ENERGY EVALUATION OF GRASS SILAGE

A Thesis Submitted to the University of Glasgow for the Degree of Doctor of Philosophy in the Faculty of Science

by

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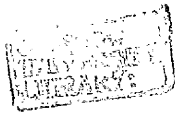

TO MY PARENTS, MY WIFE: ABEER,

MY SONS: ABDULLAH AND MOHAMMAD, MY

BROTHERS AND SISTERS FOR ALL THEIR LOVE

AND UNDERSTANDING WHICH MADE THIS

THESIS POSSIBLE.

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ABBREVIATIONS

ADAS	Agricultural Development and Advisory Service
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
AP	Acid Pepsin
API	Acid Pepsin Washing followed by rumen Incubation
APIAP	Acid Pepsin Washing followed by rumen Incubation followed by Acid Pepsin Washing.
CP	Crude Protein
DANI	Department of Agriculture for Northern Ireland.
DDM	Digestible Dry Matter
DE	Digestible Energy
DM	Dry Matter
DOM	Digestible Organic Matter
DOMD	Digestible Organic Matter in the Dry Matter
ED	Energy Digestibility
ESCA	East of Scotland College of Agriculture
FEU	Feed Evaluation Unit
GE	Gross Energy
IAP	Rumen Incubation followed by Acid Pepsin Washing
IND	Rumen Incubation followed by Neutral Detergent
IVDMD	In vitro Dry Matter Digestibility
IVDOMD	In vitro Digestible Organic Matter in the Dry Matter
IVOMD	In vitro Organic Matter Digestibility
IWP	Rumen Incubation followed by Washing Powder Washing.
k_f	Efficiency of Utilisation of ME for gain.
kg	Kilo Gram
k_l	Efficiency of Utilisation of Me for milk production
k_m	Efficiency of Utilisation of ME for maintenance
LIGA	Acetyl Bromide Lignin Method
MADF	Modified Acid Detergent Fibre
MAFF	Ministry of Agriculture, Fisheries and Food
ME	Metabolisable Energy
MJ	Mega Joule
Mt	Million Tonne
N	Nitrogen
NB48 OMD	Nylon Bag 48 hours Incubation OMD.

NCD	Neutral Detergent Cellulase DOMD
NCDOMD	Neutral Detergent Cellulase DOMD
NCOMD	Neutral Detergent Cellulase OMD
ND	Neutral Detergent
NDA	No Data Available
NDF	Neutral Detergent Fibre
NE	Net Energy
NDI	Neutral Detergent Washing followed by Rumen Incubation
NDIND	Neutral Detergent Washing followed by Rumen Incubation followed by Neutral Detergent Washing.
NIR	Near Infrared Reflectance
NOSCA	North of Scotland College of Agriculture
NR	Nitrogen Concentration in Bags Residue
NS	Not Significant
ODM	Oven Dry Matter
OM	Organic Matter
OMD	Organic Matter Digestibility
PCA	Principal Component Analysis
PCDOMD	Pepsin-Cellulase DOMD
PCOMD	Pepsin-Cellulase OMD
POP	Population
RRI	Rowett Research Institute
RSD	Residual Standard Deviation
SAC	Scottish Agricultural Colleges
SD	Standard Deviation
SEC	Standard Error of Calibration
SED	Standard Error of Difference
SEP	Standard Error of Prediction
TDM	Toluene Dry Matter
WP	Washing Powder
WPI	Washing Powder Washing followed by Rumen Incubation
WPIWP	Washing Powder Washing followed by Rumen Incubation followed by Washing Powder Washing.
WSC	West of Scotland College.

SUMMARY

1 The areas covered in the literature review include:

a) Metabolisable energy as a measure of the nutritive value of grass silages;

b) The importance of digestibility as a useful index of nutritive value;

c) Factors affecting the digestibility measurements;

d) Laboratory methods for predicting the organic matter digestibility of grass silages.

2 The effect of different washing procedures on the losses of organic matter and nitrogen from samples of hay incubated in polyester bags within the rumen of sheep was investigated. For organic matter, post-incubation detergent washing reduces variability without altering the form of the degradation curve. For nitrogen, post-incubation detergent washing might remove contaminating bacteria which could otherwise lead to the underestimation of protein degradability. Washing the bags after rumen incubation with domestic washing powder in the washing machine is both cheap and convenient.

