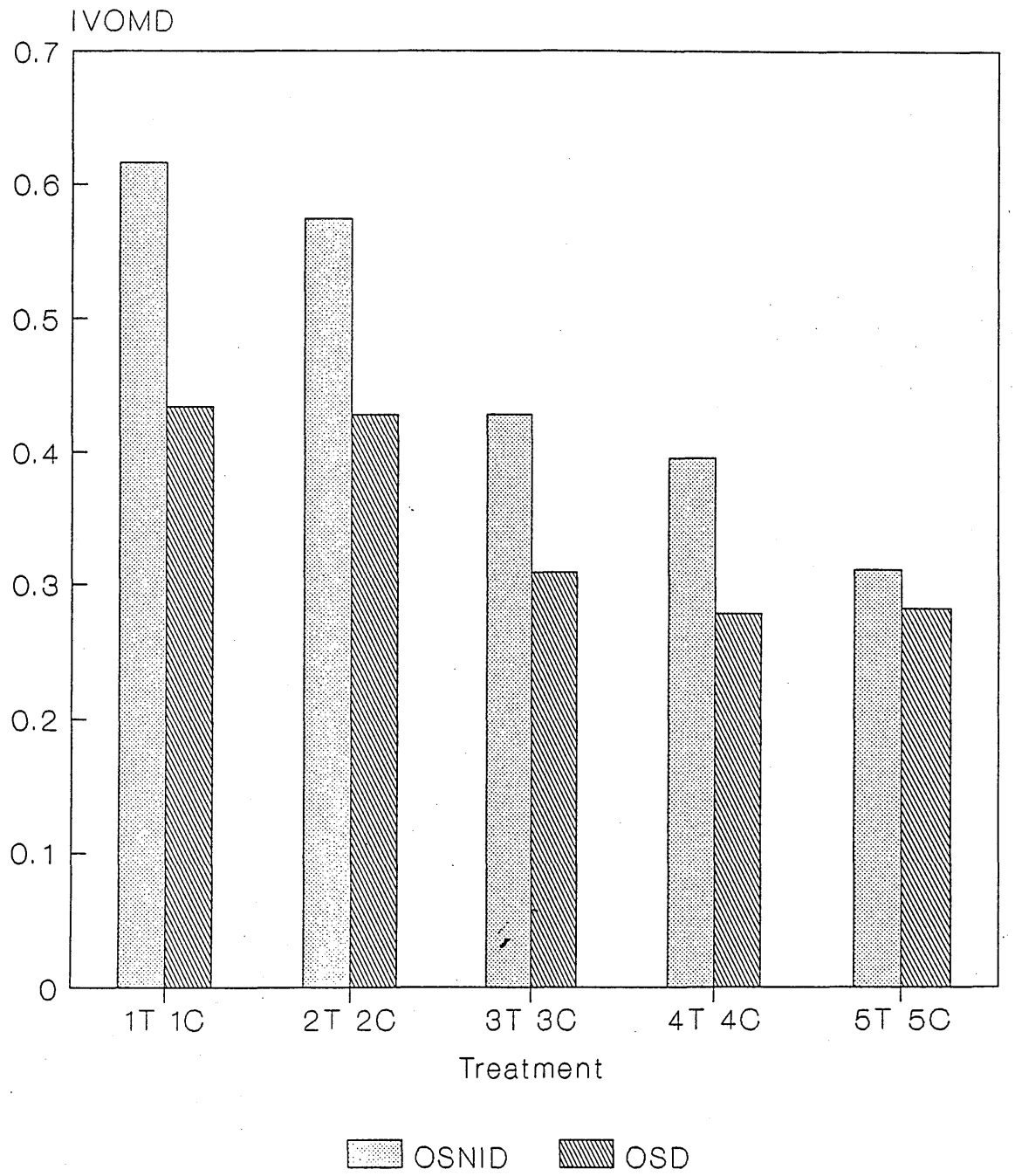
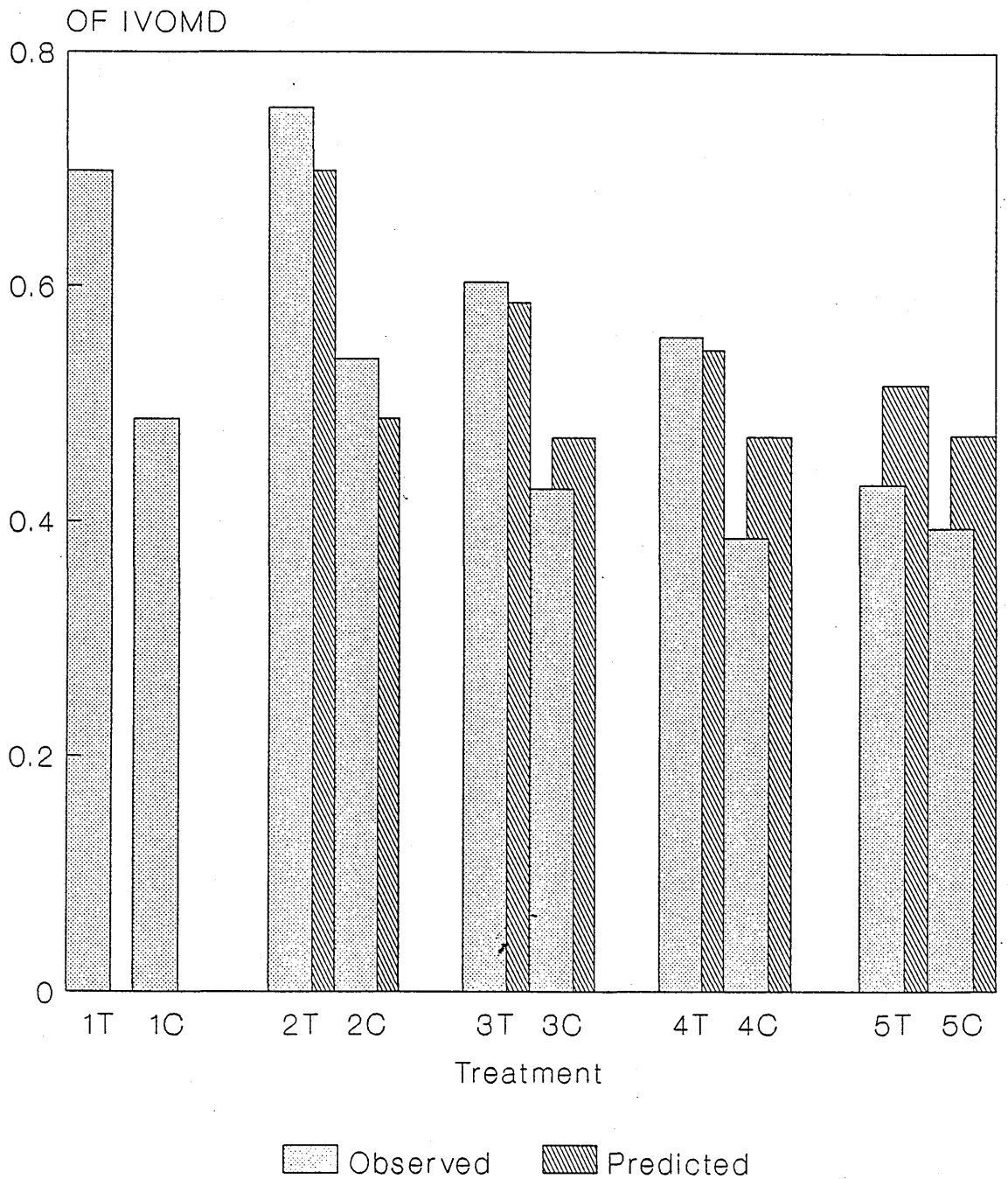


Figure 7.2 Effect of Supplementary Oil on Oil-Free IVOMD of MDG



unsupplemented NID. This assumption may be criticised in that oil-supplemented NID was treated with higher levels of $Ca(OH)_2$ than the unsupplemented which may have increased the digestibility of the non-oil fraction. However, much of the additional $Ca(OH)_2$ would have reacted with the added oil, and the digestibility response to $Ca(OH)_2$ levels above 80 g/kg DM is reduced (see Chapter 3.)

Table 7.4 The *In Vitro* Organic Matter Digestibility

Code	Mean IVOMD	IVOMD on Oil Free Basis	
	Observed	Observed[a]	Predicted[b]
1C	0.434	0.487	
2C	0.428	0.538	0.487
3C	0.309	0.427	0.471
4C	0.278	0.386	0.472
5C	0.282	0.394	0.473
1T	0.616	0.698	
2T	0.574	0.752	0.698
3T	0.428	0.603	0.586
4T	0.395	0.557	0.546
5T	0.311	0.431	0.516

[a] Calculated from observed IVOMD values

[b] Predicted from IVOMD values of components assuming no interactions

The results for the actual IVOMD and the observed and the predicted oil-free IVOMD are given in Table 7.4.

For the OSD treatments, the predicted oil-free IVOMD did not vary greatly because the digestibility of the untreated MDG did not differ greatly from the digestibility of the hay. But for the OSNID treatments, the IVOMD of the $Ca(OH)_2$-treated MDG is greater than for the hay, and therefore, as the proportion of hay in the *in vitro* tube

was increased (at the higher levels of oil supplementation) the predicted overall oil-free IVOMD was also reduced.

For the OSNID treatments, the predicted and observed OFIVOMD values were similar except at the very highest level of oil supplementation (T5). This suggests that at lower levels of supplementation (treatments T2, T3 and T4) the inhibitory effects of oil on fermentation were negated by the treatment process. For the OSD treatments, the observed OFIVOMD values were lower than those predicted reflecting the inhibitory effect of the supplementary oil on oil-free OM (probably fibre) digestion in the absence of added Ca. For the OSNID treatments, the predicted OFIVOMD exceeded the observed only in the case of the highest oil level (T5).

The depression in digestibility at the higher levels of oil supplementation (4C, 5C) for the OSD treatments suggests that the MDG became saturated at higher levels of oil supplementation, leaving free oil in the *in vitro* system to inhibit digestion of the oil-free organic matter. This provides tentative evidence that the adsorption of oil onto spent grains may reduce the toxicity of oil to rumen micro-organisms.

The results from this experiment suggest that the theories put forward concerning supplementation of MDG with unsaturated oil and the benefits of $Ca(OH)_2$ treatment in this respect were correct. However, from this experiment it is not possible to distinguish between benefits due to soap formation or due to absorption of fat onto MDG fibre.

In conclusion, this experiment provides evidence that with $Ca(OH)_2$-treatment, levels of oil supplementation of MDG can be up to 50% DM without depressing IVOMD. The OFIVOMD observed for treatment C2 (observed > predicted) suggests that adsorption of oil onto the spent grains may reduce the inhibitory effects of unsaturated oil even in the absence of $Ca(OH)_2$. This was not substantiated at higher levels of oil-supplementation (treatments C3, C4 and C5) perhaps because the MDG became saturated, leaving some oil in the unabsorbed state.

These findings suggest that $Ca(OH)_2$-treated MDG supplemented with oil (up to 50% DM) may provide a means of increasing the unsaturated fat intake of ruminants without causing digestive upset. It has been shown by Palmquist (1984) and Chalupa *et al.*, (1986) that the total intake of fatty acids in the ruminant diet may be increased by the use of calcium soaps of fatty acids, without the harmful effects on rumen fermentation normally associated with high levels of dietary fat. The use of calcium soaps, particularly soaps of saturated fatty acids such as those present in palm oil, is now common in energy dense ruminant diets such as those fed to high yielding dairy cows.

In Chapter 5 the fatty acid profiles of the depot fat of lambs fed NID were shown to be different to those fed a proprietary concentrate. It was suggested that this was due to the formation of calcium soaps in the MDG oil, leading to increased ruminal by-pass of unsaturated fatty acids and reduced saturation of the perirenal and subcutaneous fat. The present *in vitro* experiment showed that supplementary oil, added to MDG before the $Ca(OH)_2$ treatment, reduced the inhibitory effects of oil on IVOMD suggesting that the oil was not available to the rumen micro-organisms and may therefore by-pass biohydrogenation if included in the diet. Supplementation of NID in this way with an oil of similar fatty acid profile may therefore enhance its effects on the fatty acid composition of ruminant fat. To test this hypothesis, and to assess the palatability and safety of oil-supplemented products as ruminant feeds, an *in vivo* experiment was carried out in which fistulated sheep were offered diets containing two levels of MDG supplemented with approximately 30% oil. In order to assess the effects of $Ca(OH)_2$-treatment of oil-supplemented MDG *in vivo*, the treated grains were prepared both with and without $Ca(OH)_2$ treatment.

7.3 Experiment to Investigate the Effects of Feeding Oil-Supplemented MDG to Sheep.

7.3.1 Preparation of Oil-Supplemented Feeds

Oil-supplemented MDG treated with (OSNID), and without (OSD) $Ca(OH)_2$ were prepared as described in Section 7.2. An emulsion consisting of 4 parts maize oil to one part water was prepared using a few drops of the emulsifier Tween 20. To each kg of MDG was added 156 mls of emulsion, 37 g of $Ca(OH)_2$ as industrial lime (Limbux, ICI) and 16 g of minerals formulated to balance the high level of calcium present in the final product and to supply the sheeps' other mineral, trace elements and vitamin requirements whilst on the experimental diets. The mixture was blended together in a cement mixer, held in a sealed polythene bag at 60°C for 48 hours before drying at the same temperature. OSD was produced in a similar manner with the omission of $Ca(OH)_2$ and with a different mineral mixture. The analysis of the two products is shown in Table 7.5.

Table 7.5. Composition of Oil-Supplemented MDG (g/kg DM unless specified)

Parameter	OSNID	OSD
Organic Matter	850	957
Crude Protein	110	135
Total Oil	310	349
Soap (% Total Oil)	67	10
GE (MJ/kg DM)	22:9	26.2
NCD	808	697
ME (MJ/kg DM)*	20.0	18.5

* Calculated using Equation E3 (Thomas *et al.*, 1988)

7.3.2 Experimental Design

The experiment was carried out according to the design shown in Table 7.6 using 4 rumen-fistulated wether sheep. The design reflected one of the purposes of the experiment which was to test the safety of feeding high levels of the oil supplemented products to ruminant animals. Therefore the control supplement was fed to each animal in the first period to give basal rumen measurements which could be compared to those obtained when the oil-supplemented feeds were offered. The highest levels of oil were left until the final two periods to avoid any carry-over effects between treatments due to level of oil supplementation.

Table 7.6. Experimental Design.

Sheep		1	2	3	4	Concentrate (g/day DM)	Supplement (g/day DM)
Period	I	C	C	C	C	300	0
	II	OSNID	OSNID	OSD	OSD	100	200
	III	OSD	OSD	OSNID	OSNID	100	200
	IV	OSNID	OSNID	OSD	OSD	0	500
	V	OSD	OSD	OSNID	OSNID	0	500

C = Control diet
OSNID = Oil-Supplemented NID
OSD = Oil-Supplemented Draff

The concentrate was a proprietary compound feed consisting mainly of molassed sugar beet pulp (MSBP) and had an oil content of less than 1%. The concentrates and supplements were fed with 700g DM/day of chopped hay in two equal feeds. The oil-supplemented feeds were analysed for OM, CP, GE, NCD total oil and % soap formation as described in Chapter 2.

Each period lasted 14 days. Daily measurements included DM intake of supplement and rate of cellulose digestion in the rumen as given by cotton strip dry matter digestibility and as described in section 2.4.3. On the final day of each period, 4 hours after the morning feed, samples were taken of rumen contents and blood. The blood was taken by venepuncture from the jugular vein into evacuated, heparinised 10 ml blood tubes. The blood samples were centrifuged at 3,000 rpm for 15 minutes. The supernatant was taken off and stored in small plastic vials prior to analysis. The fatty acid content of the plasma lipids was measured as described in sections 2.5.

Rumen contents were taken by removing approximately 500 g of rumen contents through the cannula using a small ladle. The sample was mixed and a 100 g subsample was taken and immediately placed in a deep freeze at -20°C. The pH was measured using a portable pH meter (Cranwell CR 99-digital), and a sample of rumen liquor was taken and analysed for volatile fatty acids as described in section 2.6.4. The frozen subsamples of rumen contents were freeze dried and milled through a 1 mm screen and kept in an air tight container. The lipids from the rumen contents were extracted, derivatised and the fatty acid composition was determined as described in section 2.5.

7.3.3 Statistical Analysis

All results were analysed statistically for the analysis of variance using Genstat V. The analysis of the VFA data are given in Appendix 17 as an example. Significant differences between means were identified by Least Significant Differences (LSD) method (Steel and Torrie, 1980). It should be noted however, that because of the unbalanced design of the experiment the statistical analysis carried out could be considered invalid since the effects of treatment were confounded by the effects of period. Effects of period were ignored in the analysis of variance and the treatment means and standard errors are given. The experiment was designed to give an indication of the toxic effects of the treatments. A balanced changeover design was not applicable for this experiment since large carry-over effect were anticipated from feeding high levels of unprotected unsaturated oil.

7.4 Results and Discussion

7.4.1 Dry Matter Intake

There were no refusals of hay or concentrate and all animals ate all that they were offered. The only refusals of supplement occurred when sheep were offered 500g DM of OSD when the mean DM intake was 345g DM/day. The effects of treatment on the dry matter intake of supplements can be explained by the lower cellulose digestibility measured by the cotton strip DM loss. As shown in Section 7.4.2, cellulose digestibility was depressed most when the sheep were offered the highest level of OSD. This suggests that the low dry matter digestibility was the cause of the refusals observed with this diet. It is of interest that only the supplement was refused on this diet and all the hay offered was consumed by the sheep. If the high level of oil reduced fibre digestion then a reduction in hay intake would have been anticipated.

7.4.2 Rumen Parameters

7.4.2.1 24 Hour Cotton Strip DM Loss

Table 7.7. Cotton strip DM digestibility (%) Sheep x Treatment

	Control	Low OSNID	Low OSD	High OSNID	High OSD
Sheep 1	0.160	0.195	0.201	0.171	0.175
2	0.181	0.166	0.171	0.156	0.141
3	0.205	0.219	0.191	0.166	0.069
4	0.204	0.175	0.117	0.132	0.069
Mean	0.187^{a}	0.189^{a}	0.170^{a}	0.156^{ab}	0.114^{b}

SE individual values = 2.782, SE means = 1.391

Values with different superscripts differ significantly ($p<0.05$).

The analysis of variance for these results is given in Appendix 18. The results in Table 7.7 show that there was no significant ($p<0.05$) depression in cellulose digestibility (compared to the control diet) for the lower levels of oil supplementation. Although 500g of OSNID gave a small, non-significant depression in cotton strip digestibility, only high levels of OSD gave significant ($p <0.05$) reductions. Large fluctuations in the daily DM loss from the cotton strips were observed and these were particularly noticeable for diets containing the OSD supplements. Thus the coefficient of variation for these measurements was large (18.64%) reflecting the unstable conditions in the rumen caused by the oil.

Due to the nature of the design of the experiment, no meaningful statistical analysis could be done to prove any carry-over effects between treatments, as period and treatment were confounded. Nevertheless, the large depression in cotton strip DMD (cf. control) for sheep 3 and 4, which received the high level of OSD in period 4 after receiving a low level of OSNID in period 3, suggests that there were substantial carry-over effects. Depression in cellulose digestibility for sheep 1 and 2, which received the high level of OSD in period 5 after a high level of OSNID in period 4, was much less than for sheep 3 and 4.

The differing patterns of response to diet in the two pairs of sheep may be explained by the levels of non-soap oil reaching the rumen, which, as shown in Table 7.8, were similar for low level of OSD and the high level of OSNID. When sheep 3 and 4 were changed abruptly from a diet containing 21 to one providing 101 g non-soap oil/d, cotton strip digestibility was depressed. For sheep 1 and 2, the change to the highest level of non-soap oil was more progressive and consequently the rumen micro-organisms were not so severely affected. These observations would suggest that soap formation due to $Ca(OH)_2$ treatment was affording some protection to the rumen micro-organisms against the inhibitory effects of the unsaturated oil.

Although the depression in cotton strip DMD was less for the OSNID than for the OSD at high levels of inclusion in the diet, mean

cellulose digestibility for the 500g OSNID diet was still lower than for the control diet. This suggests the incomplete reversal of the inhibitory effects of fatty acids on digestibility as noted by El Hag and Miller (1972). It may also have been attributable to incomplete soap formation. Intakes of soap and non-soap are given in Table 7.8.

Table 7.8. Mean Daily Intake of Oil and Soap

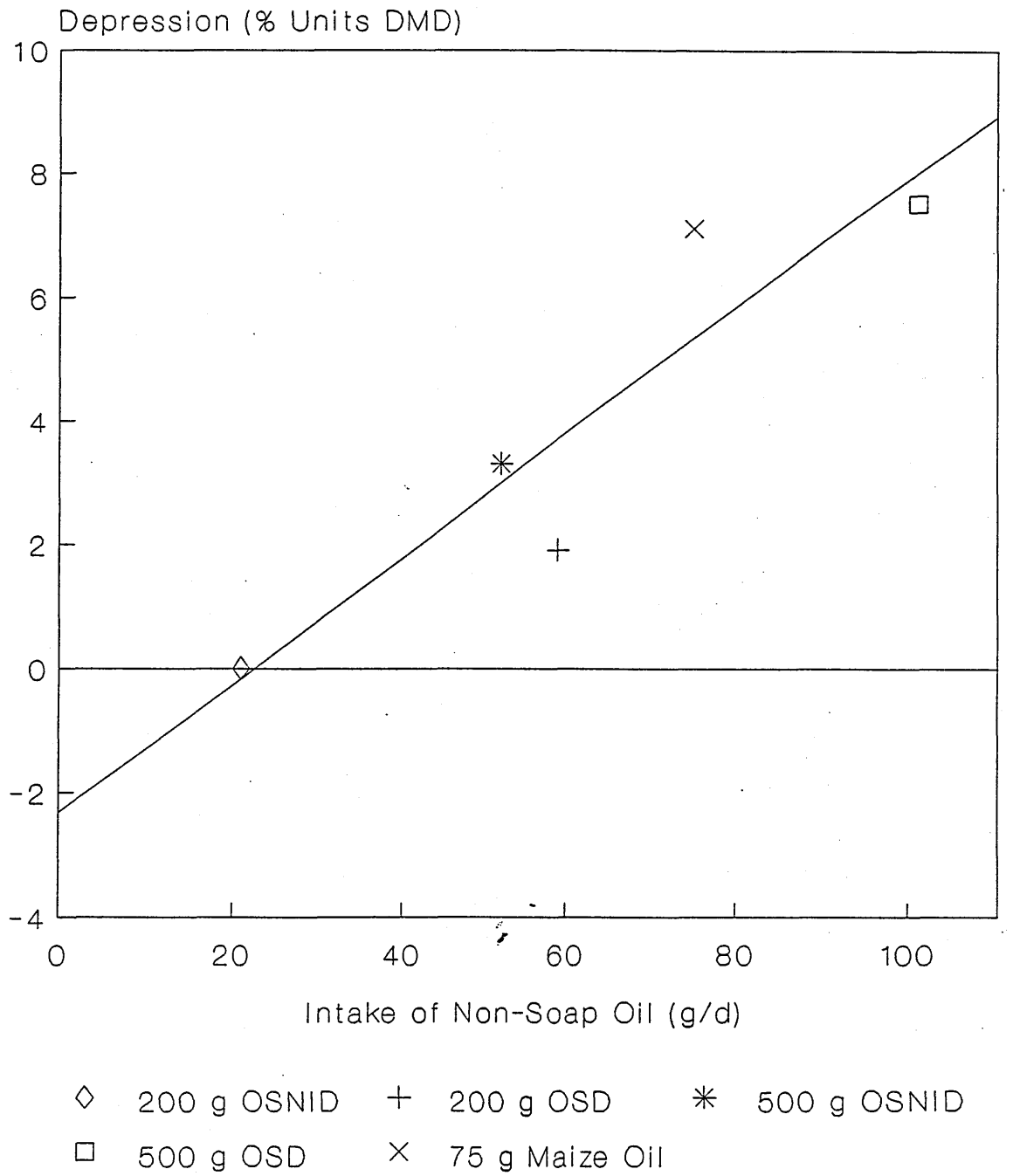
Intake	Oil (g/day)	Soap (g/day)	Non-Soap (g/day)
200g OSNID	62	41	21
200g OSD	66	7	59
500g OSNID	155	103	52
345g OSD	113	12	101

It has been shown many times that calcium soaps of fatty acids reduce the inhibitory effects of fatty acids on rumen cellulolysis (Jenkins and Palmquist, 1980, 1981, 1982). In the present experiment calcium soaps made up 10.2 and 66.7% of the total lipid in the OSD and OSNID products respectively. The sheep were consuming similar levels of non-soap oil when eating 200 g of OSD or 500 g of OSNID. Sheep receiving these diets showed similar cellulose digestibility values.

Figure 7.3 shows the relationship between intake of non-soap-fat and cotton strip DMD for three methods of oil supplementation. Included are results obtained from a parallel experiment in which the same basal diet was supplemented with 75 g/d of free maize oil given in two equal amounts directly into the rumen. There was a significant ($p<0.05$) relationship between the amount of non-soap oil in the diet and the mean depression in cotton strip DMD compared to the values obtained for the control diet.

$$\text{Depression in DMD} = -2.31 + 0.102 \times \text{mg non-soap oil} \qquad (n = 5,\ R^2 = 0.89)$$

Figure 7.3 Depression in Cotton Strip DM Digestibility with Non-Soap Oil



These results provide no evidence that the absorption of oil onto MDG reduced the inhibitory effects of oil on cellulose digestibility, but they suggest that it is the formation of calcium soaps that is more important in this respect. However, it is interesting to note that the data point for the free maize oil occurs above the regression line indicating that this treatment was more inhibitory than those to which it was compared.

Palmquist and Jenkins (1984) in a review of fat in lactation rations for cows, noted that fat may be added to diets at 3% of the total ration DM without qualification but that levels of up to 5% may be beneficial for highly productive cattle. Levels of fat in the sheep diets in this experiment were 6.2 and 12.9% of the diet DM for the low and high levels of OSNID treatments respectively and 6.6 and 10.8% diet DM for the OSD treatments. These results suggest that supplementation of diets with oil-supplemented NID may be an effective way of increasing lipid intake without compromising diet digestibility.