3 One hundred and seventy dried samples of grass silages which had been evaluated in vivo for organic matter digestibility (OMD), were collected from different sources around the UK. These sources include:

a) Agricultural Development and Advisory Service - 100 silages;

b) Rowett Research Institute - 43 silages;

c) School of Agriculture, Aberdeen - 27 silages.

All silages were subjected to seven laboratory predictors of *in vivo* OMD, including those used routinely by advisory services in the UK. These methods are:

a) Modified Acid Detergent Fibre (MADF) [Clancy and Wilson, 1966];

b) Acetyl Bromide Lignin (LIGA) [Morrison, 1972];

c) Neutral Detergent Cellulase OMD (NCOMD) [Dowman and Collins, 1982];

d) Pepsin-Cellulase OMD (PCOMD) [Jones and Hayward, 1975];

e) *In vitro* OMD (IVOMD) [Alexander and McGowan, 1966].

f) Nylon Bag 48 Hours Incubation OMD (NB48 OMD) [Kridis *et al*, 1989];

g) Near Infrared Diffuse Reflectance Spectroscopy (NIR) [Norris *et al*, 1976].

To avoid between-laboratory differences, each method was performed by a laboratory which makes routine use of the particular method.

Of all the 170 silages, 122 silages were selected to derive prediction equations (calibration silages) and the remaining 48 silages were reserved for subsequent validation purposes.

The aim of this work was to investigate the robustness of each method as a predictor of *in vivo* OMD and then explore the possibility of establishing an improved

technique which could be used by all of the advisory services in the UK.

4 The in vivo OMD of 122 calibration silages was not precisely predicted by the MADF (R^2 = 0.34 and RSD% = 5.1), LIGA (R^2 = 0.52 and RSD% = 4.4), NCOMD (R^2 = 0.54 and RSD% = 4.3) and PCOMD methods (R^2 = 0.55 and RSD% = 4.2). All gave significant between-population differences in the regression equations obtained.

The in vivo OMD was more precisely predicted by the rumen liquor methods (NB48 OMD and IVOMD) [R^2 = 0.68, 0.74; RSD% = 3.6, 3.2 respectively]. In each case their application require one single regression equation to describe all silage populations.

Of the methods tested in this work, the NIR method was the best predictor of in vivo OMD (R^2 = 0.85 and SEC% = 2.5). It also gave single regression line provided that more than five terms were used in the multiple regression equation.

The best prediction of in vivo OMD of a blind test of 48 in vivo silages was obtained by the NIR method using a multiple linear regression involving eight terms (R^2 = 0.76, SEP% = 2.6).

5 The effect of external factors in the relationship between in vivo OMD and its predictors was investigated. The IVOMD and NCOMD methods were significantly affected by the year of harvest. Cut number was also found to significantly affect the relationship based on the MADF and LIGA methods. The method of ensiling, wilting time, additive application and nitrogen fertilisation were found not to affect the regression equations of any predictor studied in this work.

6 The use of the NIR method to predict *in vivo* DOMD was examined. This method can predict directly *in vivo* DOMD, however with lesser precision than predicting *in vivo* OMD (R^2 = 0.64, SEP% = 2.97 and R^2 = 0.76, SEP% = 2.6 respectively). The calculation of *in vivo* DOMD by NIR prediction of *in vivo* OMD and then measuring ash content, a parameter useful to indicate soil contamination, gave more precise prediction than the direct prediction of *in vivo* DOMD by NIR (R^2 = 0.78, SEP% = 2.43 and R^2 = 0.64, SEP = 2.97 respectively).

7 The laboratory methods tested in this work were used to predict the *in vivo* DE of 140 grass silages. Digestible energy was not predicted with sufficient precision by the conventional methods tested in this work (for the MADF, LIGA, NCOMD, PCOMD and IVOMD methods the R^2 and RSD were 0.09; 1.65, 0.22; 1.53, 0.16; 1.60, 0.32; 1.43 and 0.31; 1.44 respectively). Only the NIR method makes a significant improvement in DE prediction (R^2 = 0.72 and SEC = 0.91). The gross energy of silages can account for much of the variation in DE prediction, suggesting the importance of gross energy in the energy evaluation of silages.