7.4.2.2 Rumen pH and VFA

The results shown in Table 7.9 show how the pH, molar percentages and the total concentrations of the rumen fermentation products were affected by diet. Rumen pH was significantly ($p<0.05$) increased from the control value for all oil-supplemented diets and was significantly higher (<0.05) for the higher levels of inclusion. The other rumen fermentation products were also affected by the inclusion of oil but not by $Ca(OH)_2$ treatment since differences between OSD and OSNID diets at the same level of inclusion were not significant. The main significant effects of oil supplementation were:-

1. Increased pH

2. Lower concentrations of total VFAs

3. Lower molar proportions of acetate, isobutyrate, butyrate, isovalerate and valerate and increased molar proportions of

propionate at higher levels of dietary inclusion.

Table 7.9 Mean Rumen pH and VFAs (molar % unless otherwise stated) 4 Hours After Feeding

	Control	Low OSNID	Low OSD	High OSNID	High OSD	(SE)
pH	6.20^{a}	6.30^{ab}	6.44^{bc}	6.51^{c}	6.50^{c}	(0.062)
Total VFA (mM)	81.73^{a}	60.79^{b}	62.58^{b}	67.63^{ab}	55.41^{b}	(6.079)
Ethanol	0.14	0.05	0.08	0.06	0.29	(0.70)
Acetate	72.93^{a}	71.34^{a}	71.34^{a}	64.00^{b}	64.77^{b}	(0.703)
Propionate	17.59^{a}	18.95^{a}	19.32^{a}	26.84^{b}	26.51^{b}	(0.689)
Isobutyrate	0.59^{a}	0.61^{a}	0.61^{a}	0.35^{b}	0.37^{b}	(0.049)
Butyrate	7.80^{a}	7.71^{a}	7.21^{ab}	7.31^{a}	6.52^{b}	(0.254)
Isovalerate	0.20^{a}	0.19^{a}	0.18^{a}	0.03^{b}	0.07^{b}	(0.029)
Valerate	0.75^{a}	1.15^{b}	1.26^{bc}	1.40^{bc}	1.46^{c}	(0.084)

Means in the same row with the same superscripts do not differ significantly ($p<0.05$)

The change in the proportions and the level of production of the rumen VFAs with increasing levels of unsaturated dietary oil has been noted by other workers (Shaw and Ensor, 1959; Varman *et al.* 1968;). A possible explanation for this effect is that unsaturated oils inhibit fibre digestion, thereby reducing the amounts of VFAs produced. Since the molar proportion of acetate is strongly related to the amount of dietary fibre digested in the rumen (Bath and Rook, 1963), it is envisaged that the reduction in total VFA production and change in molar proportions are largely due to a greater reduction of acetate production compared to propionate rather than an increase in production of propionate. However, in an experiment in which 250 mls

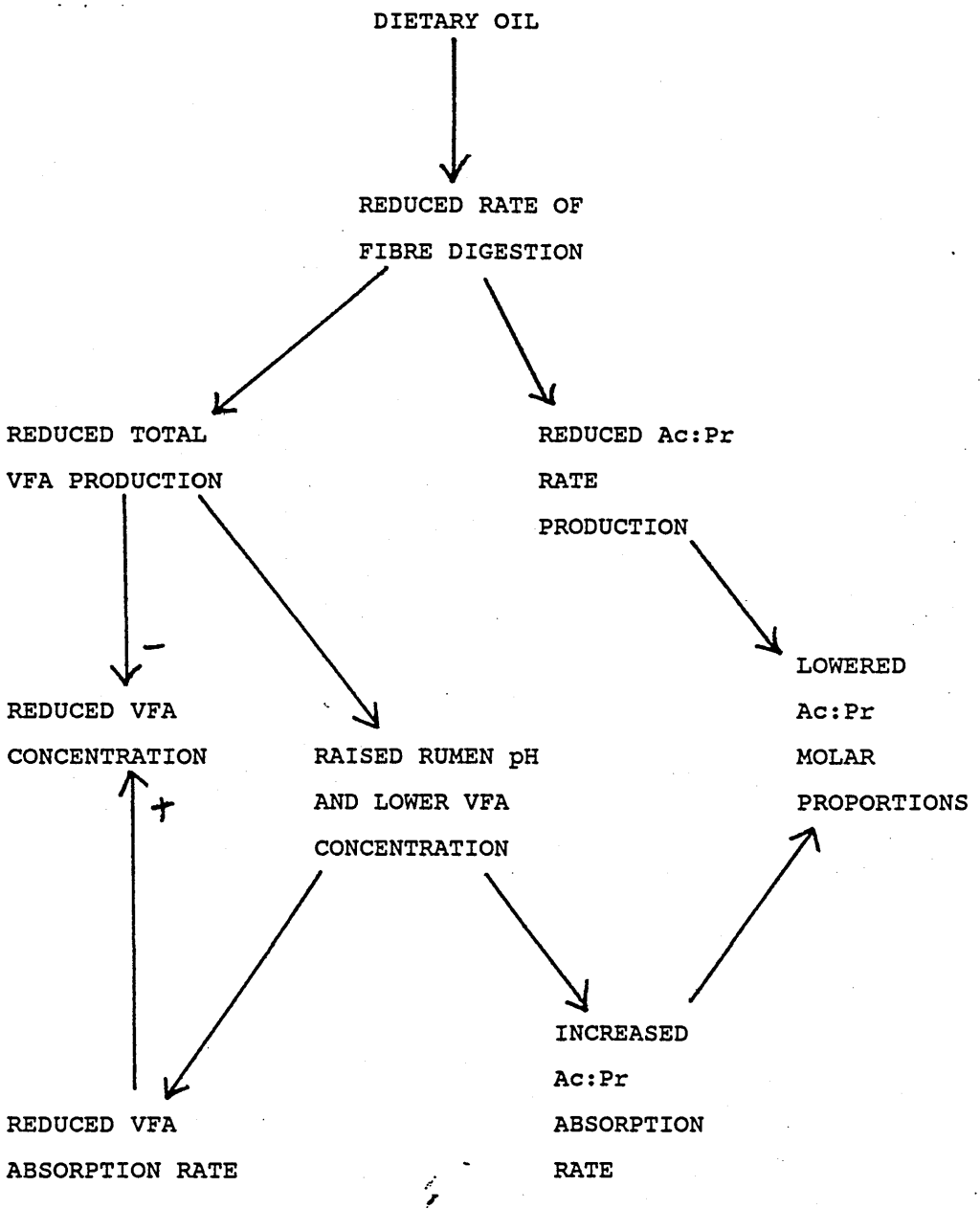
of either safflower oil or cod liver oil were fed to dairy cows (Varman *et al.*, 1968), levels of acetate in the plasma were significantly increased. Superficially, this observation is inconsistent with the generally held view that unsaturated oil depresses acetate production rates but may be explained by the indirect interactions of oil, fermentation rate, rumen pH and VFA absorption rate as discussed below.

Orskov and Ryle (1990) point out that rumen pH may affect the rates of absorption of total and individual VFAs. VFA absorption is more rapid at low rumen pH when a higher proportion of the acids are in an unionised form. At low rumen pH, propionate is more rapidly absorbed than acetate, whilst at higher pH values the reverse is true. These relationships may in part explain the observed effects of oil on rumen fermentation. Oil supplementation depresses rumen fermentation rate leading to an increase in pH which would enhance the relative rate of acetate absorption compared to propionate. This would cause a reduction in the acetate:propionate concentration ratio in the rumen.

The observed rumen fermentation characteristics for sheep fed diets containing OSNID or OSD suggest that $Ca(OH)_2$ treatment does not prevent the oil from interacting with the rumen micro-organisms. This does not support the *in vitro* findings of the previous experiment and does not explain the observed reduction of the inhibitory effects of oil on cotton strip dry matter digestibility.

The observed effects of including high levels of oil supplements on rumen fermentation may thus have occurred by either one, or more likely, by a combination of the two mechanisms described above. The interactions of the two proposed mechanisms are summarised in the following flow chart:-

The Effects of Dietary Oil on Rumen Function.



7.4.2.3 Rumen Lipids

Table 7.10 Rumen Lipid Fatty Acid Composition (% Total Lipid)

	Control	Low OSNID	Low OSD	High OSNID	High OSD	(SE)
C16:0	29.23^{c}	23.00^{b}	17.45^{a}	17.04^{a}	24.62^{bc}	(1.520)
C18:0	48.53^{b}	49.92^{b}	56.66^{c}	44.17^{a}	44.36^{a}	(1.269)
C18:1	12.40^{a}	17.66^{b}	16.82^{b}	30.03^{d}	22.26^{c}	(0.534)
C18:2	4.99^{a}	7.02^{ab}	7.48^{b}	6.77^{ab}	7.27^{ab}	(0.783)
C18:3	2.19^{a}	1.11^{b}	0.84^{b}	1.10^{b}	0.96^{b}	(0.141)
C20:4	0.80	0.62	0.50	0.16	0.19	(0.229)

Means in the same row with similar superscripts do not differ significantly ($p<0.05$).

The fatty acid profiles of the total rumen lipids are given in Table 7.10. The long chain fatty acid profile of rumen contents showed that all oil-supplemented diets gave an increase in the proportion of C18:2 indicating that biohydrogenation was incomplete. There was an increase in the proportion of C18.1 and a reduction in C18:0 when the high level of OSNID was eaten compared to other treatments. This indicates that a higher proportion of the oil included in this diet was hydrogenated only as far as the C18:1 fatty acid than for the other diets for which hydrogenation was more complete. This finding is in accord with that of Noble *et al.* (1974) who observed that the extent of hydrogenation was greater when linoleic acid was added as a substrate *in vitro* in triglyceride form, than when it was supplied as the free fatty acid. Addition of linoleic acid as the free acid decreased the proportions of the substrate hydrogenated to stearic acid and gave increased accumulation of the *trans*-11 octadecanoic acid whereas addition of the triglyceride resulted the in higher levels of stearic acid. A possible explanation for this could be the

toxicity of high levels of free fatty acids when the linoleic acid was added as such, limiting microbial hydrogenation activity, rather than lower levels which would be experienced with the progressive liberation of free fatty acids from triglycerides by lipolytic enzymes. In the $Ca(OH)_2$-treated supplements the oil would be present as the free fatty acid (or their calcium salts), whereas without $Ca(OH)_2$-treatment the oil would remain in the triglyceride form.

The results do not suggest that the formation of calcium soaps of unsaturated fatty acids protected the fatty acids from biohydrogenation in the rumen. One explanation for this observation may be that the polyunsaturated fatty acids are substantially dissociated under rumen conditions and are therefore readily hydrogenated. The mono-unsaturated (C18:1) calcium soap would remain relatively undissociated and would be hydrogenated to a lesser extent. A similar observation was noted by Palmquist (1984) who reported that there was little transfer of C18:2 into the milk fat of cows fed diets which contained high levels of calcium soaps of unsaturated fatty acids. This was explained by the higher pKa value of the unsaturated calcium soaps compared to the saturated calcium soaps. The relatively low rumen pH (<6.0) due to the feeding regime of the cows caused dissociation and therefore hydrogenation of the unsaturated but not the saturated soaps. This is in agreement with the observations of Sukhija and Palmquist (1990) who showed that the less saturated calcium soaps dissociate in rumen fluid at higher pH than saturated soaps and that significant dissociation of polyunsaturated soaps can occur at pH levels normally found in the rumen.

The source of the C18:1 in the rumen contents could either have been dietary C18:1 or it could have arisen from the partial biohydrogenation of dissociated C18:2 and C18:3 soaps. Analysis of the C18:1 from rumen lipid for *cis* and *trans* isomers would give an indication of the origin of rumen C18:1 since polyunsaturated fatty acids would be biohydrogenated via the *trans* isomer as shown on page 29. From the studies of Noble *et al.* (1974) it would be expected that the $Ca(OH)_2$-treated oil supplements would yield higher proportions of *trans* isomers than the unlimed treatments. They reported that

addition of linoleic acid as a triglyceride yielded higher proportions (approximately 30%) of the *cis* isomers whereas the free fatty acids gave larger proportions of the *trans* isomers.

Measurements of the levels of soap formation in the fat supplemented products used in the present experiment and the dry matter intakes of these products were used to calculate the amounts of oil as soap and non-soap consumed each day. These values are given in Table 7.8. There were no discernible trends in the fatty acid profiles of the rumen lipids which could be attributed to increased levels of soaps in the rumen. If soap formation was affording substantial protection against biohydrogenation then the fatty acid profile of the rumen lipids would be more similar to that of the oil supplements (ie. approximately 50% C18:2). The rumen lipid measurements suggest that polyunsaturated fatty acids are extensively biohydrogenated wether in triglyceride form or calcium soap form. The higher proportion of rumen C18:1 acid observed when 500 g of OSNID was fed suggests partial restriction of hydrogenation for this treatment. Because of the lower total oil intake of OSD, and the observations of Noble *et al.* (1974), it is not possible to argue that this was caused by calcium soap formation. The increased levels of C18:1 for the OSNID treatment may simply have been caused by the higher oil intake for this diet or the lower proportion in triglyceride form.

7.4.3 Plasma Lipids.

The results for the plasma lipids were analysed in the same way as all other variates (as shown in Appendix 17) and the same comments concerning the validity of the statistical analysis apply as before. The means and standard errors for the plasma fractions are shown in Table 7.11.

Table 7.11 The Total Lipid Content (mg/10ml) and Proportions (%) of Different Lipid Fractions in Sheep Plasma Lipids

	Control	Low OSNID	Low OSD	High OSNID	High OSD	(SE)
Cholesteryl Esters	26.15^{a}	41.41^{ab}	44.07^{b}	40.73^{ab}	42.95^{b}	(5.276)
Phospholipids	49.58	37.76	36.36	40.57	42.02	(4.604)
Triglycerides	5.89^{ab}	8.40^{ab}	9.92^{b}	8.14^{ab}	4.93^{a}	(1.265)
Free Fatty Acids	2.17	1.86	1.60	2.03	2.16	(0.436)
Free Cholesterol	16.22	10.58	8.05	7.20	7.95	(4.604)
Total Lipid	11.09^{a}	18.93^{b}	30.35^{c}	36.78^{d}	34.88^{cd}	(1.897)

Values in the same row with different superscripts differ significantly ($p<0.05$)

Table 7.11 shows that the total amount of lipid in the sheep plasma increased significantly ($p<0.05$) when oil-supplemented MDG was introduced into the diet. Generally, concentrations of total lipid were significantly higher at higher levels of inclusion. One unexplained result is the significantly lower total lipid content of plasma from sheep fed 300g OSNID compared to 300g OSD. The 300g OSNID treatment also gave intermediate results for the proportions of the different lipid fractions in the plasma, i.e. where oil supplementation changed the proportion of a particular fraction, 300g OSNID gave smaller changes than 300g OSD. These changes could have been due, in part, to the higher oil content of the OSD supplement compared to the OSNID (349 g/kg DM vs. 310 g/kg DM total oil). The effect of oil supplementation was significantly to increase ($p<0.05$) the proportion of cholesteryl esters (CE) and triglycerides (TG) and to reduce (but not significantly so) the proportions of other plasma lipid fractions.

Table 7.12 Mean Fatty Acid Composition of Plasma Lipid Fractions (% total lipid)

	Control	Low OSNID	Low OSD	High OSNID	High OSD	(SE)
Cholesteryl Esters						
C16:0	15.42^{b}	10.12^{a}	9.29^{a}	7.98^{a}	9.41^{a}	0.940
C16:1	6.81^{c}	4.93^{b}	4.96^{b}	3.14^{a}	3.56^{a}	0.220
C18:0	3.88^{b}	1.35^{a}	2.22^{a}	1.50^{a}	2.18^{a}	0.420
C18:1	41.13^{c}	32.07^{b}	33.48^{b}	22.91^{a}	26.73^{ab}	1.899
C18:2	28.89^{a}	43.50^{bc}	41.43^{b}	56.02^{c}	51.20^{bc}	2.873
C18:3	2.51	2.12	1.89	0.61	1.59	0.769
C20:4	1.37	1.60	1.41	2.59	1.51	0.768
Phospholipids						
C16:0	19.46^{c}	17.11^{b}	17.12^{b}	14.27^{a}	16.99^{b}	0.739
C16:1	3.81^{b}	0.69^{a}	0.44^{a}	0.78^{a}	0.78^{a}	0.586
C18:0	26.55^{b}	26.90^{b}	27.86^{b}	21.97^{a}	25.82^{b}	0.953
C18:1	22.28^{a}	23.78^{a}	23.09^{a}	29.01^{b}	22.32^{a}	0.791
C18:2	10.46^{a}	20.10^{b}	19.52^{b}	28.06^{c}	25.68^{c}	1.243
C18:3	2.13^{c}	1.81^{bc}	1.55^{bc}	0.68^{a}	1.28^{ab}	0.257
C20:3	2.47^{ab}	2.86^{b}	2.31^{ab}	1.07^{a}	1.21^{ab}	0.542
C20:4	6.72^{c}	5.39^{bc}	4.67^{b}	3.06^{a}	4.51^{b}	0.463
C22:5	3.19^{b}	1.04^{a}	1.67^{a}	0.83^{a}	0.86^{a}	0.409
C22:6	1.68	0.33	1.67	0.15	0.78	0.623
Triglycerides						
C16:0	28.85^{b}	26.91^{ab}	23.53^{ab}	21.70^{a}	29.84^{b}	2.106
C18:0	36.30^{ab}	41.85^{bc}	45.79^{bc}	29.67^{a}	40.56^{bc}	2.259
C18:1	19.20^{a}	25.53^{b}	23.72^{b}	41.19^{c}	23.91^{b}	1.454
C18:2	2.29^{a}	2.88^{ab}	2.73^{ab}	6.49^{c}	4.33^{b}	0.534
C18:3	0.12^{a}	0.47^{ab}	0.66^{b}	0.95^{b}	0.36^{ab}	0.157
Free Fatty Acids						
C16:0	35.97^{b}	31.13^{b}	25.50^{ab}	19.09^{a}	23.44^{a}	2.178
C18:0	31.73^{a}	36.15^{ab}	42.04^{a}	34.24^{ab}	38.23^{b}	1.774
C18:1	24.87^{a}	25.24^{ab}	26.19^{ab}	35.62^{b}	31.55^{b}	2.160
C18:2	4.51^{a}	4.94^{a}	4.94^{a}	6.66^{b}	4.31^{a}	0.324
C18:3	0.39	0.54	0.42	0.05	0.94	0.214
Proportion Saturated						
CE	19.12^{a}	11.41^{b}	11.52^{b}	9.48^{b}	11.59^{b}	1.167
PL	46.01^{a}	44.00^{a}	44.98^{a}	36.24^{b}	42.81^{a}	1.448
TG	65.15^{a}	68.76^{a}	69.32^{a}	51.38^{b}	70.40^{a}	2.598
FFA	67.75^{a}	67.28^{a}	67.54^{a}	53.34^{b}	61.68^{a}	2.649

Values in the same row in with similar superscripts do not differ significantly ($p<0.05$)

The mean percentage of saturated fatty acids in each fraction is given at the bottom of Table 7.12 This shows that the main effect of including maize oil in the diet was to reduce the degree of saturation of the plasma lipids. The most significant effects occurred when the sheep were fed high levels of OSNID. For this treatment, the degree of saturation of all fractions, except the CE, was significantly ($p<0.05$) lower. The fatty acid profiles for the separate plasma lipid fractions shows the cause of this effect. The 500g OSNID treatment gave the least C18:0 and the most C18:2 in all fractions. C18:1 levels for this treatment were lowest in the CE but were highest in the phospholipids (PL), free fatty acids (FFA) and TG. Generally, the effects on the higher fatty acids between treatments for the different plasma lipid fractions were small and variable.

The effect of feeding protected polyunsaturated fats to ruminant animals on the plasma fatty acid levels has been reviewed by Christie (1981). He noted that in experiments in which protected unsaturated fatty acids were fed, the polyunsaturated nature of all lipid fractions was increased. When dietary fat was 'unprotected' some escaped biohydrogenation, but little if any appeared in the plasma TG fraction since the unsaturated components were selectively taken up by the CE and PL fractions. This was explained by the high metabolic activity and therefore rapid rate of turnover of the TG fraction.

In an experiment in which maize oil or linoleic acid were infused directly into the rumen of sheep (Noble *et al.*, 1969) it was observed that infusion of maize oil resulted in increased concentrations of C18:0 in the plasma TG, whereas infusion of linoleic acid increased the C18:1. When sheep were fed the high OSNID diet, levels of C18:1 were increased in all plasma lipid classes except in the CE suggesting that the oil in the OSNID was present as the free fatty acids rather than the triglyceride. This concurs with the rumen lipid data.

These studies of the effects of feeding OSD and OSNID to sheep showed that there were significant changes towards reduced saturation of plasma lipids. The 500g OSNID treatment gave significantly increased levels of C18:2 in the TG and FFA fractions of the plasma lipids, and

increases in the proportions of unsaturated long chain fatty acids in all fractions were observed. The distribution of these changes were proportionally greater in the TG and FFA fractions. Polyunsaturated acids which escape ruminal biohydrogenation are preferentially esterified to the PL and the CE fractions which are turned over relatively slowly, rather than the TG and the FFA fractions which are turned over more rapidly. It is the latter fractions which are most metabolically active and which supply fatty acids to other tissues in the body such as the adipose tissues and mammary glands. (see Christie, 1981).

Thus the observed reductions in saturation of the TG and FFA fraction in the present experiment suggest that OSNID may serve as an effective means of manipulating the fatty acid composition of ruminant fat. The experiment suggests that conversion of dietary polyunsaturated fatty acids to calcium soaps is not a highly effective method for protection from rumen biohydrogenation. Nevertheless, the experiments do provide evidence that high levels of unsaturated fat may be fed as OSNID without serious inhibition of rumen function and that potential benefits may result.

To investigate whether the increases in plasma unsaturated fatty acids observed in the feeding experiment would affect the composition of the milk fat an experiment in which the diets of lactating goats were supplemented with a similar OSNID was designed. In this experiment six lactating goats were fed diets with an approximately 70:30 concentrate:forage ratio. The concentrate mixtures included fat supplements so that the total level of oil in the was approximately 160 g/kg DM. The fat was included either as a proprietary protected fat supplement or as the $Ca(OH)_2$-treated oil supplemented draff product. These were compared to a basal low oil concentrate mixture. The fatty acid profiles of milk and plasma were measured.

7.5 The Effect of Feeding $Ca(OH)_2$-Treated Oil Supplemented MDG on Plasma and Milk Fatty Acid Content of Lactating Goats.

7.5.1 Method and Materials

The experiment was a 3 x 3 latin square design using three pairs of lactating feral goats with kids at foot. The goats were taken from the SAC hill farm at Kirkton, Crianlarich approximately one month after kidding. The mothers were approximately 35 kg liveweight and were randomly assigned to three treatment groups each receiving different concentrate mixtures; control (C), proprietary fat supplement (Megalac; Volac Ltd) (M) and $Ca(OH)_2$ treated oil supplemented draff (40% oil on a DM basis) (O). The goats were offered 400g (DM) twice daily of the concentrate mixtures given in Table 7.13.

Table 7.13 Composition of Concentrate Mixtures Offered to Goats.

Dry Matter Composition (g/kg mix)			
Diet	C	M	O
Flaked Maize	245	240	232
Molassed Sugar Beet Pulp	245	240	232
Rolled Barley	343	192	
White Fishmeal	51	50	49
Soya Bean Meal	100	98	95
Megalac		164	
OSNID (40% fat)			359
Minerals			
Scotmin Sheep Minerals	14	14	
Mineral mix			32

The OSNID was prepared as described in Section 7.3.1 except that soya acid oil was substituted for maize oil. On a fresh weight basis, 150 g

of soya acid oil (Intermol Ltd, Hull) was added to 1 kg of MDG and treated with 40 g of $Ca(OH)_2$. Soya acid oil was had a similar fatty acid content to MDG oil and maize oil used in the previous experiment as shown in Table 7.14. The oil, fibre and ash contents of the dried OSNID supplement were measured to be 391, 80 and 110 g/kg DM.

Table 7.14 Fatty Acid Composition of Soya Acid Oil and MDG Oil.

Fatty Acid (% total fat)	MDG Oil	Soya Acid Oil
C14:0		1.0
C16:0	22.5	12.0
C18:0	1.7	4.0
C16:1	1.4	
C18:1	21.5	27.0
C18:2	48.6	50.0
C18:3	4.8	6.0

The minerals included in diet O were made up from the following mixture (g/kg DM):-

Monodical	550
Magnesium Phosphate	300
Sodium Chloride	100
TE + Vits	50

The special mineral mixture for diet O was designed to supply the requirements of the goats and to balance the high levels of calcium in the ration. The calcium to phosphorus ratio in the final product was 2.9:1.

The goats were penned individually and were bedded on straw. They were offered 800g DM/d of the concentrate mixture in two equal feeds at 08.30 and 16.30h with 300g/d of hay. When the goats were fed the kids were restrained for 30 minutes to allow the mothers to eat all the concentrate. Any supplement refusals after the thirty minutes were removed and the dry weight consumed was recorded. Dry matter intakes of hay were not measured.

Each period of the experiment lasted 14 days. On day 14, the goats were blood sampled by venepuncture of the jugular vein. Blood samples were taken into 10 ml heparinised evacuated blood tubes. Five blood samples were taken, one before the morning feed and the other four at 2 hourly intervals thereafter (the evening feed was delayed until the final blood sample had been taken). The fatty acids content of the concentrate feeds and of the plasma samples was determined as described in Section 7.3.2.

On days 12, 13, and 14 of each period, milk samples were taken from the goats. This was done by restraining the kids as usual at feeding time, stripping any milk from the mothers and then milking them out again an hour later into plastic sample containers. No attempt was made to measure the volume of milk since this could not be accurately related to the actual milk yield of the goats.

A 10 ml subsample of milk from each day's milking was added to a separate container containing a Lactab milk preservative pellet and was shaken well. A further 10 mls was kept in a separate container without preservative. All samples were stored in the refrigerator and on day 14 milk samples were bulked to give 30 mls of preserved and 30 mls of untreated milk sample from each goat.

Towards the end of the third period, by which time some initial statistical analysis of the measurements of concentrations of plasma lipids from the first two periods had been completed, it was evident that the oil in the 40% OSNID was reducing plasma lipid levels. A further period at the end of the experiment was therefore introduced using a the fat source of different formulation containing

approximately 20% oil DM. The lower oil supplement mixture (L) was made in the same manner as supplement O containing OSNID. The analysis of the concentrate mixture was (g/kg DM) 90 oil, 80 fibre and 104 ash). The fourth period was carried out in the same manner as the previous three periods, with all goats receiving the concentrate mixture containing the 20% oil OSNID. Plasma and milk samples were taken at the end of the period.

Statistical Analysis

The values measured for the constituents of milk, the milk fat fatty acid profiles and all plasma lipid data were analysed using Genstat V. Examples of each analysis are given in Appendix 19.

7.5.2 Results and Discussion

7.5.2.1 Analysis of Concentrate Mixtures

The fatty acid analysis of the feeds is shown in Table 7.15.

The total soap formation was calculated from the difference between the two methods of extraction adjusted for the soap extracted by ether calculated from measurements of the calcium present in the ether extracted oil. This showed that the percentage of total oil saponified was 29.8, 86.5, 45.3 and 47.3 for the supplement mixtures C, M, O and L respectively. Thus soap formation for 20% and 40% oil OSNID supplements was only partially achieved and protection was not increased at lower levels of oil supplementation as may have been anticipated. The reasons for the reduced level of soap formation compared to that achieved in the previous experiment when maize oil was used (67%) was unexplained. One possibility may have been the acidic nature of the soya acid oil, however this was unlikely since from the original calculation, sufficient $Ca(OH)_2$ was used to give alkaline conditions (< pH 11) to upgrade the MDG fibre and increase its absorbtive capacity. Optimisation of level of $Ca(OH)_2$ for maximal soap formation with different sources of oil represents an area which would require more study if oil-supplemented draff products were to be

developed further.

Table 7.15 Total Lipid Concentration and Fatty Acid Composition of the Concentrate Mixtures (% Total Lipid)

	Concentrate Mixtures								%
Fatty	C	M	O	L	C	M	O	L	Extracted
Acid	Ether Extract				Acidified Extract				in Ether
C12:0	-	1.42	0.33	0.44	0.95	0.56	0.08	0.54	44.3
C14:0	0.72	0.62	0.26	0.33	0.68	1.21	0.41	0.33	39.5
C16:0	17.24	19.54	11.20	14.94	27.81	46.25	16.41	16.80	32.8
C16:1	0.63	0.39	0.52	0.62	0.58	0.16	0.60	0.27	60.0
C18:0	3.26	2.90	3.62	3.53	3.79	4.09	4.45	3.65	40.3
C18:1	28.22	35.26	22.87	21.56	23.37	32.28	21.87	18.99	51.3
C18:2	45.65	34.94	51.13	50.29	39.64	13.41	48.01	48.66	54.8
C18:3	3.18	3.74	4.51	4.92	2.87	0.86	4.27	4.59	59.8
C22:6	-	0.35	-	0.33	-	0.14	-	-	34.5
Total Fat (g/kg DM)	11.0	22.3	97.6	55.3	22.3	161.4	158.9	93.5	

C = Control

M = Megalac

O = 40% fat Oil Supplemented NID

L = 20% fat Low Oil Supplemented NID

The analysis of the lipids extracted from the concentrate feeds by the two different extraction procedures shown in Table 7.15 shows the differential soap formation between the different fatty acids. The proportion of each fatty acids extracted by 40°C - 60°C petroleum ether tended to increase with chain length and degree of unsaturation. This trend was similar for both the M, O and L supplements suggesting that the saturated and short-chain fatty acids are preferentially saponified by $Ca(OH)_2$ as Ca soaps are only poorly extracted by this solvent. The total fatty acid composition of the concentrate mixtures

are those measured following acid hydrolysis. Intakes of fat from the dietary supplements for each treatment (g/d) 20, 114, 114 and 65 for diets C, M, O and L respectively. Intakes of non-soap oil were 14, 15, 62 and 34 respectively.

7.5.2.2 Plasma Lipid Constituents

Table 7.16 Concentration of Plasma Total Lipid and Proportions of Lipid Fractions

	C	M	O	L	(SED)
Total Lipid (mg/10ml)	40.3	36.8	34.2	38.8	3.62
Proportions (%)					
Cholesteryl Esters	43.60^{a}	45.67^{ab}	46.04^{b}	45.98^{ab}	1.112
Triglycerides	4.68^{ab}	5.50^{b}	5.44^{ab}	4.56^{a}	0.411
Free Fatty Acids	2.05^{a}	1.81^{ab}	1.84^{ab}	1.31^{b}	0.217
Free Cholesterol	8.96^{b}	7.85^{a}	8.06^{ab}	8.47^{ab}	0.420
Phospholipids	40.77^{b}	39.20^{ab}	38.63^{a}	39.59^{ab}	0.871

Values in the same row with common superscripts do not differ significantly ($p<0.05$).

Statistical analysis of the results for plasma for the 3 x 3 latin square design, showed that there was no significant ($p < 0.05$) effect of period on plasma lipid proportions or concentrations. The final period was therefore included with the other data from the first three periods despite the unbalanced design of the experiment. Similarly, the effect of sampling time was not significant and the values reported are means of the five sampling times. The lack of response of plasma lipids to time of sampling was due to the animals having received the diet for 13 days prior to sampling. If the goats had been maintained on a basal, low fat diet and had then received just

one meal of the fat supplemented concentrate then the effect of sampling time would have been greater (Noble, 1990, personal communication) as equilibration would not have been achieved.

Concentrations of the total lipid in plasma were not significantly ($p<0.05$) affected by treatment although values tended to be lower for the diets providing the most oil. The results of the plasma analyses given in Table 7.16 show that there were significant differences in the distribution of lipid between the different fractions. When the goats were fed diet C, the proportions of CE were lower and of FFA, free cholesterol (FC) and PL were higher than when the goats were offered the fat-supplemented diets.

This observation was unexplained and could not be supported by similar findings by other workers reporting the effects of dietary fat supplementation on plasma lipids. Increases in the concentrations of total fat, TG, FC and FFA in the plasma have been reported consistently when dairy cow diets were supplemented with protected saturated lipids. (for example, Bines *et al.* 1978; Kronfeld *et al.* 1980). Typical increases in levels of plasma lipids were: (g/l per kg of protected tallow) 2-3 g total lipid, 0.1-0.2 g TG, 1.0-2.5 g FC and 0.02-0.06 g FFA. Although proportions of TG in the total plasma lipid were increased in the present experiment when the goats received the supplements M and O, the values were not significantly greater than for the control. Also as total lipid concentrations were lower for treatments M and O than for C, there was little effect on the absolute concentrations of plasma TG (1.9, 2.0, 1.9, and 1.8 mg/10ml for C, M, O and L respectively).

Concentrations of FC were highest when the goats were fed the control diets (3.6, 2.9, 2.8 and 3.3 for C, M, O and L respectively). Fat supplementation leads to increased amounts of fatty acids absorbed from the intestine and transported around the body and would be expected to increase levels of plasma cholesterol as reported by many authors (for references see McDonald and Scott, 1977). In an experiment in which lactating goats were fed safflower oil protected with formaldehyde-treated casein, Mills *et al.* (1974), showed that

plasma cholesterol levels were approximately doubled. However in the present study, levels of cholesterol both in absolute concentration and as a proportion of the total plasma lipid tended to be highest for the control diet for which the oil intake was least.

The proportions of the plasma lipid classes in ruminant animals is affected by age, pregnacy and the stage of lactation and in most instances it is the FFA proportion which is most greatly affected (see Christie, 1981). Moore *et al.* (1969) observed a positive curvilinear relationship between the concentrations of FFA in the plasma and the yield of total milk fat in lactating dairy cows. This finding is contrary to what would be expected (see Moore and Christie, 1981) since the plasma TG are the fraction that make the greatest contribution to milk fat. The findings of the present experiment were also contrary to this observation, since the highest levels of FFA (both in concentration and proportion) were observed when milk-fat percentage was lowest (on the control diet). Plasma TG observed in this experiment were of the same order as those observed in the lactating goat by Annison *et al.* (1967) who reported levels of approximately 20mg/100 ml of plasma or 5 g/100g of total plasma lipid.

The reasons for the depressed levels of circulating plasma lipids for the oil-supplemented diets are not known, however it could be linked to the low milk fat syndrome which would have reduced yields of milk fat. Feeding fat supplements in such circumstances may have stimulated increased extraction of lipid from the blood either into the milk or into adipose tissue. Levels of fat in the milk were significantly higher in the dietary fat supplemented treatments (See section 7.4.2.3). However, no measurements were made of the yield of milk or milk fat or of the deposition of adipose tissue, and it is only possible to speculate about the reasons for the observed results.

Tables 7.16 and 7.17 show the fatty acid composition of the main plasma lipid fractions for the four treatments expressed as a percentage of the total lipid and as absolute concentration (mg/l). Although variation between the lipid compositions of the individual samples was high, many significant differences due to diet were

Table 7.16 Mean Fatty Acid Compositions of the Different Plasma Lipid Classes (% total lipid)

	C	M	O	L	(SED)
Cholesteryl Esters					
C16:0	15.02^{b}	16.51^{c}	10.45^{a}	10.71^{a}	0.645
C16:1	9.53^{d}	5.39^{a}	7.29^{c}	6.43^{b}	0.265
C18:0	6.50	4.75	5.48	5.88	0.914
C18:1	32.78^{b}	31.96^{b}	29.25^{a}	28.74^{a}	0.651
C18:2	32.34^{a}	38.74^{b}	42.95^{c}	44.51^{c}	1.134
C18:3	1.72^{b}	1.06^{a}	2.91^{c}	1.89^{b}	0.190
C20:3	0.08	0.06	0.01	0.06	0.048
C20:4	1.60^{b}	1.02^{a}	1.04^{a}	1.54^{b}	0.089
C22:5	0.26	0.07	0.48	0.02	0.234
C22:6	0.14	0.57	0.16	0.19	0.237
Phospholipids					
C16:0	19.94^{b}	22.10^{c}	15.56^{a}	16.29^{a}	0.407
C16:1	0.73^{a}	0.04^{b}	0.09^{b}	0.08^{b}	0.190
C18:0	25.10^{c}	21.89^{b}	20.75^{a}	24.49^{c}	0.331
C18:1	23.50^{a}	23.34^{a}	29.86^{b}	23.84^{a}	0.394
C18:2	20.72^{a}	25.35^{b}	26.96^{c}	26.75^{c}	0.317
C18:3	1.75^{b}	0.94^{a}	1.83^{b}	1.62^{b}	0.099
C20:3	0.59^{b}	0.78^{c}	0.32^{a}	0.47^{ab}	0.078
C20:4	4.60^{d}	3.35^{b}	2.65^{a}	4.09^{c}	0.157
C22:5	1.62^{b}	1.13^{a}	1.05^{a}	1.20^{a}	0.096
C22:6	1.45^{b}	1.08^{a}	1.09^{a}	1.10^{a}	0.096
Triglycerides					
C16:0	32.72^{b}	42.35^{c}	22.68^{a}	24.11^{a}	1.018
C18:0	29.97^{b}	19.88^{a}	21.57^{a}	30.93^{b}	1.016
C18:1	28.21^{a}	31.14^{b}	43.61^{d}	34.09^{c}	0.953
C18:2	4.17^{a}	4.31^{a}	7.42^{b}	7.01^{b}	0.554
C18:3	0.96^{b}	0.53^{a}	2.72^{d}	1.18^{c}	0.193
C20:3	1.10^{c}	0.47^{b}	0.15^{a}	0.50^{b}	0.098
C20:4	0.96^{b}	0.49^{a}	0.61^{a}	1.08^{b}	0.126
C22:5	0.69^{b}	0.30^{a}	0.61^{b}	0.47^{ab}	0.130
C22:6	0.97^{b}	0.41^{a}	0.63^{ab}	0.61^{ab}	0.165
Free Fatty Acids					
C16:0	23.31^{b}	26.05^{c}	16.75^{a}	18.66^{a}	1.154
C18:0	29.71^{b}	24.94^{a}	24.46^{a}	30.66^{b}	1.119
C18:1	29.03^{a}	33.41^{b}	40.35^{c}	32.02^{b}	0.911
C18:2	10.97^{a}	12.15^{ab}	11.89^{a}	14.04^{b}	0.984
C18:3	1.64^{a}	1.11^{a}	2.85^{b}	1.53^{a}	0.345
C20:3	0.12	0.05	0.14	0.15	0.109
C20:4	2.21	1.28	1.45	1.63	0.431
C22:5	0.64	0.44	0.50	0.38	0.161
C22:6	2.17^{a}	1.67^{b}	1.61^{b}	0.99^{b}	0.442

Means with common superscripts in the same row do not differ significantly ($p<0.05$).

Table 7.17 Mean Concentrations of Plasma Lipids (mg/l)

	C	M	O	L	(SED)
Cholesteryl Esters					
C16:0	271.7^{a}	274.8^{a}	161.5^{b}	190.9^{b}	25.48
C16:1	178.3^{a}	88.9^{b}	113.3^{b}	113.3^{b}	16.09
C18:0	137.4^{a}	80.7^{b}	86.9^{ab}	109.1^{ab}	23.79
C18:1	583.2^{a}	535.0^{ab}	456.0^{b}	507.0^{ab}	50.53
C18:2	552.1^{a}	651.3^{a}	672.0^{a}	809.0^{b}	61.92
C18:3	30.8^{b}	18.1^{a}	47.1^{c}	35.4^{b}	4.87
C20:3	1.7	0.9	0.2	1.1	0.79
C20:4	25.2^{b}	17.0^{a}	16.4^{a}	28.5^{b}	2.57
C22:5	2.7	1.5	6.8	0.5	3.23
C22:6	1.5	4.7	2.2	3.4	2.08
Phospholipids					
C16:0	320.1^{a}	319.2^{a}	205.1^{b}	248.3^{b}	29.34
C16:1	11.1^{a}	0.6^{b}	1.6^{b}	1.0^{b}	3.36
C18:0	408.0^{c}	315.2^{ab}	278.4^{a}	376.2^{bc}	39.48
C18:1	388.4	339.2	393.3	359.1	37.70
C18:2	336.9	368.3	355.8	412.1	36.93
C18:3	27.2^{b}	13.5^{a}	24.4^{b}	24.8^{b}	2.87
C20:3	9.0^{bc}	11.3^{c}	4.2^{a}	7.3^{b}	1.32
C20:4	72.1^{b}	47.5^{a}	35.7^{a}	62.8^{b}	6.67
C22:5	23.6^{c}	16.6^{ab}	14.3^{a}	18.4^{b}	2.28
C22:6	20.8^{b}	15.5^{a}	14.7^{a}	16.9^{ab}	2.25
Triglycerides					
C16:0	57.3^{a}	85.7^{b}	42.7^{a}	41.5^{a}	7.39
C18:0	52.8^{b}	38.2^{a}	42.3^{ab}	53.8^{b}	6.03
C18:1	50.8^{a}	62.9^{a}	84.2^{b}	59.2^{a}	8.52
C18:2	7.6^{a}	8.7^{ab}	12.9^{b}	11.5^{b}	1.47
C18:3	1.7^{a}	1.1^{a}	5.2^{b}	1.9^{a}	0.50
C20:3	1.8^{c}	0.9^{b}	0.3^{a}	0.9^{ab}	0.25
C20:4	1.7^{ab}	0.9^{a}	1.2^{ab}	1.7^{b}	0.36
C22:5	0.8^{ab}	0.5^{a}	1.1^{b}	0.9^{ab}	0.25
C22:6	1.5^{a}	0.8^{b}	1.0^{b}	1.0^{ab}	0.25
Free Fatty Acids					
C16:0	17.3^{a}	17.0^{a}	10.1^{b}	8.9^{b}	2.29
C18:0	23.1^{b}	16.5^{ab}	15.6^{a}	15.5^{a}	3.11
C18:1	22.8^{ab}	22.2^{ab}	26.1^{b}	15.8^{a}	3.32
C18:2	8.3	8.2	7.9	7.0	1.39
C18:3	1.2^{a}	0.7^{a}	1.8^{b}	0.8^{a}	0.27
C20:3	0.09	0.04	0.05	0.08	0.06
C20:4	1.53	0.91	0.95	0.93	0.32
C22:5	0.45	0.36	0.46	0.27	0.17
C22.6	1.69^{a}	1.16^{b}	1.19^{b}	0.63^{b}	0.44

Means with common superscripts in the same row do not differ significantly ($p<0.05$).

identified. The main effects of oil supplementation of the diet on plasma fatty acid composition (ie. compared to the values for diet C) were:-

1. Diets O and L (OSNID) reduced both the proportion and the concentration of C16:0 in all fractions compared to C or M.

2. The oil-supplemented diets (M, O and L) increased the proportion of C18:1 in all fractions except CE. The effect was greatest for diet O.

3. Diet O (40% oil OSNID) increased the absolute concentrations of C18:1 in the TG fraction compared to the other diets.

4. Diets O and L (OSNID) increased the proportion of C18:2 and C18:3 in all lipid fractions. The effect was greatest for C18:2 in the TG fraction, for which absolute concentration was increased.

5. Diet O (40% oil OSNID) increased the concentrations of C18:3 in all lipid fractions.

6. Diet M (Megalac) reduced both the concentrations and proportions of C18:3 in all plasma fractions.

In absolute terms, the majority of polyunsaturated fatty acids which escaped ruminal biohydrogenation when the OSNID diets were fed appeared in the CE and PL fractions. This is in accord with the mechanism thought to be responsible for conserving polyunsaturated fatty acids in ruminants mediated via a lecithin cholesteryl acyl transferase enzyme system known to exist in the plasma (Noble, 1978).

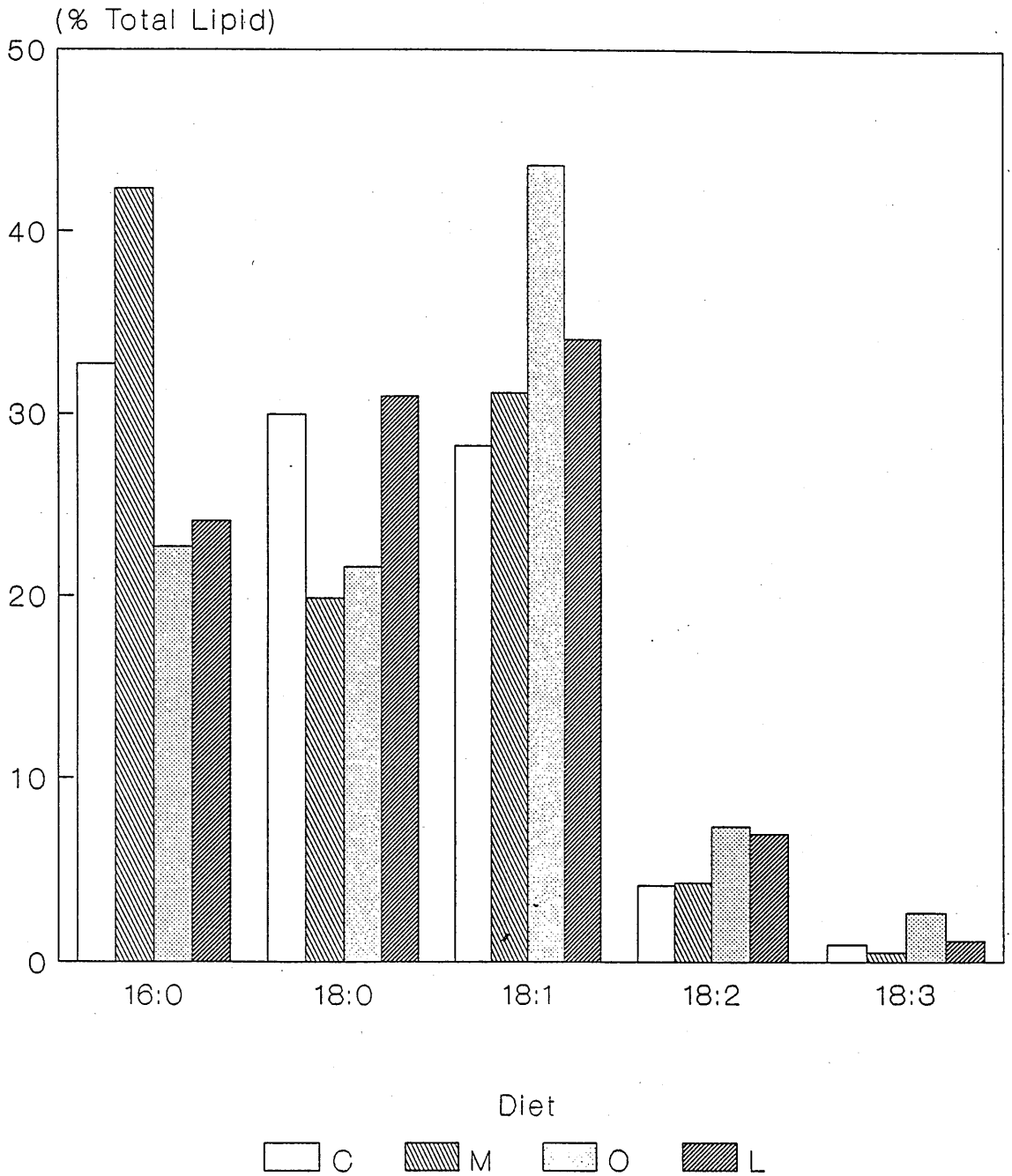
The changes in the profiles of the different lipid fractions are not as great as those seen in experiments when polyunsaturated fatty acids protected with formaldehyde treated casein were fed. In these studies the polyunsaturated fatty acid content of each lipid class was increased, particularly the TG fraction (for references see Christie, 1981). Even when unprotected lipids were fed, those fatty acids

escaping biohydrogenation in the rumen can still produce significant changes in the degree of saturation of the different lipid fractions although the largest changes occur in the CE and PL fractions (Scott *et al.*, 1971; Marchello *et al.*, 1972; Noble *et al.*, 1977).

Cook *et al.* (1972) fed unprotected and formaldehyde-treated-casein protected safflower oil to lactating dairy cows. They observed increased proportions of C18:2 with the protected supplement and increased C18:1 with the unprotected supplement in both plasma triglycerides and milk fat. Levels of C18:2 in the plasma triglycerides were increased from approximately 5% to 30% of total lipid by weight when the protected supplement was fed and to approximately 8% with the unprotected supplement. As shown in Figure 7.4; the increase in C18:2 in plasma TG in the present experiment (from approximately 4% to 7%) was of the same order as observed for the unprotected supplement.

The pattern of response of the plasma lipid composition observed in the present experiment suggests that the degree of protection provided by $Ca(OH)_2$-treatment was limited. This effect was similar at both levels of oil supplementation (diets O and L). This could have been because the soap formation was only partial or because, as discussed in section 7.4.3, conversion to calcium soaps does not afford protection of polyunsaturated fatty acids from rumen biohydrogenation.

Figure 7.4 Plasma Triglyceride
Fatty Acid Composition



7.5.2.3 Milk Constituents

Table 7.18 Effect of Diet on Mean Milk Constituents (%)

	C	M	O	L	(SE)
Fat	1.96^{a}	3.62^{c}	2.55^{ab}	3.18^{bc}	0.213
Protein	3.29^{b}	3.04^{a}	3.48^{b}	3.30^{b}	0.078
Lactose	4.80^{b}	4.90^{b}	4.94^{b}	4.58^{a}	0.071

Means in the same row with the same superscript do not differ significantly p(0.05).

The constituents measured in the goat milk are shown in Table 7.18. The levels of milk protein and lactose measured were within the normal range for goats, however, values for the fat percentage are low for all treatments. When the goats were fed the control diet the milk fat percentages were significantly lower than when fed the fat supplemented treatments. The results for the milk fat determination suggest that the goats were affected by low milk fat syndrome.

Low milk-fat syndrome (Storry *et al.*, 1974) is thought to be caused by the effects of a high concentrate, low roughage diet causing a shift in rumen fermentation towards a low acetate:propionate ratio. Depression in milk fat content is thought to be mediated via two mechanisms; reduced acetate and butyrate for *de novo* milk fat synthesis in the mammary gland, and indirectly by an insulin-mediated glucogenic response resulting from increased propionate uptake. The latter causes adipose tissue to compete more strongly with the mammary gland for lipogenic substrate. Thus adipose tissue takes up and esterifies increased amounts of dietary lipid and decreases the mobilisation of adipose lipids for milk fat synthesis. Feeding protected fats has been shown to alleviate this condition (Storry *et al.*, 1974) by providing sufficient lipid in the blood to meet the increased demands of the adipose tissue and also to compensate for the diminished intramammary synthesis of fatty acids.

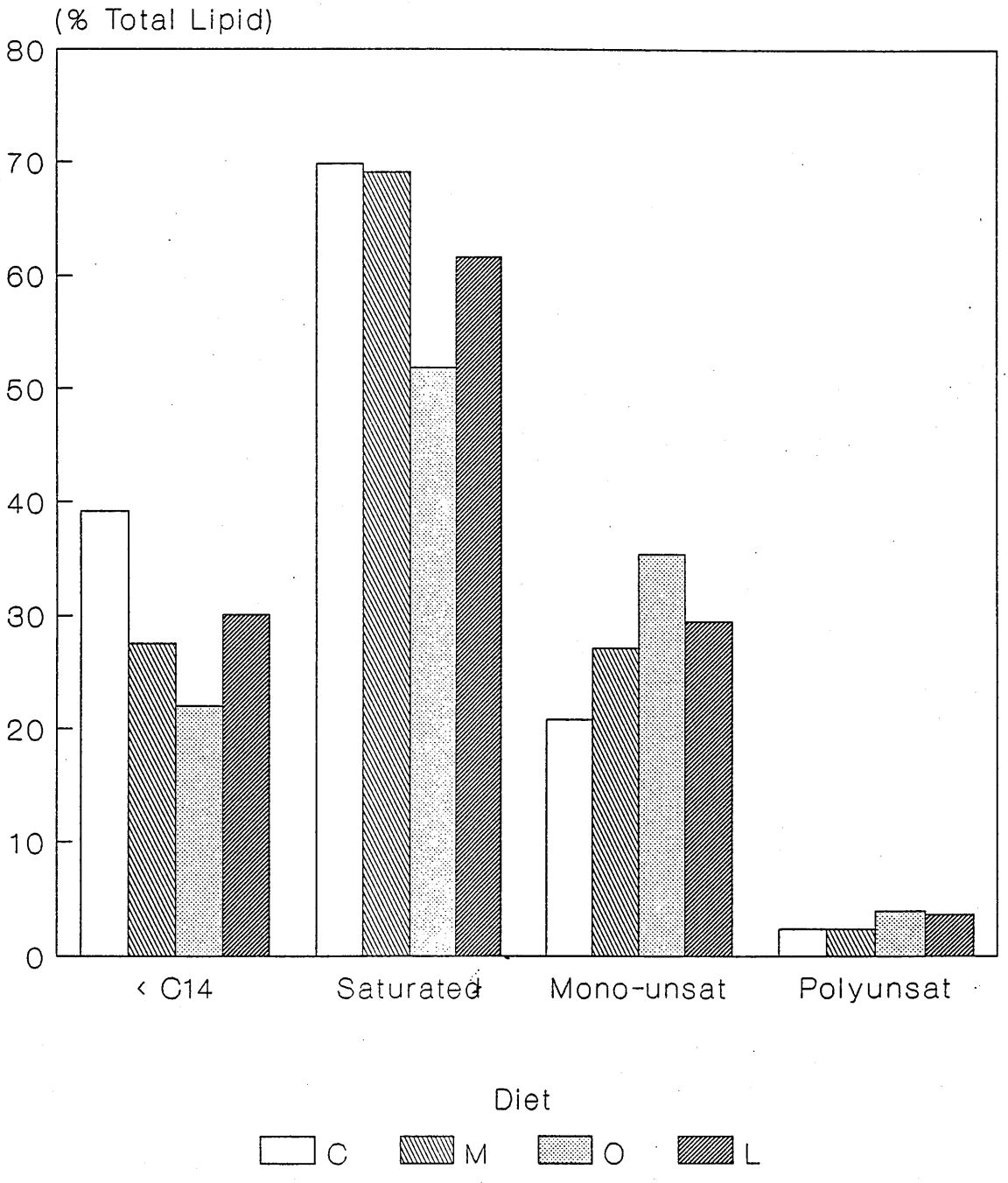
The milk fat results from this experiment suggest that a similar effect occurred with the goats. Due to the high level of concentrates that the animals received, milk fats were depressed. This was demonstrated by the observed milk fat percentages when goats were fed the control diet. Including protected fats in the diet at least partially alleviated this. The reduction in milk-fat depression was greatest when the goats were fed the diet M containing Megalac, slightly lower for diet L and lower still for diet O. Thus the oil in the OSNID diets was able to alleviate the low milk fat percentage seen with the control diet suggesting that this was a safe method of including fat in ruminant diets which does not.