GENERAL INTRODUCTION

Agriculture is considered to be one of the oldest activities of mankind. It is not clear when people started agricultural cultivation and domestication of animals, but it is believed that it may have taken place gradually from 40,000 to 10,000 B.C. (Flannery, 1965). Before that time, people were principally food gatherers, but the time came when they settled and started to become food producers. Nowadays, food production is the world's most vital primary activity without which neither modern civilisation nor primitive cultures can survive.

Due to the huge expansion of the world's population and increased demand for food in the last 100 years, it became imperative to maximise the output of meat, milk and wool from animals - and yet minimise the costs of its production.

Traditional methods of preserving food, such as salting, drying, syruping, pickling, smoking and fermenting, were established by early civilisations. For example, ensiling the plant material in the absence of air results in the production of a fermented crop which is known as silage.

History of Silage Making

Silage is a material of high moisture content stored anaerobically and produced by a controlled fermentation. The process is called ensilage and the container or the structure is called a silo.

It is believed that silage making was practised 3,000 years ago (Schukking, 1976). The ancient Egyptians were believed to be familiar with silage making as long as 1000-1500 B.C. However, it wasn't until the latter part of the nineteenth century that interest in this process became more widespread. Practical modernisation of ensilage was undoubtedly credited to the French farmer Goffart in 1877. Five years later, silage making received interest among British farmers. In 1883 it was reported that no

more than half a dozen silos were found in the whole of the UK, and as the interest increased dramatically there were about 1,605 silos found in the country by 1886 (Rew, 1888). The publication of a book "Sweet Ensilage" by Fry in 1885 was a major hindrance in accepting this technique for some 50 years. This is because of Fry's remarks of allowing the crop to heat up in the silo prior to sealing which resulted in producing a silage of a poor nutritional quality.

After the second World War, a wide general interest in silage-making was revived. This was owing to the escalating costs of feed concentrates, necessity of more intensive animal production and improvements in mechanisation. Nowadays, silage-making is an accepted method of food conservation world-wide. The principles of ensilage are now more fully understood and the conditions necessary for obtaining a good product are well defined.

In many countries silage has superseded hay as the principal conserved forage, as the figures for European countries show (Table 1).

Conserved Forages in the UK

In the UK, ensilage of a grass has become an increasingly important method of conservation. Despite the fact that this method has been practised for many centuries, as mentioned earlier, it is less than 20 years since UK farmers started to adopt the technique. For example, in the West of Scotland silage making is now considered to be the most important method of conservation for feeding livestock in winter.

Although haymaking has long been the traditional technique of preserving forage, it was not until 1981 that the acreage of grass conserved as silage exceeded the acreage conserved as hay (Figure 1.1). According to the UK agriculture census (Henderson, 1987) in England in 1985, 667,000 hectares of grass were cut for hay, 1,018,000 hectares were cut for silage and 10,100 hectares were