The reduced response for the OSNID diets compared to Megalac in terms of alleviating the milk fat depression may have been caused by the presence of unsaturated oil. Feeding unprotected polyunsaturated oils has been shown to reduce milk fat synthesis (Davis and Brown, 1970), this is thought to be due to the shift in rumen fermentation towards a low acetate:propionate ratio. Such a shift in rumen fermentation pattern was observed in the previous experiment (see Section 7.4.4.2). If the calcium soaps of polyunsaturated fatty acids were dissociating in the rumen as suggested by Sukhija and Palmquist (1990) then free polyunsaturated fatty acids would have been liberated and may have reduced milk fat synthesis further.

The fatty acid profiles of the milk samples are given in Table 7.19 which shows that there were significant differences due to treatment. The results in a summary form are given at the bottom of the same Table and in Figure 7.5. There was an overall reduction in the proportion of short and medium chain fatty acids in the milk fat for the oil-supplemented diets and a corresponding increase in the proportions of longer chain acids.

The degree of saturation of the milk fat when the oil-supplemented diets were fed was reduced. This effect was much less pronounced for Megalac although an increase in the proportion of the mono-unsaturated acids was observed. For Megalac there was a significant increase in the C16:0 reflecting the fatty acid composition of Megalac. There were

Figure 7.5 Effect of Diet on Goat Milk Fatty Acid Composition



large increases in the proportions of C18:1 and smaller but still statistically significant increases in the proportions of C18:2 when either OSNID supplement (O and L) was included in the diet compared to values for the control diet.

Table 7.19. Fatty Acid Content of Goat Milk Fat (% total fat)

Fatty Acid	C	M	O	L	(SE)
C4	2.86^{a}	3.75^{b}	4.05^{c}	3.58^{b}	0.081
C6	3.10^{b}	3.06^{b}	2.67^{a}	3.23^{b}	0.121
C8	3.33^{c}	2.64^{ab}	2.07^{a}	3.01^{bc}	0.195
C10	12.20^{c}	7.55^{b}	5.27^{a}	8.51^{b}	0.643
C12	6.79^{c}	2.98^{ab}	2.14^{a}	3.33^{b}	0.301
C14	9.93^{b}	7.07^{ab}	5.47^{a}	7.65^{ab}	1.090
C14:1	1.02^{b}	0.40^{a}	0.28^{a}	0.80^{a}	0.127
C16:0	24.74^{c}	33.71^{d}	18.07^{a}	20.11^{b}	0.633
C16:1	2.10^{b}	1.67^{b}	0.00^{a}	1.66^{b}	0.172
C18:0	6.92^{a}	8.30^{a}	12.04^{b}	12.19^{b}	0.492
C18:1	17.71^{a}	25.06^{b}	35.07^{c}	27.08^{b}	0.841
C18:2	2.04^{a}	2.34^{a}	3.83^{c}	3.36^{b}	0.153
C18:3	0.27^{bc}	0.04^{a}	0.16^{ab}	0.36^{c}	0.044
C20+	0.12	0.04	0.00	0.00	0.064
< C16	39.2	27.5	22.0	30.1	
Saturated	76.8	70.5	60.6	66.8	
Monounsats.	20.8	27.1	35.4	29.5	
Polyunsats.	2.4	2.4	4.0	3.7	

Means in the same row with similar superscripts do not differ significantly ($p<0.05$).

Estimates of efficiency of transfer of dietary long chain fatty acids to milk fat vary widely (see Palmquist 1984a). The levels of linoleic acid in the milk fat in the present experiment were very low, and although no measurements of milk yield and therefore yield of C18:2 in the milk were made, it is likely that they represented only a very small transfer from the diet. This suggests that this fatty acid was not well protected by $Ca(OH)_2$ treatment. This is in accord with the results from an experiment reported by Palmquist (1984b) in which lactating dairy cows were fed calcium soaps of varying degrees of saturation. It was noted that unsaturated soaps were less satisfactory at maintaining normal rumen function, because unsaturated soaps in rumen fluid dissociate at a higher pH than saturated soaps. This was

supported by later studies which measured the dissociation of different calcium *in vitro* (Sukhija and Palmquist, 1990). The results suggest that manipulation of milk fat by feeding calcium soaps of unsaturated fatty acids is not a feasible proposition.

Other methods used for protecting dietary polyunsaturated fatty acids have produced much larger changes in fatty acid profiles. In a review of experiments in which milk of elevated polyunsaturated fatty acid content was measured (McDonald and Scott, 1977) levels of C18:2 in the milk ranged from 5 - 6 % of the milk fat (Goering *et al.*, 1973) to 35% of the milk fat (Bitman *et al.*, 1973). The changes in milk fatty acid composition in this experiment were not of this order, however they were similar to those observed by Banks *et al.* (1987) when the diets of Friesian cows were supplemented with 500g/d of soya-bean oil. In their experiment saturated acids were reduced by supplementation (mmol/mol) from 774 to 605, mono-unsaturated acids were increased from 205 to 354 and the polyunsaturated acids were increased from 21 - 42. In this experiment they measured the extrusion value of the butter produced from these milks and the proportion of the fat which was solid at 5°C. Both were reduced by oil supplementation. They concluded that dietary manipulation of the milk fat would enhance the spreadability of the butter.

By comparison the goats in the present experiment received 127 g/d of oil in the supplements on diet O when changes in the milk fat were (g/kg total fat) from 768 to 606 saturated acids, 208 to 354 mono-unsaturated acids and 24 to 40 polyunsaturated acids. It is probable therefore that the changes in milk fatty acid content caused by the oil supplemented diet would have enhanced the spreadability of butter produced from this milk as they were of the same magnitude as observed by Banks *et al.* (1987)

The findings of these experiments can be summarised:-

1. OSNID is a safe method for oil-supplementation of ruminant diets, minimising the inhibitory effects of oil on rumen cellulolytic activity although leading to increased levels of propionate in

the rumen.

2. $Ca(OH)_2$ treatment proved an inefficient method of protecting polyunsaturated fatty acids from rumen biohydrogenation.

3. The high intakes of unsaturated oil made possible by the use of OSNID caused substantial changes in the plasma and milk fatty acid contents, particularly by increasing the proportions of C18:1. Reversal of low-milk fat syndrome by this approach seems feasible.

4. OSNID may provide an economical means for fat supplementation of ruminant diets although the use of added fat with a high polyunsaturated fatty acid content is not justified.

These experiments have shown that supplementation of the ruminant diets with oil-supplemented NID is a safe method of feeding unsaturated oil. Whilst the degree of protection against biohydrogenation conferred by $Ca(OH)_2$-treatment is limited, it does reduce the inhibitory effects of oil on rumen function and has been shown to increase milk fat content of milk in animals with low-milk-fat syndrome. This treatment process may provide a means of incorporating a wide variety of cheap sources of oil into ruminant diets at levels which would otherwise cause disruption of rumen function.

CHAPTER EIGHT

THE EFFECT OF $Ca(OH)_2$ ON THE CELL WALL CARBOHYDRATES OF SPENT GRAINS

8.1 Introduction

In Chapter 4 it was seen that $Ca(OH)_2$ treatment increased the digestibility of spent grains in two ways; by forming soaps with the unsaturated fatty acids thus reducing their inhibitory effects on rumen bacteria, and by increasing the digestibility of the fibre. The latter was thought to occur by the solubilisation of the ligno-hemicellulose and also by increased availability of the fibre particles to rumen micro-organisms due to reduction of particle size and hydrolysis of alkali-labile bonds in the ligno-carbohydrate complex of the plant cell walls.

The effects of alkali on plant fibre are well documented in the case of sodium hydroxide treatment of cereal straws and have been reviewed by Jackson (1977). However, there is little information on the effect of alkali, and in particular of $Ca(OH)_2$, on the fibre components of cereal grains. An improvement in the digestibility of spent grains when treated with sodium hydroxide was noted by Box (1979) and it was suggested that other hydroxides may have similar effects. However the detailed mechanism of the digestibility response was not discussed.

The aim of the work described in this chapter was to characterise the effects of alkali treatment on the carbohydrate fraction of spent grains. The scope of the work was to identify the effects of different treatment factors on the yield of soluble material from alkali treated barley and maize spent grains and then to characterise the changes in the soluble and insoluble carbohydrate fractions due to treatment.

8.2 The Effect of Treatment Conditions on the Yield of Soluble Material From Alkali Treated Spent Grains

8.2.1 Quantitative Measurements

Since measurements of the effect of treatment conditions for the barley and maize spent grains were carried out at different times the experimental procedures for each were slightly different. Malt distillers grains (MDG) used as the barley spent grain and maize draff from Port Dundas (Glasgow) grain distillery was used for the maize spent grain.

8.2.1.1 Barley Spent Grains

Method

Fresh MDG were treated with 80g $Ca(OH)_2$/kg DM. The samples were held at 20, 40, 60 or 80°C for 0, 2, 4, 8 or 12 hours. Untreated MDG served as a control. Subsamples (300 g) were taken and mixed in a large food mixer (Hobart Manufacturing Co. Ltd., Glasgow) with 600 mls of warm water (approximately 70°C) for 2 minutes. The slurries were then centrifuged at 4,200 rpm for 30 minutes. The supernatant containing the soluble material was taken off and lyophilised. The insoluble residue was dried at 60°C. When completely dry, the OM of the soluble fraction was measured as described in Section 2.1.2. The OM of the $Ca(OH)_2$ treated draff was also measured so that the OM lost in the soluble fraction could be expressed as a percentage of the original OM treated.

Results and Discussion

The full results are given in Appendix 20 and are shown in Figure 8.1a. This Figure shows the increases in yield of soluble OM with treatment at 80 g $Ca(OH)_2$/kg DM at different temperatures and times. Solubilisation of the OM showed a similar pattern to those observed for other parameters (e.g. increases in *in vitro* and *in sacco* OMD with treatment time and temperature) in Chapter 3.

Figure 8.1a Effect of Treatment Time and Temperature on Solubilisation of OM from NID.

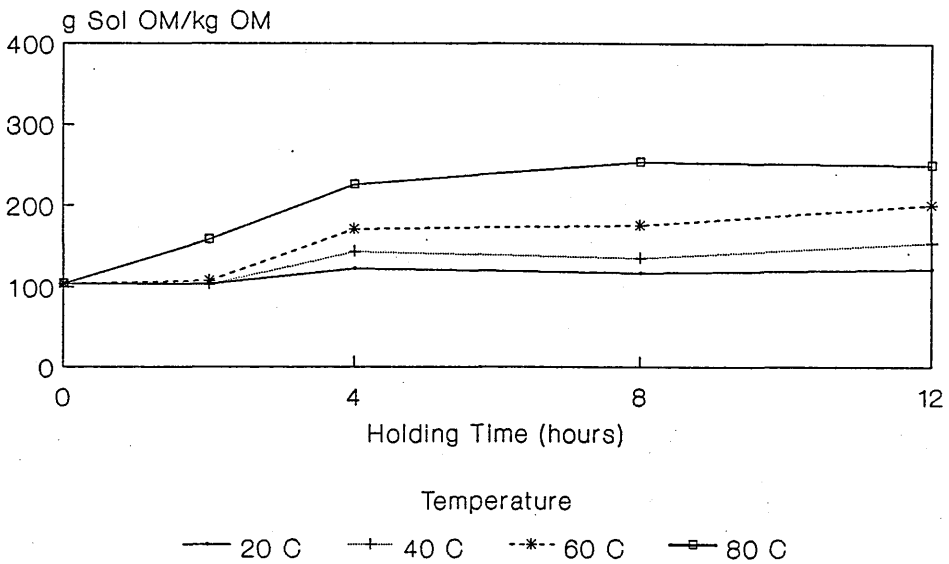
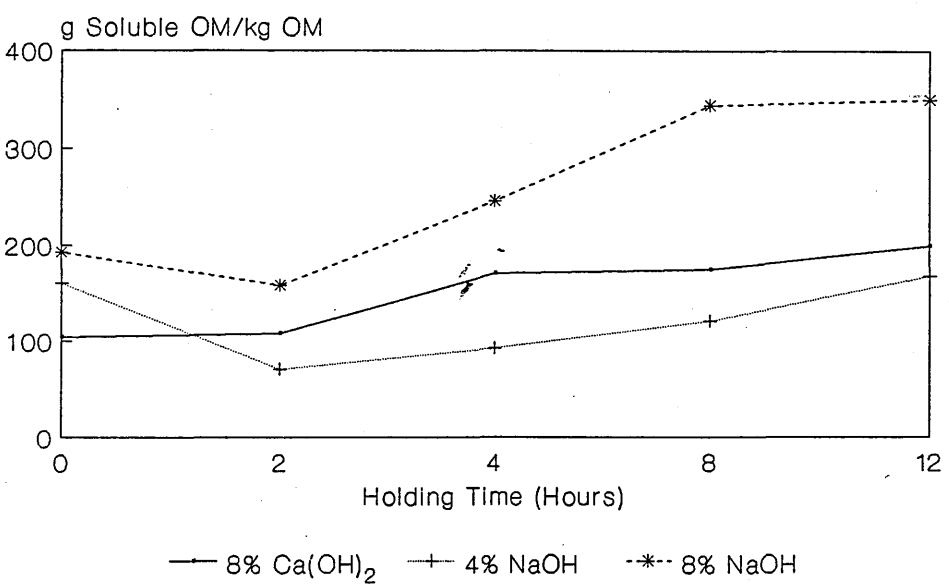


Figure 8.1b Effect of Treatment Time on Yield of Solubles from $Ca(OH)_2$- and NaOH-Treated MDG.



The yields of OM measured were used to derived an equation to predict the yield of soluble OM from the treatment conditions for MDG treated with 80g $Ca(OH)_2$/kg DM. The prediction equation was:-

$$Y = 4.66 + 0.135C + 0.660T$$

$$r^2 = 70.1\%$$

Where Y = Yield of soluble OM (% original OM)
T = Holding time (Hours)
C = Temperature (°C)

The equation shows that the holding time (in hours) had a much greater effect on the yield of soluble OM than the treatment temperature. This result supports the earlier findings in Chapter 4 where is was shown that the soluble fraction of NID was not formed instantaneously but increased with holding time.

The effect of NaOH on the yield of soluble OM from MDG was measured concurrently with this experiment. Two levels (40 and 80 g NaOH/kg DM) were tested over the same range of holding times as for $Ca(OH)_2$-treatment at 60°C. Untreated MDG served as a control. The full results for these measurements are also included in Appendix 20 and a comparison of the yields of soluble OM yields from $Ca(OH)_2$ with NaOH treated draff is given in Figure 8.1b.

Treatment with 80 g NaOH/kg DM gave the highest yield of soluble OM under all treatment conditions. When the MDG were treated with alkali and the soluble material was extracted immediately (i.e. without holding at temperature) the 80 g/kg DM $Ca(OH)_2$ gave lower yields than 40g NaOH/kg DM. This reflects the relatively slow rates of reaction associated with $Ca(OH)_2$, and the greater titratable alkalinity of NaOH. At longer holding times, 80g $Ca(OH)_2$/kg DM was more effective than 40g NaOH/kg DM but much less effective than 80g NaOH/kg DM. Generally, more soluble organic matter was released with longer holding times for all treatments. For NaOH at 80 g/kg DM, the rate of increase in solubilisation with treatment times decreased at longer holding times. This suggests that the solubilisation reactions were

reaching completion for that level of NaOH.

The soluble and insoluble fractions of draff after treatment with NaOH were much darker in colour that those from $Ca(OH)_2$-treated draff. This may indicate a difference in the modes of action of the two alkalis on the carbohydrate fractions. Differences between the soluble fractions produced by the action of the two alkalis were also observed when the materials were incubated *in vitro* with rumen liquor (see Chapter 4).

8.2.1.2 Maize Spent Grains

Method

The effects of NaOH and $Ca(OH)_2$ on the yields of soluble OM from maize draff were also studied. The experiment was carried out in a similar manner to that described for the MDG that two treatment levels (40 and 80 g/kg DM) of $Ca(OH)_2$ and NaOH were tested at a treatment temperature of 60°C for 0, 2, 4, 8, and 16 hour holding times. The effect of treatment temperature was also investigated for the 80g $Ca(OH)_2$/kg DM treatment. For these treatments, spent maize grains were held at 20, 40, 60 and 80°C for 2, 4, 8 and 16 hours. The yields of OM for each part of the experiment were measured in the same way as described for the MDG.

Results and Discussion

The full results for both parts of the experiment are given in Appendix 21 and are shown in Figures 8.2a and 8.2b.

The effect of different treatment conditions on the yield of solubles from maize draff were very similar to those observed for MDG. An equation was derived to predict the effect of holding time and temperature on the yield of soluble OM from maize draff treated with 80 g $Ca(OH)_2$/kg DM from the observed values measured in this experiment:-

Figure 8.2a Effect of Treatment Time and Temperature on the Yield of Soluble OM from $Ca(OH)_2$-Treated Maize Spent Grains

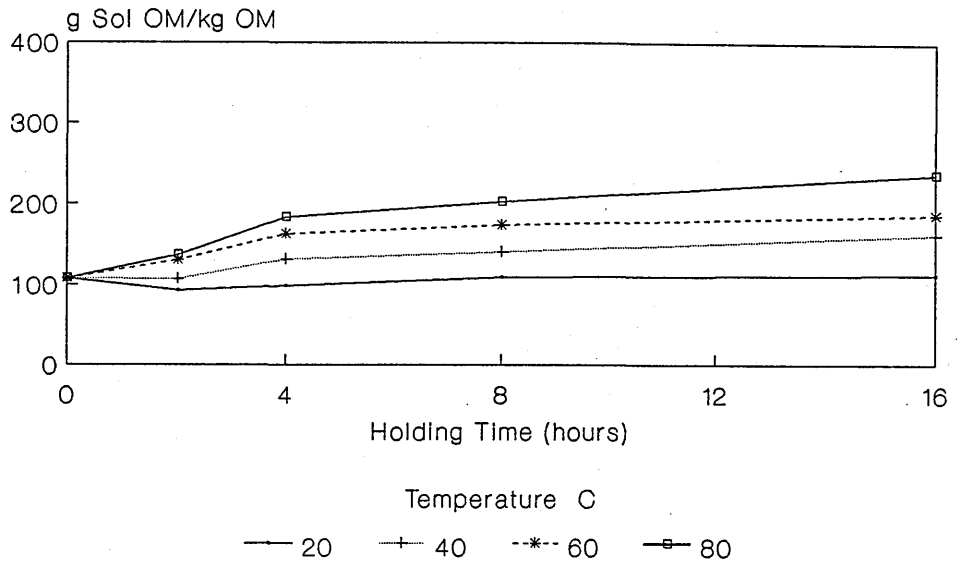
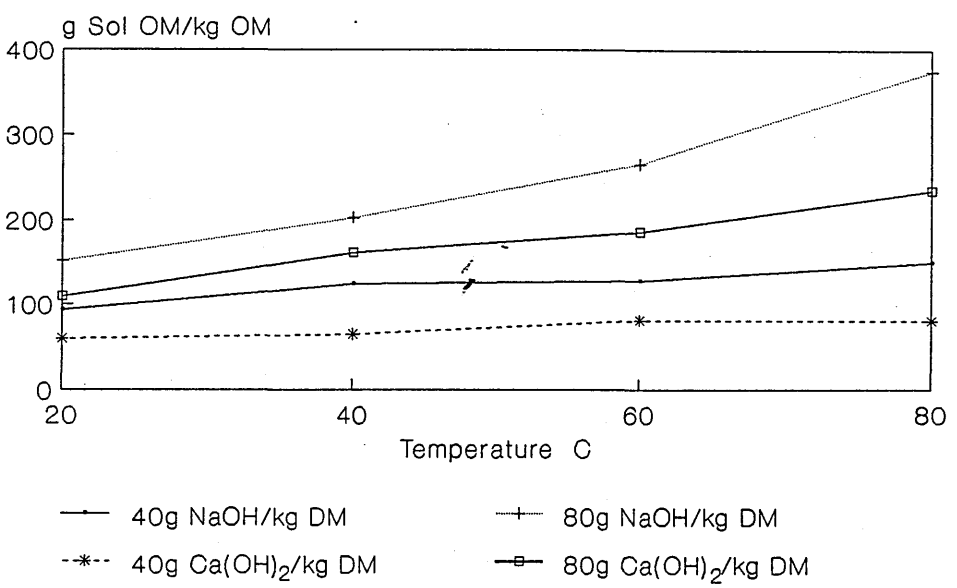


Figure 8.2b Effect of NaOH or $Ca(OH)_2$ Treatment and Temperature on Yield of Soluble OM from Maize Spent Grains



Holding Time: 16 Hours

$$Y = 5.78 + 0.116C + 0.406T$$

$$r^2 = 75.8\%$$

Where Y = Yield of soluble OM (% original OM)

T = Holding time (Hours)

C = Temperature (°C)

The effect of treatment conditions on the OM yield from $Ca(OH)_2$-treated spent grains was thus similar for maize spent grains and MDG.

Conclusions

This section of the work determined the effects of the different treatments (temperature, holding time, level and type of alkali) on the yield of soluble organic matter. The main findings were:-

1. Yields of soluble OM from both types of grain were increased with both treatment temperature and holding time.

2. Under the standard treatment conditions (80 g $Ca(OH)_2$/kg DM, 60°C for 16 hours) MDG and maize draff yield 23.3 and 19.2g respectively of soluble organic matter for per kg of organic matter treated.

3. NaOH gave higher yields of soluble OM than $Ca(OH)_2$ at all holding times, however the soluble fraction appeared darker with the NaOH treated grains indicating possible differences in the nature of the soluble and insoluble substances.

Having quantified the key factors influencing yield of solubilised material from alkali-treated draff, the remainder of this Chapter is devoted to qualitative characterisation of the chemical changes that occur during alkali treatment.

8.3 Qualitative Measurements

8.3.1 Analysis of Component Monosaccharides and Lignin Content

The effect of alkali treatment on the composition of the soluble and insoluble carbohydrate fractions of spent grains was investigated by measuring the constituent monosaccharide residues of these fractions and of the whole feed. This was done using two different hydrolysis procedures followed by derivatisation and G.C. analysis to quantify the proportions of the monosaccharides. The two hydrolysis techniques used were 72% sulphuric acid or 2M trifluoroacetic acid (TFA) and the methods are given in Section 2.6. The former gives the total monosaccharide residues present while the latter specifically depolymerises the hemicelluloses. The lignin content and UV spectra, (indicating phenolic acids) of the different fractions were also measured.

A frozen 15 kg sample of MDG was allowed to thaw. This was divided into three 5 kg portions which were treated either with 80g $Ca(OH)_2$/kg DM or 80g NaOH/kg DM (held at 60°C for 16 hours), or was left untreated. A 1 kg subsample of each was taken and was dried in a forced draught oven at 60°C. These samples of the whole feeds were then milled through a 1 mm screen and were kept in airtight containers awaiting further analysis.

The soluble and insoluble fractions were prepared from the remaining 4 kg of each treatment immediately on removal from the oven. This was done by mixing with hot water (approximately 70°C, 1:2 grains:water by weight) for 2 minutes in an industrial food mixer to make a slurry. The slurries were centrifuged at 4,200 rpm for 30 minutes. The soluble supernatants were poured into large metal trays and placed in a deep freeze at -20°C until frozen. When frozen, the soluble fractions were freeze dried. The insoluble residues were spread out onto mesh drying trays and were dried at 60°C. When dry, the insoluble material was milled through a 1 mm screen. These large batches of soluble and insoluble materials were stored in air tight containers and were used for all the analyses described in subsequent sections.

Samples of the whole feed and the soluble and insoluble fractions for untreated, $Ca(OH)_2$-treated and NaOH-treated grains were then analysed for component monosaccharides and lignin content. The ultra violet absorption spectra of the soluble fractions were measured with particular reference to absorption at 280 and 315 nm using a scanning UV spectrometer (Shimadzu 420) to determine the presence of free lignin and phenolic substances. All analytical methods are described in section 2.6.

8.3.3 Results and Discussion

8.3.3.1 Malt Distillers Grains

Hydrolysis with 72% Sulphuric Acid

The 72% sulphuric acid procedure caused complete hydrolysis of all carbohydrates present to constituent monosaccharide residues. Alkali treatment had little effect on the monosaccharide proportions of the whole feeds. Glucose made up approximately half of the total sugars, a large part of which would have resulted from hydrolysis of the cellulose present in the cell wall. The remaining 50% was made up largely of arabinose and xylose which originated from the cell wall hemicelluloses.

Alkali treatment did affect the monosaccharide composition of the soluble and insoluble fractions. For all samples, most of the glucose appears in the insoluble fraction reflecting the insoluble nature of the cellulose. The proportions of glucose in the soluble fraction were lower than in the whole feed or in the insoluble fractions but were increased by $Ca(OH)_2$ treatment and still further by NaOH treatment. The low proportion of glucose and the high proportion of arabinose plus xylose residues in all soluble fractions suggested that this fraction was composed mainly of hemicellulose. The effect of alkali was to increase the soluble content of draff without great effect on its monosaccharide composition. The arabinose:xylose ratios where higher in the soluble fraction and lower in the insoluble fraction compared to those in the whole feed samples. Ratios observed

in the whole feeds were of a similar order to those observed by Henry (1985) who noted total arabinose:xylose ratios of 0.81 and 0.82:1 for malt and barley respectively. Arabinose:xylose ratios were lower in the insoluble fractions for all treatments, particularly for the alkali-treated draff insolubles where the ratios were 0.61:1 and 0.69:1 for $Ca(OH)_2$- and NaOH-treated MDG respectively. This resulted from the removal of carbohydrate of high arabinose:xylose ratio in the soluble fractions.

Table 8.1 Component Monosaccharides Following Hydrolysis with 72% Sulphuric Acid (% total sugars) for MDG

	Rha	Fuc	Ara	Xyl	Man	Glc	Gal	Ara/Xyl
Whole Feed								
Untreated Draff	0.5	-	21.7	25.8	-	49.8	tr	0.84
$Ca(OH)_2$-treated	0.5	-	21.5	24.9	-	53.1	tr	0.86
NaOH-treated	0.5	-	20.7	27.8	-	51.0	tr	0.74
Insoluble Fraction								
Untreated Draff	0.4	-	20.2	27.2	-	52.2	tr	0.74
$Ca(OH)_2$-treated	-	-	16.0	26.1	-	57.9	tr	0.61
NaOH-treated	-	-	21.1	30.8	-	48.1	tr	0.69
Soluble Fraction								
Untreated Draff	1.6	-	48.5	41.6	-	8.2	tr	1.17
$Ca(OH)_2$-treated	-	-	45.9	42.4	-	11.7	tr	1.08
NaOH-treated	1.6	-	45.2	37.4	-	15.8	tr	1.21

Rha = Rhamnose
Fuc = Fucose
Ara = Arabinose
Xyl = Xylose
Man = Mannose
Glc = Glucose
Gal = Galactose

Alkali treatment appears to act by greatly increasing the yield of soluble carbohydrate without great effect on its monosaccharide content. For untreated MDG, 3.53% of the OM could be extracted with hot water as described in Section 8.2.1. This was increased to 23.3% and approximately 37% with $Ca(OH)_2$ and NaOH treatment (16 hours at 60°C) respectively.

Hydrolysis with 2M Trifuoroacetic Acid

Table 8.2 Component Monosaccharides Following Hydrolysis with 2M Trifluoroacetic Acid (% total sugars)

	Rha	Fuc	Ara	Xyl	Man	Glc	Gal	Ara/Xyl
Whole Feed								
Untreated Draff	0.4	-	36.1	44.7	-	18.9	-	0.81
$Ca(OH)_2$-treated	-	-	36.1	45.1	-	18.9	-	0.80
NaOH-treated	-	-	36.5	45.8	-	17.8	-	0.80
Insoluble Fraction								
Untreated Draff	1.1	-	38.0	46.3	-	14.5	-	0.82
$Ca(OH)_2$-treated	0.3	-	32.6	49.0	-	18.1	-	0.66
NaOH-treated	0.2	-	38.4	48.2	-	13.2	-	0.80
Soluble Fraction								
Untreated Draff	1.2	-	45.8	34.3	-	8.2	10.5	1.33
$Ca(OH)_2$-treated	0.6	0.7	44.0	36.9	-	12.1	5.6	1.19
NaOH-treated	0.7	-	56.9	42.3	-	-	-	1.35

Table 8.2 shows the results from the milder hydrolysis procedure using 2M TFA. This method does not hydrolyse cellulose and therefore is largely a measure the monosaccharides associated with the hemicellulose fraction of the samples. It shows that the hemicellulose of the whole feeds are made up of approximately 18 - 19% glucose and arabinose and xylose in the ratio of 0.8 to 1. This ratio is similar to that observed under more severe hydrolysis conditions

and to that measured in extracts from whole barley and malt (Henry, 1985).

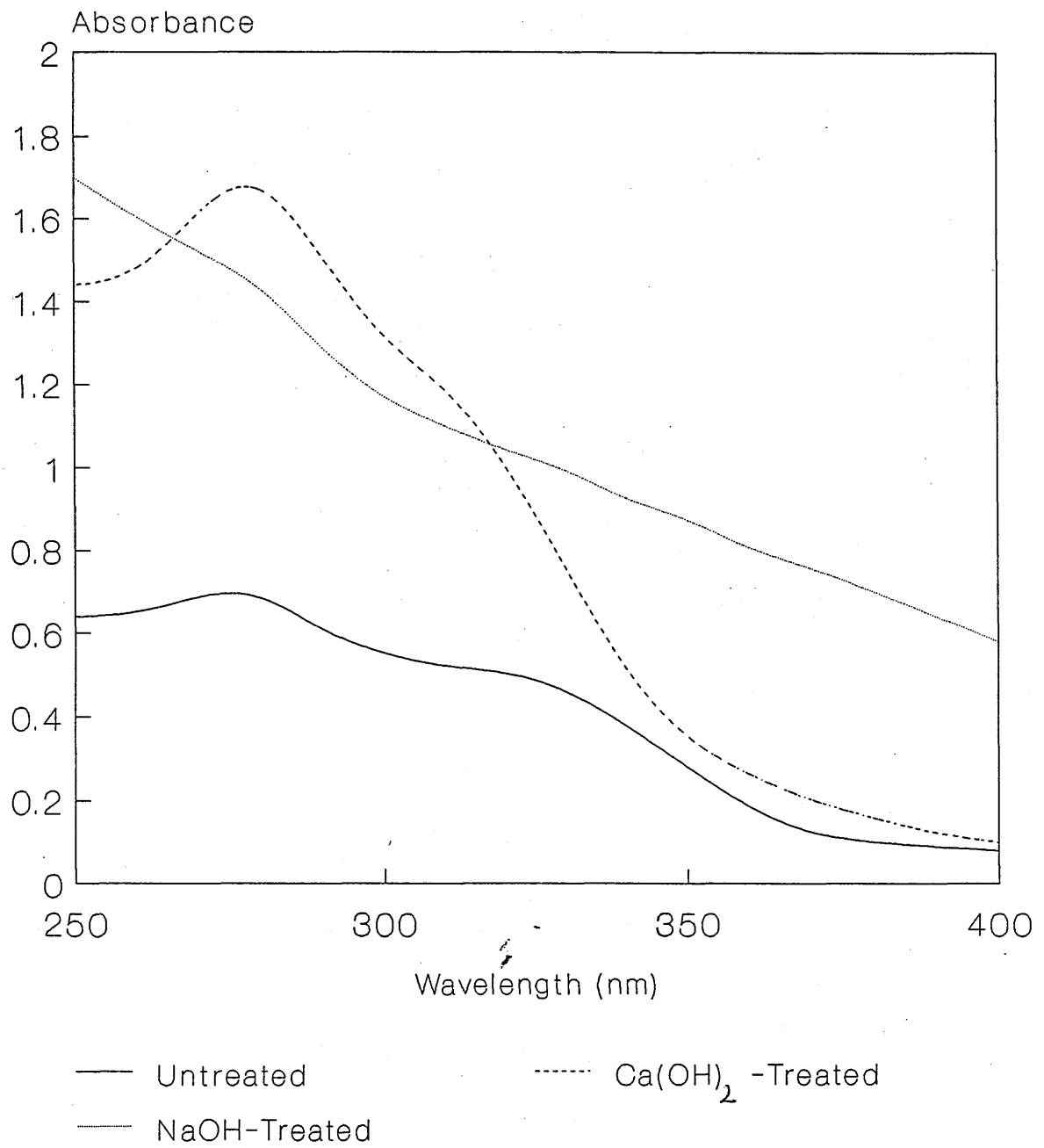
The whole feed and the insoluble fractions under the milder hydrolysis conditions yield much lower proportions of glucose than seen with 72% H_2SO_4, supporting the view that most of the glucose residues originated from cellulose. As a result, the proportions of arabinose and xylose measured in the whole feeds and the insoluble fractions are increased, but for the soluble fractions remain unchanged compared to levels found following hydrolysis with 72% H_2SO_4.

The soluble fraction of the NaOH-treated grains contained no glucose when subject to 2M TFA hydrolysis whereas with 72% H_2SO_4 hydrolysis glucose made up 15.8% of the total sugars of the soluble fraction indicating that cellulose could have been present in the NaOH solubles. A possible explanation for this could be the presence of colloidal material rich in cellulose which remained in the supernatant after centrifugation. Alkali treatment of the MDG increases the friability of the grains and was shown in Section 4.1 to increase the amount of small particles. Such small particles could have remained in colloidal suspension and may have remained in the liquid phase after centrifugation and therefore be assumed to be part of the soluble fraction.

The results from the analysis of the lignin content of the samples are given in Table 8.3 and show that only treatment with sodium hydroxide reduced the lignin content of the spent grains. This is notable both in the whole feed and in the insoluble fraction of the feeds. A slight increase in lignin content was seen when MDG were treated with $Ca(OH)_2$ under the conditions stated. This suggests that sodium but not calcium hydroxide partially solubilises the lignin content of the cell wall material. This may reflect the greater titratable alkalinity of NaOH compared to $Ca(OH)_2$ (as measured in Section 3.4.

This observation is supported by the UV spectra of the soluble fractions given in Figure 8.3. The absorbance at 280 nm and at 315 nm

Figure 8.3 Effect of Alkali on UV Spectra of MDG Soluble Fraction



give an indication of the content of lignin and phenolic acids such as ferulic and p-coumaric acid which have been released by hydrolysis of the lignocellulosic complexes in the cell walls. These spectra show that there is an increase in these substances due to alkali treatment. NaOH-treated draff solubles produced an unusual absorption spectra with relatively high absorbencies at all wavelengths perhaps indicating a high content of lignin-related substances. If, as suggested earlier, this sample contained material which was not truly soluble then this may be an explanation for the unusual absorption spectra observed.

Table 8.3 The Lignin Content (% Dry Matter)

Whole Feed	
Untreated Draff	7.74
$Ca(OH)_2$-treated	8.20
NaOH-treated	5.91
Insoluble Fraction	
Untreated Draff	7.06
$Ca(OH)_2$-treated	7.49
NaOH-treated	6.12

The presence of lignin is a major factor associated with the poor digestibility of low quality forages (Van Soest, 1981). Morrison (1974) showed the presence of ester crosslinks between phenolic groups and polymers of xylan and cellulose in lignin-carbohydrate complexes isolated from rye grass. These crosslinks are broken by alkali agents by saponification (Tarkow and Feist, 1969). However, the total lignin content of feeds is a poor predictor of digestibility since it is only affected by lignin which is bound to the lignocarbohydrate complex of the plant cell wall. Lau and Van Soest (1980) showed that only bound lignin was negatively correlated to the digestibility of alkali treated straw and suggested that the negative effect of lignin on digestibility was dependent upon the covalent linkage between lignin

and the carbohydrate residue that it protects. Chesson (1981) showed that the extent of solubilisation of p-coumaric acid produced by NaOH showed a linear relationship with cellulose digestibility as the p-coumaric acid is covalently bonded to the core lignin.

These results suggest that the effects of alkali treatment on spent grains are similar to those observed when other fibrous feeds are treated with alkali. The increase in the level of free phenolic material in the soluble fractions due to alkali treatment, as measured by the increases in absorbance, suggests that the cross links between polysaccharide polymers have been saponified. Although no direct measurements of the digestibility of cell wall polysaccharides were made, it is likely that the solubilisation of phenolic material measured in the present experiment is the cause of the increased digestibility of the NDF of spent grains measured in previous Chapters. Gas production measurements described in Chapter 4 show that the soluble carbohydrates, the yield of which is increased by $Ca(OH)_2$ treatment, are rapidly metabolised by rumen micro-organisms and contribute to the increase in digestibility of the whole feed.

8.3.3.2 Maize Spent Grains

The analysis of the dried maize draff and $Ca(OH)_2$-treated maize draff was carried out in a similar manner to that described for MDG. It included the two different hydrolytic procedures followed by GC analysis, acetyl bromide lignin measurements and determination of the UV spectra. This was done both for comparison with MDG, and to try to elucidate possible reasons for the poor intake and digestibility characteristics of $Ca(OH)_2$-treated maize draff observed in Chapter 6.

Results

Values from G.C. analysis after hydrolysis with 2M TFA were very low and therefore are not given. The reason for this was thought to be due to insufficient hydrolysis of the polysaccharides. The results for the analysis of the component monosaccharides after hydrolysis

with 72% H_2SO_4 are given in Table 8.4.

Table 8.4. Component Monosaccharides of Maize Draff (% total sugars)

	Rha	Fuc	Ara	Xyl	Man	Glc	Gal	Ara/Xyl
Whole Feed								
Untreated Draff	0.4	0.4	9.5	13.4	5.8	65.8	4.9	0.71
$Ca(OH)_2$-treated Draff	0.5	0.6	11.3	13.0	7.2	63.0	4.7	0.86
Insoluble Fraction								
Untreated Draff	0.3	0.8	9.9	13.2	4.3	68.3	3.3	0.75
$Ca(OH)_2$-treated Draff	0.4	0.7	13.9	21.9	6.1	52.0	5.2	0.63
Soluble Fraction								
Untreated Draff	0.5	0.8	14.0	13.8	9.9	57.8	3.3	1.01
$Ca(OH)_2$-treated Draff	0.7	0.7	27.3	33.4	10.1	20.4	8.6	0.82

Maize draff contains a wider range of monosaccharides with slightly more glucose but less arabinose and xylose than MDG. The effect of $Ca(OH)_2$ treatment is to change markedly the monosaccharide composition of both the soluble and insoluble fractions. This was particularly so for the soluble fraction and contrasts with the finding for MDG where alkali treatment had little effect on the proportions of monosaccharides present in the soluble fraction. The arabinose:xylose ratios for the soluble fractions of maize draff were much lower than for MDG. The change in ratio in the whole feed due to $Ca(OH)_2$-treatment was not expected and is unexplained since nothing has been removed from the grains and only $Ca(OH)_2$ had been added.

The acetyl bromide lignin measurements showed that, for the whole feeds, $Ca(OH)_2$ treatment reduced the lignin content from 4.36 to 2.53 g/kg DM. For the insoluble fractions, the $Ca(OH)_2$ treated material was a little higher than for the untreated (5.20 vs 4.16). These results suggest that $Ca(OH)_2$ treatment solubilised some of the lignin

fraction. The lignin remaining in the insoluble fraction is concentrated by the removal of the solubles and therefore gave slightly higher values than for the whole feed.

The maize soluble fractions from the untreated and $Ca(OH)_2$-treated grains were scanned in the UV range using a scanning spectrophotometer as described in section 2.6. The spectra are given in Figure 8.4. The untreated maize draff solubles gave a typical spectrum but the $Ca(OH)_2$-treated maize solubles gave an unusual spectrum with a high level of absorption at around 335 nm. The reason for this is unexplained.

$Ca(OH)_2$ treatment of maize draff thus solubilises some of the lignin and some of the hemicellulose. In contrast to the $Ca(OH)_2$-treated MDG, the hemicellulose released from maize draff is different in monosaccharide composition to the soluble fraction of the untreated grains. These findings give interesting insights into the action of $Ca(OH)_2$ on maize draff cell wall polysaccharides and lignin and show that the two sources of spent grains respond differently to $Ca(OH)_2$-treatment. The differences in digestibility response to $Ca(OH)_2$ treatment (see Chapter 6) may well be related to these observations.

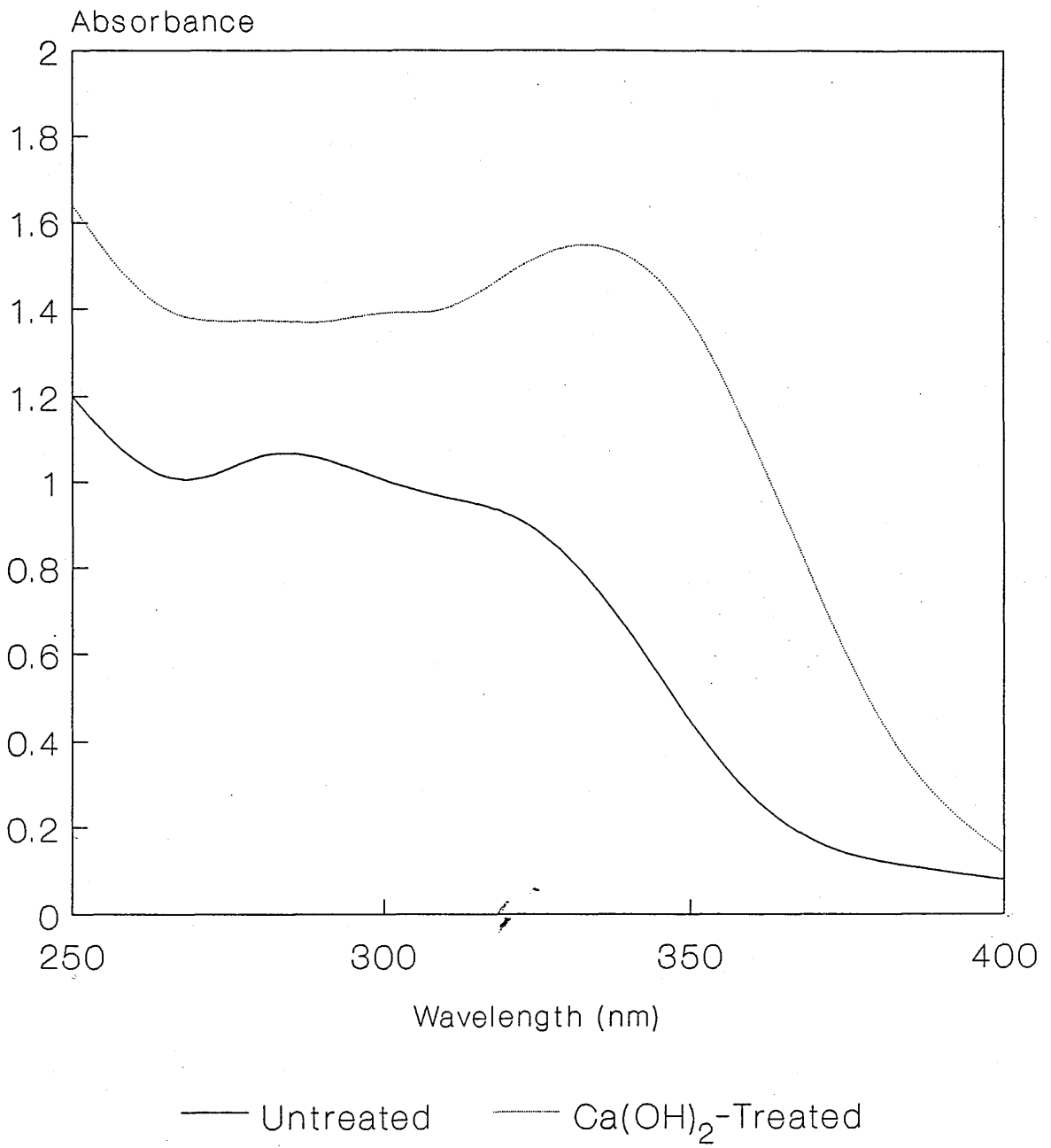
8.4 Analysis of the Soluble Fraction of $Ca(OH)_2$-Treated Spent Grains

All analyses of the soluble fractions were made on a large sample of soluble material. This was prepared from several smaller extractions carried out on $Ca(OH)_2$-treated material as described in Section 8.3.2.1. The batches of solubles prepared were then thoroughly mixed together and stored in an air-tight container.

8.4.1 Analysis of Solubilised Nitrogen

A fractionation of the soluble nitrogenous components of $Ca(OH)_2$-treated MDG and maize draff was done to determine the form of the protein present. It was done by measuring the crude, true protein and the non-protein content of the soluble fractions as described in

Figure 8.4 Effect of $Ca(OH)_2$-Treatment on the UV Spectra of Maize Spent Grain Soluble Fraction



Section 2.1.3. The true protein and non-protein content were determined by fractionation using copper sulphate. Analysis for ammonia nitrogen was carried out using the Indophenol Blue colourimetric assay. The results are given in Table 8.5.

Table 8.5. Constituents of Soluble Nitrogen Fraction from $Ca(OH)_2$-Treated MDG and Maize Draff (g/kg Soluble DM)

	NID	TMD
Crude Protein	117.6	177.7
True Protein	28.8	52.4
Non-Protein Nitrogen	88.8	125.3
Ammonia Nitrogen	0	0.5

The levels of crude protein measured were higher for the $Ca(OH)_2$-treated maize draff (TMD) than for the NID. This may be a reflection of the higher crude protein content of the whole feed for the $Ca(OH)_2$-treated maize draff compared to NID. The analysis of the nitrogenous components of the soluble fractions of $Ca(OH)_2$ treated spent grains showed that for NID and TMD respectively approximately 24.5 and 29.5% of the nitrogen was made up of true protein. Protein is known to be associated with plant cell wall substances in the form of cell wall bound enzymes (Lamport and Catt, 1981) and hydroxyproline-rich glycoproteins containing arabinose residues (Lamport and Miller, 1971). The non-protein fraction contained little or no detectable ammonia and probably consisted of mainly free amino acids and short chain peptides which were not precipitated by the copper (II) sulphate. Although no measurements of the soluble protein were made in untreated draff, the levels are likely to be low because of the hot water extraction during the mashing process. The existence of the non-protein nitrogen fraction following $Ca(OH)_2$ treatment may explain the slight increase in the protein degradation observed *in sacco* (Chapter 4).

8.4.2 Fractionation of NID Solubles

The soluble fractions of $Ca(OH)_2$-treated spent grains were analysed further by fractionation of the carbohydrates present by serial precipitation in increasing concentrations of ethanol. These analyses were carried out on soluble fractions extracted from $Ca(OH)_2$-treated maize and MDG as described. The UV spectra of the precipitates was measured, with particular reference to absorption at 280 and 315 nm as an indication of phenolic material associated with each fraction.

8.4.2.1 Method

1 g of the freeze dried soluble material was dissolved in a minimum volume of distilled water. According to this volume, sufficient absolute ethanol was added drop-wise with continual stirring to give a 10% (by volume) solution of ethanol. This was placed in the refrigerator at 4°C for one hour to encourage precipitation from solution. The solution was then centrifuged at 3,000 rpm for 10 minutes to harvest the precipitate. The supernatant was taken off, the volume was measured and sufficient ethanol was added in the same manner as described previously until the concentration of ethanol in the mixture was 20% (by volume). This was repeated at ethanol concentrations of 30, 40, 50, 60, 70, 80 and 90%. For each concentration, the precipitate was washed in absolute alcohol and centrifuged again, before being deep frozen and freeze dried in preweighed foil dishes. When dry, the foil dishes were reweighed to give the dry weight yield of each fraction. The final fraction which did not precipitate in 90% ethanol was freeze dried directly in a preweighed container. Fractions were stored in screw topped glass vials until analysed

The monosaccharide compositions of the samples were analysed by hydrolysis in 72% H_2SO_4 followed by derivatisation and identification and quantification by G.C. analysis as described in Section 2.6. The absorbance of all samples in the UV range of wavelengths was measured using a scanning spectrophotometer as described in Section 2.6..

8.4.2.2 Results

Malt Distillers Grains Solubles

Table 8.6 Fractional Precipitation with Ethanol of MDG Solubles (% total sugars).

% Ethanol	Yield (mg/g)	Rha	Ara	Xyl		Glc	Gal	Ara/Xyl
10	8.5	No sugars detected						
20	14.5	-	65.0	35.0		tr	tr	1.86
30	50.0	1.6	52.4	45.9		tr	tr	1.14
40	4.0	-	48.8	51.2		tr	tr	0.93
50	149.5	1.7	66.2	32.1		tr	tr	2.06
60	129.0	tr	77.1	22.9		tr	tr	3.37
70	44.5	1.4	34.1	10.8		35.1	18.6	3.16
80	45.0	tr	35.4	10.4		34.9	19.3	3.40
90	31.0	tr	37.4	7.2		36.0	19.4	5.20
Not Ppt	94.5	tr	16.1	7.9	(34.9)*	25.6	15.5	2.30

* = Unknown Peak

Table 8.6 shows the yields and the monosaccharide compositions of the fractional precipitations of the NID solubles in ethanol. These results suggest that this material contains at least two distinct polysaccharides which occur in greater quantities than the other polysaccharides. These precipitate out at an ethanol concentration covering the range 20 - 40% and 50 - 90%. In order to study these fractions more closely, the fractional precipitation of barley draff solubles was repeated with just two precipitation steps at 45 and 90% ethanol. The results of this are shown in Table 8.7.

This larger scale fractional precipitation of NID solubles yielded two fractions of very different monosaccharide composition. Both fractions contained a wider range of monosaccharide residues than was

determined in the initial fractionation including rhamnose, fucose, mannose, glucose and galactose. The most significant difference was in the arabinose:xylose ratios. The first fraction precipitated in ethanol concentrations of less than 45%, contained a ratio of arabinose:xylose of 1.28, whilst the second, precipitated at higher ethanol concentrations, had a ratio of 2.71.

Table 8.7 Fractional Precipitation in 45 and 90% Ethanol

% Ethanol	Yield (% soluble DM)	Rha	Fuc	Ara	Xyl	Man	Glc	Gal	Ara/Xyl
45	25.7	2.2	1.1	42.5	33.3	1.2	13.7	6.0	1.28
90	44.8	1.4	0.9	52.0	19.2	4.9	14.8	6.9	2.71
Not Ppt	29.5	0.5	1.6	60.1	-	18.1	19.7	-	

Xylans have been shown to be the major hemicellulosic component of monocotyledonous cell walls. The backbone is composed of 150-200 ß-(1-4)-linked D-xylose residues (Timell, 1964). Most frequently, L-arabinofuranosyl groups are attached by α-(1-3) linkages to a backbone of xylosyl residues, while occasionally, D-glucuronic or 4-O-methyl-D-glucuronic acids are attached by α-(1-2)-linkages (Wilkie, 1979; Aspinall, 1980). The arabinose side chains are responsible for the water solubility of the arabino-xylan. The high arabinose:xylose ratios measured in the polysaccharides precipitated by ethanol suggest that the xylan molecule is branched with arabinose side chains since there are more than two arabinose molecules for every xylose.

It is noted (Meuser and Suckow, 1985) that the hemicellulose from wheat, which consists primarily of arabinoxylans, is structurally changed by the alkaline extraction procedures. They also cite Ali and D'Appolonia (1979) as suggesting that extraction time and temperature not only affect the yield but also the sugar composition of such extracts.

Arabinoxylans find application in the bread-making industry for their dough-enhancing properties. It is thought that the hemicellulose (or insoluble pentosan) in wheat flour is linked via ferulic acid to protein and that this confers gelling properties which are responsible for some of the dough enhancement characteristics of the arabinoxylan in bread making. The arabinose to xylose ratios of such arabinoxylans are much lower than those isolated from $Ca(OH)_2$-treated barley draff (0.54 compared to 1.28 and 2.71). The branched arabinoxylan extracted from barley draff could possibly find similar application in the baking industry to those present in wheat flour.

Maize Spent Grain Solubles

Table 8.8. Fractional Precipitation with Ethanol of Maize Draff Solubles (% Total Sugars)

% Ethanol	Yield (mg/g)	Rha	Fuc	Ara	Xyl	Man	Glc	Gal	Ara/Xyl
10	13	1.0	1.6	28.2	39.1	10.1	9.7	10.3	0.72
20	6	0.8	1.2	29.7	40.2	10.0	8.0	10.0	0.74
30	16	0.7	0.7	30.8	38.7	10.4	9.0	9.7	0.80
40	151	0.8	1.3	27.9	42.0	10.8	6.4	10.8	0.66
50	287	0.6	0.4	28.9	42.6	12.9	3.9	10.6	0.67
60	104	1.2	0.9	33.2	42.5	5.1	6.6	10.5	0.78
70	49	1.0	1.4	29.9	34.2	4.8	17.8	10.9	0.88
80	46	1.0	1.7	31.1	29.4	5.2	26.2	5.2	1.06
90	42	1.8	1.2	35.6	23.3	7.4	27.6	3.1	1.53
Not Ppt	195	0.6	1.2	23.6	12.2	19.9	36.6	5.6	1.90

Table 8.8 shows the monosaccharide composition of the polysaccharides precipitated from maize draff solubles in the different concentrations of ethanol. Compared to the NID solubles, these samples contained a wide range of monosaccharide residues demonstrating the more complex

nature of the maize cell wall material compared to barley. Unlike the fractionation of the NID solubles, no clear trends in the data were apparent suggesting that the $Ca(OH)_2$-treated maize draff soluble fraction is made up of many different polysaccharides with variable monosaccharide compositions.

The UV spectra of the ethanol precipitated fractions from NID and $Ca(OH)_2$-treated maize spent grains are given in Table 8.9. It shows that maize draff solubles generally had higher levels of lignin and phenolic components than the NID solubles. For both maize and barley solubles, the greatest concentration of phenolic acids and lignin appears in the fractions which were not precipitated in 90% ethanol. The ratio between the absorbencies at 280 and 315 nm gives an indication of the proportions of lignin and phenolic materials. None of the spectra for the maize draff fractions gave the high absorbance at the 335 nm wavelength observed with the whole $Ca(OH)_2$-treated maize solubles in Section 8.3.3.2.

Table 8.9 UV Spectral Data for Fractionated for $Ca(OH)_2$-Treated Barley and Maize Draff Solubles

	NID Solubles			Maize Solubles		
Ethanol	Absorbance of 10% solution					
	(280)	(315)	280/315	(280)	(315)	280/315
10	4.14	3.39	1.22	25.3	23.0	1.10
20	3.21	2.61	1.23	31.2	27.1	1.15
30	3.36	2.73	1.23	32.0	28.0	1.14
40	2.31	1.80	1.28	30.0	28.0	1.07
50	2.20	1.84	1.20	15.8	13.8	1.15
60	1.76	1.46	1.21	15.9	13.5	1.18
70	2.92	2.44	1.20	23.3	19.8	1.17
80	3.48	2.56	1.36	30.0	25.2	1.19
90	5.76	4.35	1.32	36.1	30.0	1.20
Not Ppt	76.5	52.0	1.47	28.6	18.0	1.60

The UV spectra of those fractions precipitated at 45 and 90% ethanol were also measured. For these samples the determinations were also carried out on samples which had been extracted with ether (3 x 5mls) prior to being dissolved in water and scanned on the spectrophotometer. This extraction takes out all free phenolic acids. The results are given in Table 8.10.

Table 8.10 UV Spectral Data from Major Arabinoxylans Isolated from NID

% Ethanol	Absorbance of 10mg/ml solution		
	280nm	315nm	280/315
45	31.5	24.0	1.31
90	34.0	26.7	1.27
Unppt	82.5	58.3	1.42

Ether extracted samples of the soluble fractions gave similar absorbencies to the non-extracted samples except in the fraction unprecipitated by ethanol which gave a much greater absorbency at 280 and 315 nm. This suggests that the phenolic material associated with the precipitated fractions is linked to the carbohydrate and is not present as free phenolic acids.

Smith and Hartley (1983) have shown that at least some of the ferulic acid of graminaceous plant cell walls is linked via the carboxyl group to arabinose which is in turn linked to xylose. It is possible that the polysaccharides isolated from NID solubles are linked in this way, however further studies would be required to confirm this hypothesis.

8.5 Conclusions

The experiments described in this Chapter have shown some of the effects of alkali treatment on barley and maize spent grains. They have shown the effects of treatment conditions, type of alkali, treatment temperature and holding time, on the yield of soluble

organic matter from the grains.

It cannot be assumed that the nature of the solubilised material does not change with changing treatment conditions. Indeed Ali and D'Appalonia (1979) suggest that the monosaccharide content of the hemicellulose which can be extracted by alkali is affected by treatment conditions. Since much of the work in previous Chapters has used grains treated with $Ca(OH)_2$ at 60°C for 16 hours, these treatment parameters were used for the majority of the analyses carried out. Further work is required in this area but it was felt that investigation into the effects of treatment parameters on the monosaccharide content of solubilised hemicellulose was outwith the remit of the present studies.

One of the main findings of these experiments was the solubilisation of two previously undescribed polysaccharides from NID which differ from polysaccharides isolated from cereal grains by other workers due to their high arabinose to xylose ratios. Only a preliminary characterisation of these substances was made, with further work being required to elucidate their physical and chemical properties. It is possible that they may find application in such fields as bread making. The yields of these polysaccharides from NID would be approximately 6.6% DM for the arabinoxylan precipitated at the lower concentrations of ethanol, and 11.5% DM for that precipitated between 45 and 90% ethanol.

The investigations described here do not give any indication of whether the nitrogenous components measured in the soluble fractions are associated with the polysaccharides. This would be another area worthy of further investigation.These studies have quantified the effects of alkali treatment on the carbohydrate and lignin fractions of draff and characterised the changes that occur. In this way, the data provide the basis for an explanation of the increased digestibility of the fibre fraction of MDG following $Ca(OH)_2$-treatment in terms of hydrolysis of linkages within the plant cell wall and the release of soluble polysaccharide components.

CHAPTER NINE

INVESTIGATIONS INTO THE WET PRESERVATION OF $Ca(OH)_2$-TREATED SPENT GRAINS

9.1 Introduction

Whilst many experiments have been carried out to investigate the immediate increase in the digestibility of various fibrous residues by the action of alkali (reviewed by Jackson, 1977), relatively little work has been done on the subsequent storage of the treated materials. Wilkinson and Gonzalez Santillana (1978) carried out a detailed investigation into the effects of sodium hydroxide and other alkalis on the digestibility and chemical composition of barley straw which was ensiled anaerobically for 90 days after treatment. They reported that the level of alkali used affected the profile of the volatile fatty acids. At sodium hydroxide levels of less than 5% of the DM, they found less lactic and acetic acids and more butyric acid than at higher alkali levels. After 16 days of subsequent exposure to the air mould growth was reduced with increasing concentration of sodium hydroxide and with lower levels of moisture.

The composition of silages made with sodium hydroxide treated crop residues has been reviewed by Wilkinson (1984). It was concluded that the preservation of such materials can best be achieved by anaerobic storage at high pH rather than the low pH which results from fermentation more usually associated with ensilage. It was also noted that in crops of high water content (>70%) and/or crops containing relatively high levels of fermentable carbohydrate, the addition of sodium hydroxide may buffer any fermentation thereby inducing secondary, clostridial fermentation.

Calcium hydroxide has been used to upgrade and subsequently ensile many different crop residues including; maize (Klopfenstein and Owen, 1981), wheat (Paterson *et al.*, 1980), barley (Owen and Nwadukwe, 1980; Wilkinson and Gonzalez-Santillana, 1978). It has also been applied to

spent grains in a storage and lamb feeding experiment (Abrams *et al.*, 1983). Generally, the use of calcium hydroxide to treat fibrous residues prior to ensiling has been characterised by butyric fermentations and moulding. Abrams *et al.* (1983) ensiled wet distillers grains (31% DM) with 3 g $Ca(OH)_2$/kg DM and reported butyric type fermentations. In drier residues such as maize cobs (40 -45% moisture) very little fermentation has been observed but moulding has been reported (Paterson *et al.*, 1980). This is supported by similar observations reported by Owen and Nwadukwe (1980) and Bass *et al.* (1982).

The ensilage and long term storage of spent grains without alkali treatment has been investigated (Hyslop *et al.*, 1989). In this study it was reported that storing draff uncompacted and poorly sheeted led to high dry matter losses due to moulding, effluent and invisible losses which were reduced on compaction. The long-term storage characteristics of uncompacted spent grains and the reported effects of alkali treatment on wet crop residues would suggest that the storage of calcium hydroxide treated spent grains with a high level of fermentable carbohydrate would require the maintenance of a high pH to achieve adequate preservation.

Johnson *et al.* (1987) and Johnson and Huber (1986) carried out a series of experiments investigating the short-term storage characteristics and the feeding value of ammonia-treated Brewers and Distillers wet grains. They found that ammonia treatment (4% DM) of wet grains led to effective preservation under both aerobic and anaerobic conditions with lower DM losses and less mould growth after 14 days when the grains were sampled compared to grains ensiled without ammonia. The pH of the grains treated with 4% ammonia remained above pH 9.6 in both aerobic and anaerobic storage. Addition of ammonia decreased the fall in pH over the storage period and increased the level of water soluble carbohydrates in the silages. The lack of moulding was explained by the antifungal action of ammonia and by the direct chemical action of ammonia (Britt and Huber, 1975).

The alternative to wet storage is to dry the treated product. This is expensive since spent grains are generally of the order of 25% dry matter and is something which, on an industrial scale has been resisted.

If a method of wet storage of calcium hydroxide treated draff could be found then the on-farm treatment of spent grains could be a possibility. Calcium hydroxide has the advantage over other commonly used alkalis such as sodium hydroxide or ammonia, in that it is less corrosive and safer and easier to handle. The following experiments therefore were carried out in order to characterise the long term storage of $Ca(OH)_2$-treated spent grains and to investigate treatments which may overcome the moulding and the butyric secondary fermentations reported in the literature.

Two experiments were carried out to investigate the effect of urea on the wet storage characteristics of untreated and $Ca(OH)_2$-treated spent grains. In the first experiment, MDG treated with $Ca(OH)_2$ at 80 g $Ca(OH)_2$/kg DM was stored either aerobically or anaerobically with four levels of urea. The grains were then sampled after 7, 28 or 60 days of storage at ambient temperature and the pH, NH_3 and urea N and fermentation products were measured.

In the second experiment spent barley, maize and wheat grains were treated with three different levels of $Ca(OH)_2$, with and without the addition of urea, and stored at ambient temperature in loosely sealed polythene bags. The bags were checked after 30 days and those which were completely mouldy were discarded. The remaining bags were opened after two months and measurements of pH and fermentation characteristics were made together with an assessment of mould development.

9.2 Experiment 1.

Methods and Materials

The first experiment was a 4 x 3 x 2 factorial design in which 4 levels of urea (0, 5, 10 and 15 g urea/kg FW), three storage times (7, 28 and 60 days) and two storage conditions (aerobic and anaerobic) were compared. 24 kg of MDG was mixed with 20g $Ca(OH)_2$/kg FW and sealed in a large polythene bag. This was held at 60°C for 16 hours. The grains were treated with different levels of urea and stored as shown in Table 9.1.

Table 9.1. Experimental Design.

Level of Urea (g/kg FW)	Aerobic (+)/ Anaerobic(-)
0	(+)
	(-)
5	(+)
	(-)
10	(+)
	(-)
15	(+)
	(-)

The initial pH of all samples was measured. For each treatment, triplicate amounts of approximately 1 kg were tightly sealed in polythene bags, evacuated to ensure anaerobic conditions, tied tightly with string and placed under 10 kg weights to simulate ensilage under anaerobic conditions. The remaining three 1 kg portions were loosely packed in plastic buckets (capacity 2 l) with snap-on lids to give aerobic storage conditions.

An aerobic and an anaerobic sample at each level of urea treatment was opened after 7, 28 and 60 days. The pH was measured immediately and a water extract was made by shaking a 20 g subsample of draff in 100 mls of distilled water for 30 minutes and then filtering. Water extracts were frozen until they were analysed for ammonia, urea, and fermentation products. The ammonia and urea were measured using the indophenol blue method, before and after incubation of a sample of the extract with urease. Fermentation products were measured using GC analysis. Details of analytical techniques are given in Chapter 2.

The data was analysed statistically by analysis of variance using the EDEX statistical package.

Results and Discussion

The changes in pH measured are given in Table 9.2. There were significant differences between the pH of aerobically and anaerobically stored samples ($p<0.01$), mean values were 10.33 and 10.79 respectively. The pH of treatments at each level of urea tended to drift downwards with time although there were no significant differences in pH due to level of urea or to the urea x time interactions. Interactions between level of urea and the method of storage were not significant ($p<0.05$).

Table 9.2. The Effect of Level of Urea, Storage Time and Method of Storage on the pH of MDG

Storage Time		Aerobic				Anaerobic		
(days)	0	7	28	60		7	28	60
Level of Urea (g/kg FW)	0	11.27	10.74	10.28	9.97	11.17	10.63	10.59
	5	11.04	10.78	10.56	9.98	11.10	10.89	10.71
	10	10.85	10.36	10.06	9.76	10.68	10.44	10.11
	15	10.60	10.67	10.54	10.15	10.86	10.71	10.57

Total concentrations of fermentation acids were slightly higher in aerobic than in anaerobically stored treatments (7.81 vs. 7.35 respectively) as shown in Table 9.3. There were no significant differences ($p<0.05$) in fermentation end products due to storage time, level of urea or method of storage. Concentration of fermentation end products were low compared to levels in materials preserved by fermentation (e.g. grass silage) with acetate being predominant.

Table 9.3 The Effect of Method of Storage on MDG Fermentation Characteristics

Parameter (g/kg FW)	Anaerobic	Aerobic	SE
Ethanol	0.10	0.14	0.026
Acetate	6.55	6.24	0.229
Propionate	0.06	0.03	0.015
Butyrate	<0.01	<0.01	
Iso Butyrate	0.05	0.02	0.025
Lactic Acid	1.05	0.92	0.086
Total Fermentation Products	7.79	7.24	0.340

All containers, when initially opened, smelt strongly of ammonia. The level of ammonia measured in the samples remained approximately 0.25 g/kg FW and did not vary significantly with time or level of added urea. Even grains not treated with urea had a similar ammonia level which suggests that the $Ca(OH)_2$ treatment in the absence of added urea causes the release of ammonia in the stored MDG. Ammonia may have arisen from the breakdown of draff protein under alkaline conditions with the subsequent release of gaseous ammonia. Higher levels of ammonia were observed for anaerobic than for aerobic treatments (0.26 and 0.23 respectively), which coincided with higher pH values (10.79 and 10.33) and although differences in ammonia concentration were not significantly different at $p< 0.05$. Whether the high levels of

ammonia were the cause of the high pH or the high pH caused the release of high levels of ammonia remains a matter for speculation.

Urea levels in the samples did not vary significantly with time but were closely related to the level of urea applied at the time of treatment as shown in Table 9.4.

Table 9.4 The Degradation of Urea in $Ca(OH)_2$-Treated Spent Grains.

Treatment Level (Urea g/kg FW)	Residual Urea (g/kg FW)	% Urea Undegraded
0	0	-
5	3.83	76.6
10	7.96	79.6
15	10.79	71.9

The proportion of urea degraded to ammonia was independent of the level of urea applied at the time of treatment. These results show that only approximately 25% of the added urea was degraded at all levels of application. For the rapid breakdown of urea to ammonia, a source of urease enzyme is required. Under the conditions of treatment (both at the distillery and at $Ca(OH)_2$ application) it is likely that most if not all endogenous enzymes are denatured. Addition of an exogenous source of urease may increase urea breakdown. However, the pH in $Ca(OH)_2$-treated MDG is not optimal for urease activity so that even if a source of urease were provided, urea breakdown may still be limited. Addition of urea and urease would in any case seem to be superfluous since $Ca(OH)_2$ treatment releases ammonia and optimisation of ammonia concentration in treated MDG may best be achieved by storing the product under anaerobic conditions and by preventing the immediate escape of ammonia liberated as a result of treatment.

Addition of urea to straw and other fibrous feeds which are low in endogenous rumen degradable protein (RDP) has a secondary benefit as it provides supplementary RDP. Since MDG already contain approximately 200 g/kg DM crude protein it is not RDP deficient as a feed and this benefit would not apply.

Table 9.5. The Effect of Urea on the Fermentation Characteristics of $Ca(OH)_2$-Treated MDG. (Means of aerobic and Anaerobic Samples)

Parameter (g/kg FW)	Level of Urea g/kg FW 0	5	10	15	SE
Ethanol	0.09	0.11	0.17	0.11	0.037
Acetate	7.04	6.77	5.99	5.77	0.324
Propionate	0.03	0.03	0.03	0.08	0.022
Butyrate	<0.01	<0.01	<0.01	<0.01	
Iso Butyrate	0.09	0.04	<0.01	<0.01	0.036
Lactic Acid	1.24	1.05	0.90	0.76	0.121
Total Fermentation Products	8.50	7.79	7.09	6.67	0.481

The mean concentrations of the fermentation acids for the different urea levels are given in Table 9.5. Although none of the differences due to inclusion of urea were significant ($p<0.05$) there were trends towards lower acetate and lactate at higher levels of urea. This is reflected in the concentration of total fermentation products in the fresh weight which is also reduced. Possible explanations are that urea reduces the production of these acids or alternatively, stimulates greater aerobic activity leading to greater losses of the volatile acids.

Table 9.6 shows that the concentrations of the fermentation end products declined with increasing storage time. The levels of fermentation products were generally similar to those observed in

Table 9.6 The Effect of Duration of Storage on Mean Fermentation Characteristics of $Ca(OH)_2$-Treated MDG.

Parameter (g/kg FW)	7d	28d	60d	SE
Ethanol	0.10	0.11	0.16	0.032
Acetate	6.79	6.65	5.74	0.280
Propionate	0.03	0.03	0.07	0.019
Butyrate	<0.01	<0.01	<0.01	
Iso Butyrate	0.07	0.02	0.01	0.031
Lactic Acid	1.13	0.99	0.85	0.105
Total Fermentation Products	7.95	7.80	6.79	0.417

ammonia treated brewers grains (Johnson *et al.*, 1987) and untreated draff (Hyslop and Roberts, 1988). Concentrations of fermentation products in draff are much lower than those found in grass silages. Although grass silages vary widely, concentrations for a typical lactate silage made from perennial ryegrass are shown in Table 9.7 (from McDonald, 1981).

Table 9.7 Typical Concentrations of Fermentation Acid for Lactate Silage made from Perennial Ryegrass. (McDonald, 1981)

Fermentation Products (g/kg FW)	
Ethanol	2.28
Acetate	6.84
Propionate	0.38
Butyrate	0.19
Lactate	19.38
Total Fermentation Products	29.07

By comparison, the MDG fermentation produced much lower levels of VFAs generally than grass silages (mean total VFAs 7.57 g/kg FW for draff against 29.07 g/kg FW for grass). MDG also gave proportionally higher levels of acetate than grass silages, although in absolute terms concentrations are similar. Wilkins *et al.* (1971) reported a negative correlation between silage dry matter intake and high levels of acetate in grass silages.

A notable difference between the typical fermentation characteristics of MDG and those for MDG which had been $Ca(OH)_2$ treated was that treatment reduced levels of butyrate. Butyrate levels in untreated MDG have been reported as approximately 2 g/kg FW (Hyslop and Roberts, 1988) whereas in $Ca(OH)_2$-treated MDG butyrate was barely detectable in the present experiment.

9.3 Experiment 2.

Methods and Material

This experiment was of a 3 x 4 x 2 factorial design in which three spent grains (wheat, barley and maize draff) were treated with four levels of $Ca(OH)_2$ (0, 50 75 or 100 g/kg DM), with and without urea at 40 g/kg DM. A dry matter determination was carried out on each type of grain which was used to determine the weight of $Ca(OH)_2$ and urea required to achieve the correct concentrations in the dry matter. DM contents were approximately 24, 26 and 32% for the barley, wheat and maize grains respectively. Duplicate samples of each treatment were prepared. The grains were treated with either 0, 50, 75 or 100g $Ca(OH)_2$/kg DM and with either sufficient of a 40% urea solution to give a concentration of 40 g urea/kg DM, or an equal volume of water. The pH of each treatment was measured immediately (initial pH) and the grains were sealed in polythene bags. The bags were double sealed using a heat sealing device to prevent the free movement of air into and out of the bags, however the polythene was only thin and it was unlikely that diffusion of air was totally prevented.

The bags of grains were left at ambient temperature (15°C) and were visually checked after 30 days. Any samples which were completely mouldy were discarded. The remaining bags were left for a further 30 days and after this time the pH was measured again, the percentage of mouldy grains in each bag was assessed and water extracts were made as described in experiment 1 for later analysis by GC.

Data was analysed for statistical significance using analysis of variance using the EDEX statistical package.

Results

Addition of urea had no significant effects on the parameters measured and the tabulated results are the means of samples stored with and without added urea.

pH

Initial pH values measured for each spent grain at the different levels of $Ca(OH)_2$ treatment and values are given in Table 9.8

Table 9.8. Initial pH.

		Barley	Wheat	Maize
$Ca(OH)_2$	0	3.80	3.90	3.85
(g/kg DM)	50	11.73	12.11	11.81
	75	12.27	12.41	12.34
	100	12.63	12.63	12.44

The results for the initial pH (the pH taken immediately after the addition of the $Ca(OH)_2$), show that the spent grains all started with a similar initial pH and that addition of a fixed amount of $Ca(OH)_2$ had a similar effect on each species, although the spent wheat grains gave slightly higher pH values at 50 and 75 g $Ca(OH)_2$/kg DM levels.

Table 9.9. Final pH of Stored Grains (at Day 60).

		Barley	Wheat	Maize
$Ca(OH)_2$	0	4.38	M	6.73
(g/kg DM)	50	M	M	7.85
	75	9.44	8.14	9.99
	100	10.60	10.95	11.11

SE = 0.402

M= Moulded and discarded at Day 30

The values given in Table 9.9 are means of measurements of final pH from stored bags of grains after 60 days. The 0 and 50g $Ca(OH)_2$/kg DM treatments for wheat and the 50g $Ca(OH)_2$/kg DM treatment for barley were not analysed for pH or fermentation end products because they were discarded on day 30 due to the samples being completely mouldy. Final pH not significantly affected by the inclusion of urea ($p<0.05$), however level of $Ca(OH)_2$ and type of grain and the interaction between these two factors were significant ($p<0.05$).

Percentage Mouldy

Table 9.10. Percentage of Grains Mouldy on Day 60

		Barley	Wheat	Maize
$Ca(OH)_2$	0	25	100	50
(g/kg DM)	50	100	100	75
	75	5	20	0
	100	0	0	0

The bags of grains were observed on day 30 of the trial and those which were badly moulded at that stage were discarded. These included all the bags which contained wheat draff treated at either 0 or 50 g/kg DM with $Ca(OH)_2$, and the barley draff treated with 50 g/kg $Ca(OH)_2$. The inclusion of 40 g/kg DM urea (10 g/kg FW) did not prevent moulding in any of these treatments.

Table 9.10 shows the proportion of each sample found to be mouldy after 60 days storage. Mould development was totally inhibited at the 100 g/kg level of $Ca(OH)_2$ for all grains but was very great at 0 and 50 g/kg levels. $Ca(OH)_2$ at 75 g/kg DM gave effective mould control for maize but only partial control for the wheat and barley spent grains. In all cases, the mould growth was restricted to the surface, except in those samples which were recorded as 100% mouldy, where mould growth penetrated the whole sample.

Fermentation Products

Table 9.11 gives the mean pH and concentrations of fermentation end products for the 75 and 100 g $Ca(OH)_2$/kg DM treatment levels. There were no significant differences in fermentation end products due to addition of urea, and mean values are therefore presented. There were significant effects on pH and concentration of propionate due to the interaction of type of grain and level of $Ca(OH)_2$. Other significant ($p<0.05$) differences were due either to the grain or level of $Ca(OH)_2$. The wheat draff had a lower mean final pH than the other spent grains and a higher concentration of butyrate after 60 days. The maize draff produced the highest level of total fermentation products and significantly higher levels of acetate than the other grains ($p<0.05$). Barley draff produced significantly more lactate and less acetate than the other grains but, in general, concentrations of all fermentation products were low.

Although ammonia and urea measurements were not carried out, samples which were treated with the higher levels of $Ca(OH)_2$ (75 and 100 g/kg DM) smelt strongly of ammonia when they were opened for sampling. This was particularly noticeable for the barley and maize draff treatments

but less so for the wheat draff.

Table 9.11 Mean pH and Concentrations of Fermentation Products of the Barley, Wheat and Maize Spent Grains Treated with 75 and 100 g $Ca(OH)_2$/kg DM after 60 Days Storage.

Parameter (g/kg FW)	Barley		Wheat		Maize		SE
	g $Ca(OH)_2$/kg DM						
	75	100	75	100	75	100	
pH	9.44^{b}	10.60^{c}	8.14^{a}	10.95^{c}	9.99^{b}	11.11^{c}	0.329
Ethanol	0.21^{ab}	0.20^{a}	1.00^{b}	0.08^{a}	0.01^{a}	0.03^{a}	0.264
Acetate	1.94^{a}	2.50^{a}	3.08^{ab}	2.02^{a}	3.67^{ab}	4.93^{b}	0.648
Propionate	$<0.01^{b}$	$<0.01^{b}$	0.05^{a}	0.04^{a}	0.01^{ab}	0.03^{a}	0.008
Isobutyrate	$<0.01^{a}$	$<0.01^{a}$	0.02^{b}	$<0.01^{a}$	$<0.01^{a}$	$<0.01^{a}$	0.005
Butyrate	0.01^{a}	$<0.01^{a}$	0.12^{b}	$<0.01^{a}$	$<0.01^{a}$	$<0.01^{a}$	0.032
Lactate	1.48^{c}	1.98^{d}	0.60^{ab}	0.85^{b}	0.39^{a}	0.57^{ab}	0.146
Total Fermentation Products	3.63	4.67	4.89	2.99	4.09	5.57	0.923

Means with different superscripts in the same row differ significantly ($p<0.05$).

The analysis of the 0 and 50 g/kg DM $Ca(OH)_2$ treatments which were measured after 60 days is given in Table 9.12. The mean VFA concentrations show that the barley draff, without added $Ca(OH)_2$, produced only low levels of total fermentation products, approximately a third of which was butyrate or isobutyrate. Samples which were stored without $Ca(OH)_2$ were darkened, particularly around the edges, suggesting that there had been diffusion of air into the thin polythene bag in which the grains had been kept. The maize draff

Table 9.12 Mean Concentrations of Fermentation Products of Barley and Maize Spent Grains with and without 50 g/kg DM $Ca(OH)_2$ Treatment after 60 Days Storage.

Parameter (g/kg FW)	Barley	Maize		SE
	g $Ca(OH)_2$/kg DM			
	0	0	50	
pH	4.38	6.73	7.85	0.402
Ethanol	0.01	0.02	0.04	0.231
Acetate	1.40	3.34	2.72	0.567
Propionate	0.09	0.16	0.06	0.014
Isobutyrate	0.11	0	0	0.025
Butyrate	1.01	0.06	0.01	0.060
Lactate	0.88	0.58	0.35	0.135
Total Fermentation Products	3.44	4.15	3.19	0.807

stored without $Ca(OH)_2$ treatment had a final pH of 6.73, and as with the $Ca(OH)_2$ treatments, a high proportion of the fermentation products were present as acetate. Total fermentation products levels for the both the $Ca(OH)_2$-treated and non-$Ca(OH)_2$-treated maize draff were also low. Very little butyrate or isobutyrate was measured in any of the maize draff treatments.

The final pH values and the fermentation characteristics of the spent grains, as with all ensiled feeds, are not independent. At the 50 g/kg level of $Ca(OH)_2$ application, the pH of the maize grains was approaching neutrality by the time the bags were opened. Although the final pH of the 50 g/kg treatments for wheat and barley draffs were not measured, it is likely that they were similar to that observed for the maize draff. The 50 g/kg DM level of $Ca(OH)_2$ treatment gave initial pH values very similar to the higher levels but provided insufficient alkali to maintain alkaline conditions during storage.

Discussion

Ensiling spent grains after treatment with calcium hydroxide depends on preservation by anaerobic storage at high pH rather than by fermentation of sugars to short chain organic acids. Little if any fermentation occurs in alkali-treated 'silages' although some bacteria can tolerate alkaline conditions and where there is sufficient free water, a limited amount of fermentation may occur.

Treating the spent grains with a low level of calcium hydroxide (e.g. 50 g/kg DM) induces a secondary, clostridial type fermentation, resulting in elevated levels of butyrate and increased moulding. At this level of treatment the spent grains are less well preserved than the untreated grains. There are two possible reasons for this; firstly the calcium hydroxide releases fermentable substrates (sugars) from the hemicellulose fraction whereas the untreated draff has little or no fermentable substrate. Secondly, unreacted alkali may buffer fermentation to the extent that the normal decrease in pH and dominance of lactic acid bacteria is prevented (Bolsen *et al.*, 1983). The provision of fermentable substrate is in itself not detrimental to preservation. However when the fermentation is buffered by the formation of calcium salts from unreacted alkali rather than free acids, clostridial rather than lactic fermentation is favoured.

Clostridial fermentation is optimal under neutral conditions and at a dry matter content of < 300g/kg (McDonald, 1981). The 50 g/kg DM levels of $Ca(OH)_2$ treatment provided optimal final pH for clostridial fermentation and the production of butyrate and protein breakdown products. The maize draff had a dry matter of approximately 330 g/kg compared to 260 and 240 g/kg for the wheat and barley draffs respectively. This provides a possible explanation as to why the maize draff was not as susceptible to spoilage as the wheat and barley draffs.

Moulding of the untreated and alkali-treated spent grains is the major problem with their wet storage although secondary butyric fermentations can also lead to spoilage as demonstrated by the

untreated barley draff. The presence of mould in spent grains may depend on four different factors;

1. The availability of oxygen. If the grains are not stored in an anaerobic manner the degree of moulding is greater.

2. The pH. When the pH approaches neutrality mould growth is favoured.

3. The water content. Grains which were drier (such as the maize draff) showed less moulding than the other grains.

4. Availability of substrate for mould growth. This could provide an explanation for the rapid moulding of the wheat draff which had the highest protein content.

Carpintero *et al.*, (1969) suggested that pH 4.2 was the critical value below which silages are adequately preserved. This however refers to acid preservation by fermentation and not to alkaline storage. In studies carried out on a variety of alkali-treated fibrous feeds butyric acid was observed whenever the pH was higher than pH 4 except when the pH was maintained above pH 10 (Flipot *et al.*, 1976; Bolsen *et al.*, 1983; Mowat, 1976; Schultz *et al.*, 1974 and Herrod-Taylor, 1980).

In the present experiment, when the grains were treated with highest levels of alkali (75 and 100 g $Ca(OH)_2$/kg DM), fermentation was virtually prevented and preservation was adequate. This is supported by the findings of Wilkinson and Gonzalez Santillana (1983). They reported that lactate and acetate were present in all silages and butyrate was present only in those treated with less than 50 g/kg sodium hydroxide. They stated that the optimum level of alkali for treatment of barley straw prior to ensiling was 75 g/kg straw DM.

The present studies suggest that for spent grains, reliable alkaline preservation using calcium hydroxide requires a minimum concentration

of 100g/kg grain DM. The higher requirement for calcium hydroxide compared to that for sodium hydroxide is because the former is a weaker base. Measurement by titration shows their base equivalents to be in the ratio of 100:66 for NaOH:$Ca(OH)_2$. Additionally the spent grains are of a lower dry matter content than straw and may have a higher buffering capacity. At a $Ca(OH)_2$ level of 75 g/kg DM, acceptable preservation of barley and maize draff may be achieved provided that anaerobic conditions are maintained.

Further work on the characterisation of the fermentation processes in draff is required, particularly with regard to the measurement of the stage at which fermentation acids are formed. Studies into the optimal conditions for mould growth in draff and $Ca(OH)_2$-treated draff are also justified if the treated residues are to be stored and fed wet on a large scale.

Conclusions

1. Inclusion of urea in calcium hydroxide treated spent grains has little effect on the storage characteristics.

2. Treating spent grains with 100g $Ca(OH)_2$/kg DM can maintain the grains at a high pH and prevent secondary fermentation and moulding.

3. Treatment of spent grain with 75 g $Ca(OH)_2$/kg DM gave acceptable preservation for maize and barley draffs but not for wheat.

4. Treating spent grains with low levels of $Ca(OH)_2$ (<75 g/kg DM) induces clostridial fermentation and mould growth.

5. Anaerobic storage conditions enhance preservation of the spent grains.

CHAPTER TEN

DISCUSSION

Results of the experiments described in the previous chapters on the effects of $Ca(OH)_2$-treatment of spent grains are summarised in Figure 9.1. It has been shown that the effects of $Ca(OH)_2$-treatment on the nutritional characteristics of spent grains are two-fold; the fibre is upgraded by cleavage of alkali-labile bonds in the cell wall structure, and the inhibitory effects of oil are ameliorated by the formation of calcium soaps. Both of these factors change the rate and pattern of fermentation of the grains in the rumen. The reduced inhibition of fibre digestion and the increased rate of fermentation due to solubilisation of carbohydrate increase the *in vivo* digestibility and dry matter intake resulting in increased animal performance.

Commercial Exploitation of NID as a Feedstuff

The increased *in vivo* digestibility due to $Ca(OH)_2$-treatment increases the nutritional value of spent grains as a feed. Exploitation of this improvement on a commercial scale has not proved easy. Treatment of the spent grains is most cost-effective when the lime can be added to the grains as they leave the mash tun. This makes use of the residual heat in the grains from the mashing procedure. Allowing the grains to cool naturally following lime-treatment, for example, by holding in a large storage bin would result in the maximum yield of soluble material. Unfortunately, it would also result in the formation of a large mass of sticky, dough-like material which could not be handled by existing handling and drying equipment in distilleries. A feasible solution would be the installation of high pressure wet extrusion equipment discharging directly onto a horizontal conveyer prior to drying. This however, would require substantial capital investment. Alternatively, the grains could be dried after only a short holding time. It has been shown that the length of time for which the grains

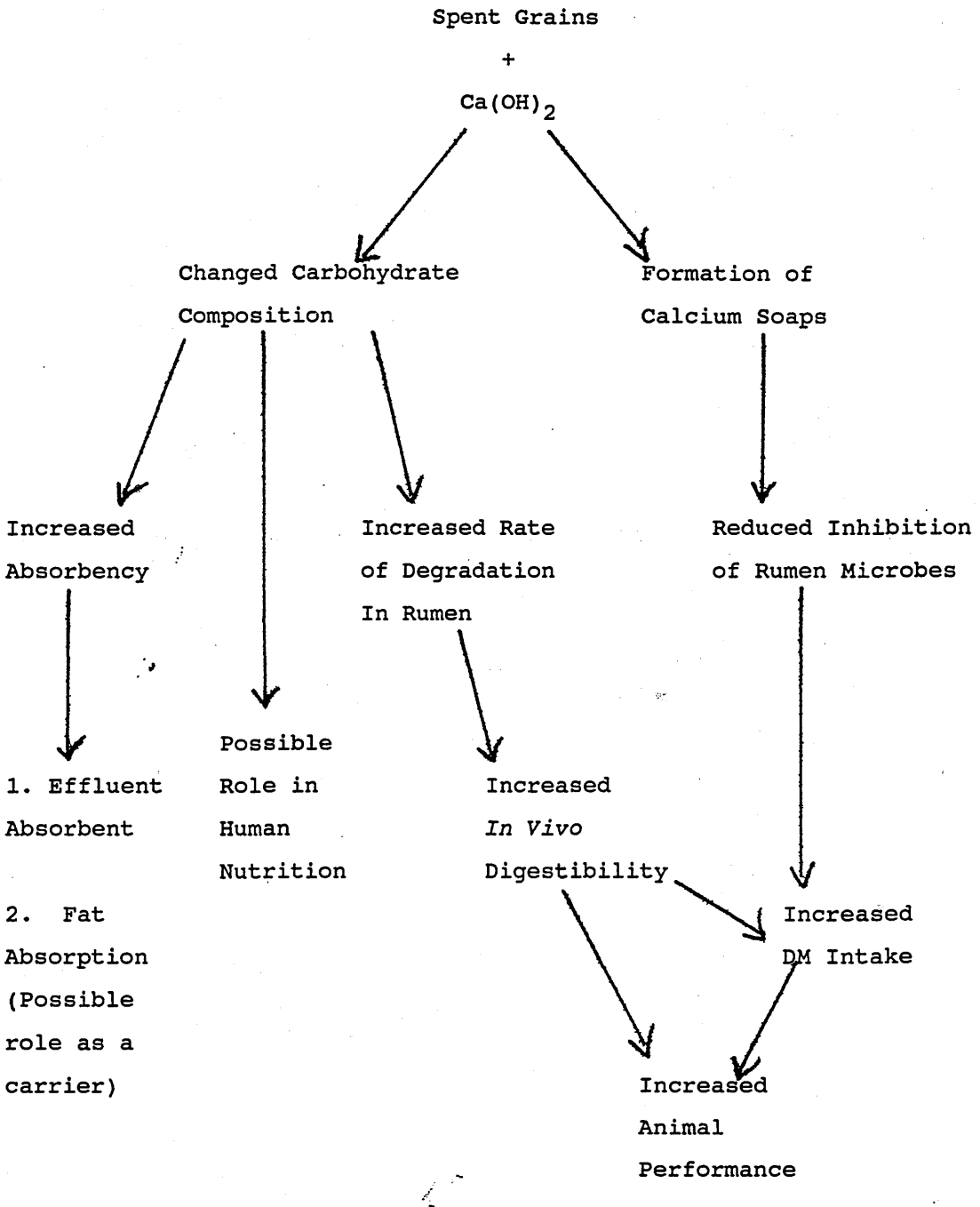
are held at elevated temperatures affects the formation of soluble material, but not the 24 hour digestibility (measured *in sacco*).

An additional commercial difficulty is the distilleries reluctance to dry draff without the incorporation of Pot ale. Pot ale syrup results from the evaporation of the yeast-rich wash remaining in the still after the alcohol has been removed by distillation. There is a limited demand for the syrup and consequently, the main method of disposal of this material is by mixing it with draff and drying it. This yields the product known as dark grains.

The possibilities of delivering wet NID directly from the distillery or even mixing draff with lime on-farm should be considered but neither option overcomes the difficulties of handling the dough-like material. If this was to be attempted on a large scale, further studies into the optimal levels of treatment for long term storage and preservation would be required to substantiate the findings of the small scale experiments described in chapter 9.

Ideally, the process should be fitted into an existing dark grains plant where treatment and drying could be carried out. The addition of pot ale would reduce the beneficial effects of $Ca(OH)_2$ treatment since the draff would be effectively diluted. The cost of drying and the practicalities involved in handling the treated material have been the main difficulties in exploiting NID. It has been suggested by commercial sponsors that the added value given by the nutritional improvements measured do not compensate for these difficulties.

The Effect of $Ca(OH)_2$-treatment of Spent Grains.



Other Potential Uses for NID

The changes in composition of the carbohydrate fraction following $Ca(OH)_2$-treatment also changed the physical properties of the grains. The dough-forming property of $Ca(OH)_2$-treated spent grain gave a substance which could be formed into different shapes and allowed the extrusion of NID through a 1/2 inch die forming the pellets which were used in the lamb feeding experiment.

A further effect on the physical properties was to increase the water-holding capacity of the spent grains. This effect is probably related to an observation by Ternrud (1987) who noted that alkali treated straw was more easily wetted than untreated straw and speculated that this effect could facilitate the colonisation of the straw by rumen micro-organisms.

The absorptive nature of $Ca(OH)_2$-treated spent grains initiated the investigations into their use as a silage effluent absorbent. This application proved not to be practical when tested in laboratory-scale silos, for two reasons: Firstly, the $Ca(OH)_2$-treated grains buffer strongly between pH 5 and 6 allowing secondary fermentations and the formation of butyric end-products in the silage. Secondly, the absorptive advantage conferred by $Ca(OH)_2$-treatment was lost after a few days, presumably due to the fermentation of the absorbent polysaccharides in NID. The use of NID as an absorbent in non-fermentative situations and where its buffering capacity is not a disadvantage may still be feasible.

The use of NID as a carrier for oils in ruminant diets is a further role which has been investigated. Although the experiments described in this study suggested that the process conferred only limited protection against biohydrogenation, they have shown that oil-supplemented NID can be fed at high levels of dietary inclusion without causing ruminal upset. Oil-supplemented NID has a further advantage in that it forms a free-flowing material when dry and would provide an ideal way of incorporating oil into a home-mix diet. It is doubtful whether this would be practical on a commercial scale in its

present form since it requires 48 hours at 60°C followed by drying. However, if it could be marketed as a high value oil supplement, at a price which exceeded the costs of processing and drying then it could offer a potential means of incorporating fat or oil safely into ruminant diets.

The experiments in which oil-supplemented NID has been fed have shown that calcium soaps of unsaturated fatty acids are not substantially protected from biohydrogenation. This is thought to be due to the dissociation of the less saturated soaps at higher rumen pHs as demonstrated by Sukhija and Palmquist (1990). Coating fat droplets with formaldehyde-treated protein has proved, in carefully controlled experiments, to be an effective method for by-passing ruminal biohydrogenation (see Scott and McDonald, 1977). However, the technology has not been widely adopted in practice due to the practical difficulties of large scale quality control and due to concern over the safety of feeding large quantities of formaldehyde-treated feed.

A safer method of protecting fat using the protein encapsulation approach may be to react the protein with sugars under such conditions that Maillard reactions occur and to coat the droplets in these complexes. The sugar-protein complexes would be resistant to breakdown in the rumen. Wallace (1989) used this method to encapsulate protein supplements and observed that their degradability *in vitro* was depressed. This method may have the disadvantage of oxidising the unsaturated oil under the treatment conditions required to form such complexes and is unlikely to be practical on a larger scale. Nevertheless, this could provide a potential method of protection against biohydrogenation and is worthy of further investigation.

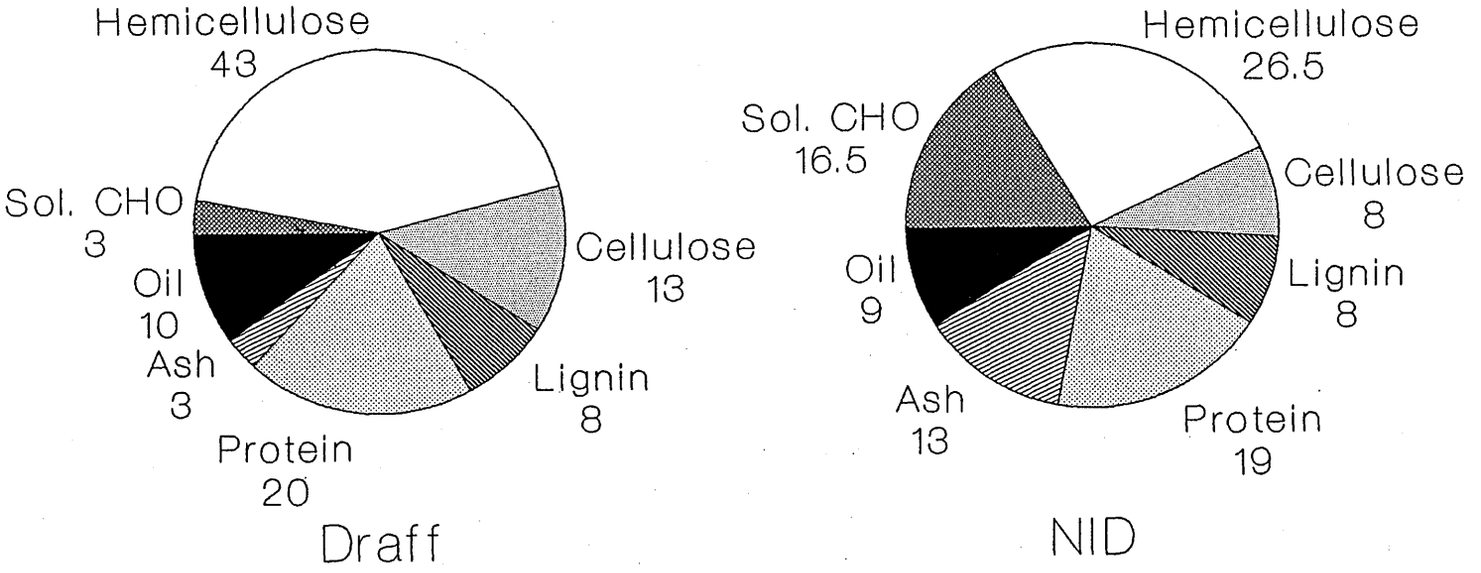
An alternative method may be to coat droplets of unsaturated oil with calcium soaps of saturated fatty acids, for example palm fatty acid distillate. Such calcium soaps have been shown to be stable in the rumen and have even been used as a method of protecting dietary protein from ruminal breakdown (Sklan, 1989). Further protection would be provided if the oil was adsorbed first onto a fibrous carrier

such as NID since this has been shown to reduce the inhibitory effect of oil on rumen function. A potential disadvantage with this would be that the fibre would also be protected from ruminal breakdown. However, if the supplement was fed in small quantities (which it would be in practical rations) then this would be of little consequence especially as compensatory fibre digestion in the hind gut may be anticipated.

The effect of treatment on the composition of dry matter of spent grains is shown in Figure 10.1 which shows values for a typical barley draff before and after $Ca(OH)_2$-treatment. The greatest effect of treatment occurs on the NDF fraction, which contains lignin, cellulose and hemicellulose. NDF is reduced from 640 to 425 g/kg DM mainly as a result of solubilisation of hemicellulose, but also due to a small reduction in acid detergent fibre (ADF, made up of cellulose and lignin). The proportion of lignin in spent barley grains was found to be unaffected by $Ca(OH)_2$-treatment but ADF was reduced from 210 to 160 g/kg DM suggesting a reduction in concentration of cellulose. The higher level of ash in NID compared to untreated draff would, in absolute terms, account for part, but not all, of this increase. As cellulose has been determined indirectly as the difference between two measurements (ADF-lignin), each subject to it own analytical error, it is not possible to be certain that there is a real reduction in cellulose content. The evidence from the literature regarding the alkali treatment of straw suggests that cellulose and ADF levels are unaffected by alkali treatment.

The apparent reductions in NDF and ADF reported may in part result from the increased friability of $Ca(OH)_2$ -treated material, increasing losses during the filtration stages of the determinations. This phenomenon was also observed in increased zero-time losses of NDF from nylon bags following $Ca(OH)_2$-treatment described in Chapter 3 when *in sacco* measurements of NID degradation were made. Theoretically this is not possible since NDF is a measure of fibre which is insoluble in neutral detergent fibre and therefore should not be washed from the bag by cold water. Thus $Ca(OH)_2$-treatment may increase the amount of very small particles in draff leading to increased filtration losses

Figure 10.1 The Effect of Lime Treatment on MDG Composition



during the measurement of the fibre fractions. Further work is required to investigate these apparent discrepancies.

A further possible application for NID may be as a source of non-starch polysaccharide (NSP) and fibre in human nutrition. There is particular interest at present in the effects of soluble NSP on carbohydrate and lipid metabolism in man. Although the precise mechanisms of the actions of materials such as guar gum are not completely understood, they find application in the management of diseases such as diabetes mellitus and some forms of hyperlipidaemia.

A commonly occurring source of fibre known to cause these effects is that found in oat bran. Oat bran contains substantial quantities of an unbranched (1-3)(1-4)-ß-D-glucan (Wood, 1986). Oats have been shown to produce a low glycaemic response (Lund *et al.*, 1989) which, it was suggested, was due to the increased viscosity of intralumenal contents of the small intestine delaying absorption of constituent carbohydrates. Oats have also been shown to exert negative influences on plasma cholesterol levels in humans (Judd and Truswell, 1981) and although this effect has been the subject of much recent research the mechanisms behind it are yet to be fully elucidated. Current theories are that viscous non-starch polysaccharides increase faecal losses of bile salts as a result of interference with their recovery in the distal small intestine (Lund *et al.*, 1989) or cause a reduction in endogenous cholesterol synthesis in the small intestine (Jonhnson, 1989). Alternatively, the increased absorption of fermentation end-products resulting from hind gut fermentation of non-starch polysaccharides may cause changes in hepatic lipid metabolism (Anderson and Bridges, 1981).

The work described in Chapter 8 showed that NID contained a high proportion of arabinoxylans. There is a possibility that this component may have the similar beneficial effects to those observed with oats. This possibility is currently under investigation.

Studies on the effects of non-starch polysaccharides in pig diets (reviewed by Low, 1989) have shown them to increase endogenous

secretions from the salivary glands, the stomach, liver, exocrine pancreas and intestinal wall. These effects were often accompanied by increased protein synthesis in the gut tissue. It was suggested that such non-starch polysaccharides had a negative effect on the energetic efficiency of conversion of animal feed to animal tissue, but often resulted in leaner carcasses from animals fed diets with high levels of NSP.

The use of NID in monogastric diets generally is an area worthy of further investigation. Although the formation of calcium soaps would not have the benefits observed for the ruminant, the upgrading of the fibre may improve diet digestibility. Such an effect would occur by increasing the digestibility of the feed in the hind gut where the transit time for digesta is generally in the range of 20 - 40 hours (Low, 1985). The monogastric animal itself has no capacity to digest complex polysaccharides but the solubilised hemicellulose would be readily fermented by micro organisms in the hind gut.

The potential energy available to the monogastric would be less than that obtained from the usual enzymic digestion of feeds in the stomach and small intestine due to losses as heat of fermentation and methane. Additionally, NID contains high levels of ash. Both of these factors would result in the ME being too low for inclusion at high levels in the diets of most highly productive livestock but it could be used in less productive diets such as dry sow rations. The use of chemically treated straw in pig diets has been reviewed by Bergner (1981). It was concluded that chemical treatment of straw was suitable for pig diets and that there would be merit in the treatment of other feedstuffs for this purpose.

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Appendix 1. Effect of Treatment Conditions on Final pH

Analysis of Variance

DF	SS	MS	f	Prob	
Time	3	3.8071	1.2690	6.601	**
Temp	3	19.6873	6.5624	34.133	***
Lime	3	482.2521	160.7507	836.117	***
L*Te	9	14.9406	1.6601	8.635	***
Ti*L	9	4.4479	0.4942	2.571	*
Ti*Te	9	0.5299	0.0589	0.306	
Error	27	5.1910	0.1923		
Total	63	530.8558			

Lime*Temp Table

Lime	20	40	60	80	Mean
0	4.33	4.22	3.80	3.70	4.01
40	8.23	6.21	7.25	7.29	7.25
80	11.22	10.65	9.65	8.84	10.09
120	11.91	11.76	10.81	9.76	11.06

SE 0.219, 0.110

Appendix 2. Effect of Treatment Time on NDF Content and In Sacco NDF Digestibility

Treatment Time (h)	NDF (g/kg DM)	In Sacco Loss 0h	24h
0	627	0.108	0.820
4	563	0.114	0.723
8	519	0.051	0.779
12	530	0.127	0.780
16	532	0.173	0.798
24	536	0.231	0.774

Appendix 3. NID and MSBP *In sacco* OM Degradation

Incubation Time (h)	OMD (SE) NID	SBP
0	0.556 (0.0052)	0.290 (0.0165)
3	0.604 (0.0045)	0.332 (0.0054)
5	0.636 (0.0082)	0.369 (0.0112)
8	0.654 (0.0328)	0.434 (0.0557)
16	0.766 (0.0302)	0.653 (0.1276)
24	0.820 (0.4150)	0.804 (0.0636)
48	0.912 (0.1428)	0.971 (0.0024)
72	0.933 (0.0096)	0.969 (0.0028)

Appendix 3 (cont) Analysis of Variance of 'a','b' and 'c' terms for *in sacco* digestibility of MSBP and NID

'a' Term Analysis-of-Variance Table

	DF	SS	MS	F	Prob
S	2	15.0726	7.5363	2.867	
F	1	1534.7204	1534.7204	583.930	***
Error	2	5.2565	2.6283		
Total	5	1555.0494			

CV = 4.16 per cent

S.F Table SE 1.621 1.146 0.936

Sheep	NID	MSBP	Mean
1	54.18	19.69	36.93
2	55.80	25.81	40.80
3	54.84	23.35	39.09
Mean	54.94	22.95	38.94

'b' Term Analysis-of-Variance Table

	DF	SS	MS	F	Prob
S	2	110.1004	55.0502	1.092	
F	1	2830.7678	2830.7678	56.133	*
Error	2	100.8596	50.4298		
Total	5	3041.7278			

CV = 11.29 per cent

S.F Table SE 7.101 5.021 4.100

Sheep	NID	MSBP	Mean
1	43.04	80.63	61.83
2	39.41	77.11	58.26
3	41.07	96.11	68.59
Mean	41.17	84.62	62.89

'c' Term Analysis-of-Variance Table

	DF	SS	MS	F	Prob
S	2	0.0019	0.0009	12.066	
F	1	0.0000	0.0000	0.053	
Error	2	0.0002	0.0001		
Total	5	0.0020			

CV = 18.74 per cent

S.F Table SE 0.009 0.006 0.005

S	1	2	Mean
1	0.06	0.08	0.07
2	0.04	0.04	0.04
3	0.03	0.02	0.03
Mean	0.05	0.05	0.05

Appendix 4. *In vitro* **OMD with added oil and alkali**

Oil (mg)	$Ca(OH)_2$ (mg)	IVOMD	Oil (mg)	NaOH (mg)	IVOMD	Oil (mg)	IVOMD
61.4	2.5	0.564	60.8	2.5	0.431	0.00	0.534
62.1		0.535	62.1		0.453	0.00	0.557
59.7	5.0	0.509	60.7	5.0	0.439	31.4	0.523
61.6		0.502	60.2		0.421	31.8	0.524
59.7	7.5	0.532	59.7	7.5	0.445	61.6	0.506
59.2		0.502	59.9		0.390	60.3	0.471
59.6	10.0	0.513	90.1	10.0	0.403	93.4	0.448
61.6		0.506	93.3		0.406	90.4	0.422

Appendix 5. *In vivo* **Digestibility Coefficients for NID**

Parameter	Sheep No. 1	2	3	4	Mean	SE
DM	0.572	0.630	0.599	0.598	0.600	0.0119
OM	0.650	0.718	0.688	0.678	0.642	0.0254
NDF	0.685	0.764	0.746	0.731	0.732	0.0169
GE	0.657	0.696	0.653	0.658	0.666	0.0101
Nitrogen	0.510	0.580	0.547	0.604	0.560	0.0204

Appendix 6. Analysis of Variance of Supplement DMI

Source of Variation	DF	SS	SS%	MS	
p Stratum					
f	2	25760926	12.79	12880463	29.014
Residual	12	5327214	2.65	443934	19.943
TOTAL	14	31088140	15.44	2220582	99.755
p*UNITS* Stratum					
d	59	149943376	74.45	2541413	114.168
d.f	118	4600333	2.28	38986	1.751
Residual	708	15760317	7.83	22260	
TOTAL	885	170304032	84.56	192434	
GRAND TOTAL	899	201392176	100.00		

GRAND MEAN 1738.7

Total Number of Observations 900

	NID	MD	CN
Feed Means	1849.1	1449.7	1867.4

Standard Errors of Difference (Feed) Rep= 300 SED= 54.40

Stratum SE and CV

Stratum	DF	SE	CV%
p	12	86.02	4.9
p.*UNITS*	708	149.20	8.6

Appendix 7. Analysis of Variance of Hay DMI

Source of Variation	DF	SS	SS%	MS	
p Stratum					
f	2	7327916	23.73	3663958	10.065
Residual	12	4368300	14.15	364025	36.057
TOTAL	14	11696215	37.88	835444	82.752
p*UNITS* Stratum					
d	59	9578204	31.02	162342	16.080
d.f	118	2457313	7.96	20825	2.063
Residual	708	7147800	23.15	10096	
TOTAL	885	19183316	62.12	21676	
GRAND TOTAL	899	30879532	100.00		

GRAND MEAN 513.0

Total Number of Observations 900

	NID	MD	CN
Feed Means	516.3	400.8	621.8

Standard Errors of Difference (Feed) Rep= 300 SED= 49.26

Stratum SE and CV

Stratum	DF	SE	CV%
p	12	77.89	15.2
p.*UNITS*	708	100.48	19.6

Appendix 8. Feed Time Course OMD (%).

Sheep	Feed	Incubation Time (Hours)						
		0	3	5	8	17	24	48
1	Min.	16.00	23.43	36.40	42.93	52.67	59.22	66.26
2	Draff	16.36	25.09	29.13	45.73	53.26	58.38	65.08
3		15.88	23.57	22.94	28.22	55.21	54.48	52.29
4		16.49	25.20	29.33	39.41	55.16	57.85	66.32
1	NID	44.52	54.61	55.19	60.23	72.62	83.14	87.80
2		45.53	54.07	55.94	62.14	72.23	79.54	82.27
3		45.20	53.69	55.57	58.11	66.98	68.58	85.77
4		42.91	54.74	56.12	61.86	75.61	77.98	86.27
1	Carrs	49.26	54.68	57.29	60.09	69.60	73.71	80.95
2	Nuts	52.72	56.59	57.40	59.48	68.10	70.13	81.48
3		48.62	56.23	56.76	57.16	67.97	70.60	70.83
4		48.86	57.63	58.17	61.91	64.66	71.84	81.44
1	Hay	7.29	10.18	10.74	14.64	30.41	38.62	55.32
2		4.61	12.32	10.89	11.55	24.68	40.94	43.85
3		6.11	9.29	10.41	12.95	14.94	21.26	29.42
4		7.71	11.80	12.23	15.56	36.05	37.20	51.61

Analysis of Variance Degradation Constants for Feed OM

A Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	1.5093	0.5031	0.154	
F	3	6196.8861	2065.6387	633.008	***
Error	9	29.3688	3.2632		
Total	15	6227.7642			

Feed	MD	NID	CN	Hay
Mean	14.55	45.45	50.97	4.77
SE	0.903			

B Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	375.9136	125.3045	1.301	
F	3	720.0971	240.0324	2.492	
Error	9	866.9854	96.3317		
Total	15	1962.9961			

Feed	MD	NID	CN	Hay
Mean	49.87	45.84	34.76	52.25
SE	4.907			

C Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	0.000086	0.000029	0.067	
F	3	0.005542	0.001847	4.325	*
Error	9	0.003845	0.000427		
Total	15	0.009472			

Feed	MD	NID	CN	Hay
Mean	0.0828	0.0560	0.0463	0.0318
SE	0.01033			

Nitrogen Loss

A Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	5.8819	1.9606	1.072	
F	3	4621.5419	1540.5140	842.546	***
Error	9	16.4556	1.8248		
Total	15	4643.8794			

Feed	MD	NID	CN	Hay
Mean	47.80	57.62	54.30	14.85
SE	0.676			

B Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	196.6050	65.5350	4.484	*
F	3	847.3650	282.4550	19.324	***
Error	9	131.5500	14.6167		
Total	15	1175.5200			

Feed	MD	NID	CN	Hay
Mean	46.53	34.02	34.55	50.67
SE	1.912			

C Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	0.001016	0.000339	4.804	*
F	3	0.009892	0.003297	47.767	***
Error	9	0.000635	0.000071		
Total	15	0.011543			

Feed	MD	NID	CN	Hay
Mean	0.1170	0.0870	0.0808	0.0470
SE	0.00420			

NDF Degradation

A Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	15.0447	5.0149	0.693	
F	3	2566.4814	855.4938	118.291	***
Error	9	65.0889	7.2321		
Total	15	2646.6150			

Feed	MD	NID	CN	Hay
Mean	8.61	18.58	25.63	-8.10
SE	1.345			

B Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	362.2356	120.7452	0.769	
F	3	516.0200	172.0067	1.096	
Error	9	1413.0227	157.0025		
Total	15	2291.2784			

Feed	MD	NID	CN	Hay
Mean	49.79	65.52	55.75	58.93
SE	6.265			

C Constant

Analysis of Variance Table

	DF	SS	MS	F	Prob
S	3	0.000252	0.000084	0.216	
F	3	0.011382	0.000389	9.747	**
Error	9	0.003503	0.000389		
Total	15	0.015138			

Feed	MD	NID	CN	Hay
Mean	0.0995	0.0493	0.0315	0.0383
SE	0.00986			

Appendix 9. Fermentation Characteristics of Different Silages

		Control	+MSBP	+NID	+MDG
Lactic Acid (g/kg Silage FW)					
Ensilage	2	0.297	1.173	0.451	0.447
Time	24	2.309	2.838	2.057	2.639
(Hours)	48	5.706	3.853	2.930	3.645
	96	4.633	4.673	3.008	3.702
	144	4.725	5.087	3.354	3.588
VFAs (g/kg Silage FW)					
Ensilage	2	0.166	0.057	0.167	0.116
Time	24	0.852	0.722	1.285	0.732
(Hours)	48	1.095	0.869	1.986	1.043
	96	2.354	1.183	2.591	1.250
	144	2.425	1.426	3.575	1.600
Amino Acid Nitrogen (g/kg Silage FW)					
Ensilage	2	0.026	0.023	0.003	0.022
Time	24	0.239	0.158	0.132	0.212
(Hours)	48	0.265	0.210	0.210	0.243
	96	0.551	0.272	0.338	0.299
	144	0.525	0.364	0.375	0.283
Total Nitrogen (g/kg Silage FW)					
Ensilage	2	0.060	0.058	0.024	0.047
Time	24	0.331	0.231	0.205	0.277
(Hours)	48	0.473	0.299	0.361	0.368
	96	0.991	0.376	0.520	0.416
	144	1.011	0.430	0.661	0.461
Water Soluble Carbohydrate (g/kg Silage FW)					
Ensilage	2	<0.001	2.170	0.485	0.422
Time	24	0.052	2.993	3.283	1.382
(Hours)	48	<0.001	2.209	4.102	1.808
	96	1.405	1.519	4.797	1.939
	144	1.175	1.622	5.329	2.110

Appendix 10. Effluent Losses From Individual Silos.

Treatment	Effluent Weight (kg/t grass)	Effluent DM (g/kg)	Effluent OM (g/kg)	% Grass DM Lost *	% Grass OM Lost *
Control	124	92.79	71.70	7.30	6.41
"	97	90.54	67.56	5.54	4.69
"	149	95.67	70.44	9.11	7.35
"	165	89.71	65.29	8.91	7.49
+ Draff	29	111.93	84.04	2.02	1.7

* Figures normalised for small differences in grass DM between clamps.

Appendix 11. Analysis of Variance of Intakes

Concentrate Dry Matter Intakes Analysis-of-Variance Table

	DF	SS	MS	F	Prob
T	3	33813.60000	11271.2000	1.065	
V2	1	23893.36878	23893.36878	2.258	
Error	15	158727.03122	10581.80208		
Total	19	216434.000			

CV = 4.46 per cent

Regression Coefficient V2 1.07041 SE 0.71234

Silage Dry Matter Intakes Analysis-of-Variance Table

	DF	SS	MS	F	Prob
T	3	1646916.15	548972.05	5.861	**
V2	1	5675379.78	5675379.78	60.588	***
Error	15	1405064.62	93670.97		
Total	19	8727360.55			

CV = 11.94 per cent Regression Coefficient V2 16.497 SE 2.11940

Table of CDM and SDM Intakes

Diet	1	2	3	4	Mean	(SE)
Concentrates	2347.00	2259.96	2347.86	2269.19	2306.00	(46.006)
Silage	2896.40	2203.70	2803.60	2348.90	2563.15	(136.879)

Appendix 12. Weekly Silage DMI Analysis of Variance

Analysis-of-Variance Table

	DF	SS	MS	F	Prob	
D	3	16464433.2927	5488144.4309	1.240		
Error(1)	16(3)	70819685.9968	4426230.3748			
W	9	68264590.6719	7584954.5191	121.830	***	
D.W	27	7413326.0551	274567.6317	4.410	***	
Error(2)	144	8965243.2000	62258.6333			
Total	199	171927279.2166				

CV = 9.73 per cent

Diet x Week Table

	Diet				
Week	1	2	3	4	Mean
1	1487.60	964.80	1535.60	1214.40	1300.60
2	1678.20	1423.60	2028.00	1785.60	1728.85
3	2812.40	1921.40	2586.20	2230.40	2387.60
4	2541.40	2323.40	2964.20	2461.80	2572.70
5	3201.80	2602.80	3124.20	2559.40	2872.05
6	3015.40	2923.20	3547.20	2708.40	3048.55
7	3215.80	2178.20	2880.20	2458.60	2683.20
8	3280.80	2282.20	2757.40	2503.80	2706.05
9	3622.60	2727.20	3271.00	2993.40	3153.55
10	4106.20	2787.00	3209.40	2608.60	3177.08
Mean	2896.40	2203.70	2803.60	2348.90	2563.09

SE H 111.587 VI 315.802 297.531
55.794

Appendix 13. Analysis of Variance Bull LWG.

All Time Analysis-of-Variance Table

	DF	SS	MS	F	Prob
T	3	0.27338	0.09113	3.124	
V2	1	0.17451	0.17451	5.982	*
Error	15	0.43757	0.02917		
Total	19	0.88546			

CV = 14.27 per cent
Regression Coefficient V2 0.00289 SE 0.00118

LWG to 27.03.90 Analysis-of-Variance Table

	DF	SS	MS	F	Prob
T	3	0.17088	0.05696	1.933	
V2	1	0.04159	0.04159	1.411	
Error	15	0.44201	0.02947		
Total	19	0.65448			

CV = 14.26 per cent
Regression Coefficient V2 0.00141 SE 0.00119

LWG from 27.03.90 to end Analysis-of-Variance Table

	DF	SS	MS	F	Prob
T	3	0.30346	0.10115	3.856	*
V2	1	0.14703	0.14703	5.604	*
Error	15	0.39353	0.02624		
Total	19	0.84402			

CV = 14.30 per cent

Regression Coefficient V2 0.00266 SE 0.00112

Table of LWG

Diet	1	2	3	4	(SE)
All Time	1.378	1.070	1.122	1.215	1.197 (0.0764)
To 27.03.90	1.284	1.107	1.117	1.308	1.204 (0.0768)
27.03.90 to End	1.324	0.996	1.060	1.151	1.133 (0.0724)

Appendix 14. Programme for Calculation of the Kf and Goodness-of-Fit Values From Bull Liveweight and Intake Data

```
DEF FNB(X)=INT(X+.5)
DEF FNA(X)=(INT(X*100+.5))/100

1.0 LPRINTER

OPEN "TEMP.DAT" AS 1

FOR F%=1 TO 4
PRINT "*****************************************************************"
PRINT "TREATMENT ";F%
PRINT

READ #1;SME,CME
PRINT "SILAGE ME = ";SME;"  CONC ME = ";CME

FOR C%=1 TO 5
READ #1;SDMI,CDMI,W,LWG


MEI=SDMI*SME+CDMI*CME
TDMI=SDMI+CDMI

KM=.019*(MEI/TDMI)+.503
KP=.0424*(MEI/TDMI)+.006

B=KM/(KM-KP):P=KM*LOG(KM/KP)
EM=1.15*(.53*((W/1.08)^.67))+.0043*W:REM BULL BULL BULL BULL
R=B-1-(B/(EXP((MEI*P)/EM)))
EP=R*EM:REM NE AVAILABLE FOR LWG

EVG=0.85*((4.1+.0332*W-.000009*W*W)/(1-.1475*LWG)):REM BULL BULL BULL BULL

LWGP=EP/(4.1+.0332*W-.000009*W^2+.1475*EP):REM LWG PREDICTED
EPA=EVG*LWG:EPRAT=FNA(EPA/EP):REM ACTUAL NE STORED/PREDICTED NE STORED

MEM=FNB(EM/KM)
KF=FNA(EPA/(MEI-MEM))
KP=FNA(KP)

LWGP=FNA(LWGP)


PRINT C%;TAB(10);W;TAB(20);SDMI;TAB(30);CDMI;TAB(40);MEI;\
TAB(50);LWG;TAB(60);LWGP;TAB(70);EPRAT;\
TAB(80);KP;TAB(90);KF;TAB(100);MEM
NEXT C%

PRINT:PRINT:PRINT
NEXT F%

CLOSE 1
```

Appendix 15. Analysis of Variance of k_f and Goodness-of-fit to ARC 1980 Model

k_f Analysis-of-Variance Table

	DF	SS	MS	F	Prob
T	3	0.1055	0.0352	4.705	*
Error	16	0.1196	0.0075		
Total	19	0.2252			

CV = 14.93 per cent SE 0.039

Diet	1	2	3	4	Mean
	0.68	0.54	0.49	0.62	0.58

Goodness-of-Fit

Calculated as Actual NE Stored

Predicted NE Stored

Analysis-of-Variance Table

	DF	SS	MS	F	Prob
T	3	0.5172	0.1724	4.721	*
Error	16	0.5843	0.0365		
Total	19	1.1015			

CV = 15.90 % SE 0.085

Appendix 16. ***In Sacco*** **OM, N and NDF Time Course Rumen Degradation**

Feed	Time (Hours)	Digestibility (%) DM (SE)	OM	N	NDF
TMD	0	53.0 (2.538)	50.3	55.9	28.4
	4	58.6 (1.166)	59.4	61.8	39.0
	7.5	63.5 (1.782)	65.7	65.4	47.5
	17	71.6 (0.345)	74.6	68.5	59.9
	24	79.3 (0.915)	83.0	77.4	70.2
	48	80.5 (0.332)	84.8	76.3	78.0
Beef Nuts	0	54.0 (0.424)	51.4	68.7	25.0
	4	58.6 (1.086)	56.5	67.4	30.1
	7.5	62.8 (2.674)	60.9	74.7	37.4
	17	72.5 (1.829)	71.3	81.7	48.7
	24	78.6 (0.950)	77.9	85.4	55.9
	48	84.5 (1.922)	84.6	92.3	71.8
UMD	0	33.9 (0.394)	31.0	32.5	11.8
	4	40.1 (0.969)	37.8	36.8	21.3
	7.5	47.2 (2.832)	44.9	46.6	33.4
	17	69.4 (6.482)	68.4	62.4	60.2
	24	69.8 (4.512)	69.1	56.8	58.1
	48	84.1 (2.922)	84.1	78.8	80.0
Control Silage	0	55.0 (2.859)	53.7	68.6	41.8
	4	52.9 (1.131)	51.1	81.2	26.9
	7.5	58.5 (1.649)	54.7	84.2	38.2
	17	77.2 (4.618)	75.9	94.2	65.4
	24	83.4 (1.094)	84.1	92.3	75.3
	48	90.6 (0.583)	91.6	94.7	88.5
Absorbent Silage	0	61.2 (0.323)	59.6	85.6	35.0
	4	64.6 (1.678)	63.6	87.9	37.5
	7.5	62.9 (0.653)	61.4	87.8	37.1
	17	82.2 (3.579)	81.6	94.3	71.1
	24	85.4 (2.242)	86.2	94.6	76.1
	48	92.8 (0.280)	93.7	98.6	88.8

Appendix 17. Example of Analysis of Variance of Rumen Volatile Fatty Acids and Other Variates in OSNID Feeding Experiment

```
                    GENSTAT V    MARK 4.03
(C)1980 LAWES AGRICULTURAL TRUST (ROTHAMSTED EXPERIMENTAL STATION)
          MS-DOS VERSION BY C.E.M.S. ( J.C. AND Y.M. )

  1  'refer' Jill
  2  'units' dpoint $ 20
  3  'factor' sheep $ 4
  4  'factor' diet $ 5
  5  'heading' hf=''a:tg.dat''
  6  'input/file=hf' 2
  7  'read/form=p' period,sheep,diet,v(1...10)
  8  'input' 1
  9  'blocks' sheep
 10  'treats' diet
 11  'for' ii=v(1...10);aa=vres(1...10)
 12  'anova' ii; res=aa
 13  'repeat'
 14  'covariates' period
 15  'for' ii=v(1...10);bb=cvres(1...10)
 16  'anova' ii; res=bb
 17  'repeat'
 18  'for' ii=v(1...10);aa=vres(1...10);bb=cvres(1...10)
 19  'print/form=p' period,sheep,diet,ii,aa,bb $ 10.0,10.0,10.0,10.2,10.2
 20  'repeat'
 21  'run'

***** ANALYSIS OF VARIANCE *****

VARIATE:  v(1)

SOURCE OF VARIATION     DF          SS       SS%          MS
VR

sheep STRATUM            3  125.62     24.81       41.87     2.669

sheep.*UNITS* STRATUM
  diet                   4  192.49     38.01       48.12     3.067
  RESIDUAL              12  188.28     37.18       15.69
TOTAL                   16  380.78     75.19       23.80

GRAND TOTAL             19  506.40    100.00

GRAND MEAN                26.17
TOTAL NUMBER OF OBSERVATIONS          20
```

***** TABLES OF MEANS *****

VARIATE: v(1)

GRAND MEAN 26.17

diet	1	2	3	4	5
	28.85	26.91	23.53	21.70	29.84

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	diet
REP	4
SED	2.801

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
sheep	3	2.894	11.1
sheep.*UNITS*	12	3.961	15.1

Appendix 18. Analysis of Variance of Cotton Strip DMD

	DF	SS	MS	F	Prob
S	3	66.3413	22.1138	2.857	
T	4	172.9905	43.2476	5.587	**
Error	12	92.8953	7.7413		
Total	19	332.2272			

CV = 18.64 per cent

S.T Table

	Control	LOSNID	LOSD	HOSNID	HOSD
1	16.00	19.52	20.05	17.10	17.50
2	18.09	16.63	17.10	15.60	14.10
3	20.51	21.95	19.10	16.60	6.90
4	20.37	17.50	11.70	13.20	6.90
Mean	18.74[a]	18.90[a]	16.99[a]	15.62[ab]	11.35[b]

SE 2.782
1.391

Appendix 19. Example of Analysis of Variance of Plasma Fatty Acids

```
                    GENSTAT V    MARK 4.03
(C)1980 LAWES AGRICULTURAL TRUST (ROTHAMSTED EXPERIMENTAL STATION)
          MS-DOS VERSION BY C.E.M.S. ( J.C. AND Y.M. )

   1  'refe' jill
   2  'units'dpoint$120=1...120
   3  'name'ntreat=control,megalac,fibrefat,lowff
   4  'factor' goat$6
   5  :        period$4
   6  :        diet$ntreat
   7  :        time$5
   8  'heading' title
   9  :         hf=''a:goattg.dat''
  10  'input/file=hf' 2
  11  'read' title
  12  'print'title
  13  'read/flev=f' period,goat,diet,time,v(1...9)
  14  'input' 1
  15  'hist' v(1):v(2):v(3):v(4):v(5):v(6):v(7):v(8):v(9)
  16  'blocks'goat
  17  'treats'diet*time
  18  'anova'v(1):v(2):v(3):v(4):v(5):v(6):v(7):v(8):v(9)
  20  'run'

***** ANALYSIS OF VARIANCE *****

VARIATE:  v(1)

SOURCE OF VARIATION  DF          SS      SS%       MS
VR

goat STRATUM          5       73.39     0.79    14.68   0.944

goat.*UNITS* STRATUM
  diet                3     7422.27    80.20  2474.09 159.196
  time                4       62.36     0.67    15.59   1.003
  diet.time          12      220.34     2.38    18.36   1.181
  RESIDUAL           95     1476.41    15.95    15.54
TOTAL                9181.38 99.21    80.54

GRAND TOTAL          119    9254.76   100.00

GRAND MEAN                 30.47
TOTAL NUMBER OF OBSERVATIONS          120
```

***** TABLES OF MEANS *****

VARIATE: v(1) C16:0

GRAND MEAN 30.47

diet	C	M	O	L
	32.72	42.35	22.68	24.11

time	1	2	3	4	5
	31.35	30.32	30.65	30.82	29.19

time	1	2	3	4	5
diet					
control	34.67	32.59	31.68	34.78	29.89
megalac	43.81	43.35	40.10	43.84	40.66
fibrefat	22.33	22.00	26.06	21.15	21.85
lowff	24.60	23.34	24.77	23.52	24.35

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	diet	time	diet time
REP	30	24	6
SED	1.018	1.138	2.276

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
goat	5	0.857	2.8
goat.*UNITS*	95	3.942	12.9

Appendix 20. Yield of Soluble OM From Barley Spent Grains

Effect of Different Treatment Temperatures and Times on Barley Spent Grains Treated with 80 g $Ca(OH)_2$/kg DM

Control (Untreated Draff) 3.53

Draff treated with 80g $Ca(OH)_2$/kg DM

Holding Time (hours)	Holding Temperature (°C)			
	20	40	60	80
0	10.4			
2	10.40	10.29	12.80	15.82
4	12.27	14.31	17.09	22.54
8	11.74	13.52	17.57	25.31
12	13.22	15.44	20.09	25.00

Effect of NaOH Compared to $Ca(OH)_2$ on Yield of Soluble OM.

% OM Solubilised by Treatment

Holding Time (hours)	NaOH		$Ca(OH)_2$
	40	80	80 (g/kg DM)
0	16.0	19.3	10.4
2	7.1	15.9	10.8
4	9.3	24.6	17.1
8	12.1	34.4	17.6
12	16.9	37.0	20.1

Appendix 21. Yield of Soluble OM From Maize Spent Grains

Effect of Different Treatment Times and Temperatures on Maize Spent Grains Treated with 80 g$Ca(OH)_2$/kg DM

% OM Solubilised by Treatment

	Treatment Temperature °C			
Holding	20	40	60	80
Time				
0	10.76			
2	9.31	10.74	13.07	13.72
4	9.82	13.16	16.30	18.36
8	10.93	14.12	17.44	20.31
16	11.19	16.13	18.62	23.60

Effects of NaOH Compared to $Ca(OH)_2$ on OM Solubilisation of Maize Draff Treated at 60°C.

Untreated Maize Draff 4.91 g OM soluble

% OM Solubilised by Treatment

	NaOH		$Ca(OH)_2$	
	40	80	40	80 (g/kg DM)
0	10.19	14.31	6.50	10.76
2	9.38	15.15	5.92	13.07
4	12.42	20.27	6.46	16.30
8	12.81	26.65	8.12	17.44
16	15.08	37.50	8.15	18.62