TABLE 1

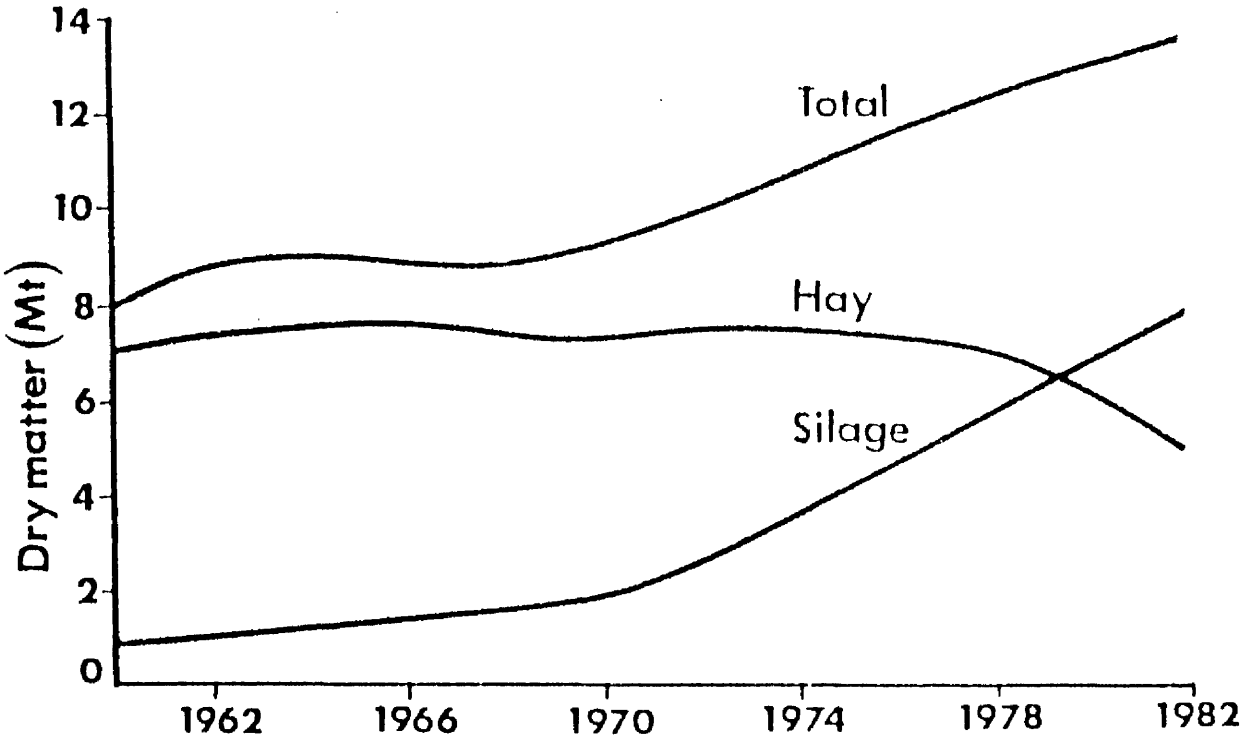
Production of Silage and Hay in Europe: Estimates for the Countries of the European Economic Community and Scandinavia (1978 - 1979) [Wilkinson, 1980]

Country	Silage	Hay	Silage of Total (%)
Belgium	1.9	1.7	52.8
Denmark	1.1	0.4	73.3
Finland	0.9	1.7	34.6
France	16.0	22.3	41.8
Germany (FRG)	10.5	9.1	53.6
Iceland	2.4	3.6	40.0
Italy	5.9	9.3	38.8
Luxemburg	0.2	0.2	50.0
Netherlands	4.1	1.3	75.9
Norway	1.1	0.7	61.1
Sweden	1.0	4.6	17.9
United Kingdom	5.5	6.9	44.4

Figures expressed as x 10^6 tonnes of dry matter.

FIGURE 1.1

Estimated Production of Conserved Grass
in the UK (From Wilkinson, 1985)



cut for artificially dried fodder. This represented an increase of 18.7% in the area cut for silage over the previous year. There was also an increase of 32.9% in other crops harvested for silage.

Silage making has its own advantages over hay making. Woolford (1984) indicated that silage making is less dependent on the weather, the crop is harvested at a less mature stage of growth than for hay and thus at the outset, is more nutritious and two or three cuts per year can be taken for silage, with hay it is usually one. In addition, silage is generally superior to hay in terms of its content of energy and crude protein (Wilkinson et al, 1976) and apparently superior to the original crop in terms of energy (Alderman et al, 1971).

The Nutritive Value of Grass Silage

Grass silage is an important forage in feeding ruminant livestock in the UK. The determination of its nutritive value is of primary concern to farmers so that ration formulation can be achieved. While the nutritive value of silage is a measure of its ability to satisfy the energy and protein needs of the animal, much of the effort has been directed to characterise silage as an energy source.

In the UK, metabolisable energy has been used to estimate the nutritive value of silages. While this parameter is difficult to measure, digestibility is more frequently used as a useful index of the nutritive value.

Factors Affecting the Digestibility and Energy Value of Grass Silages

A **Composition of The Parent Crop**

There are many factors which affect the composition of the harvested crop. These include, species and strain of the

plant, stage of growth, application of fertilisers, climate, type of soil and growth condition. The stage of growth is the most important factor influencing the composition and nutritive value of the standing crop. As plants grow, there is a greater need for structural tissue, and therefore the structural carbohydrates (cellulose, hemicellulose) and lignin increase. This increase in cell wall components is coupled with a parallel decrease in the digestible cell contents. Figure 1.2 demonstrates how the cell components change as the plant advances in maturity.

B The Effect of Ensiling on Digestibility and Metabolisable Energy of Grass Silages

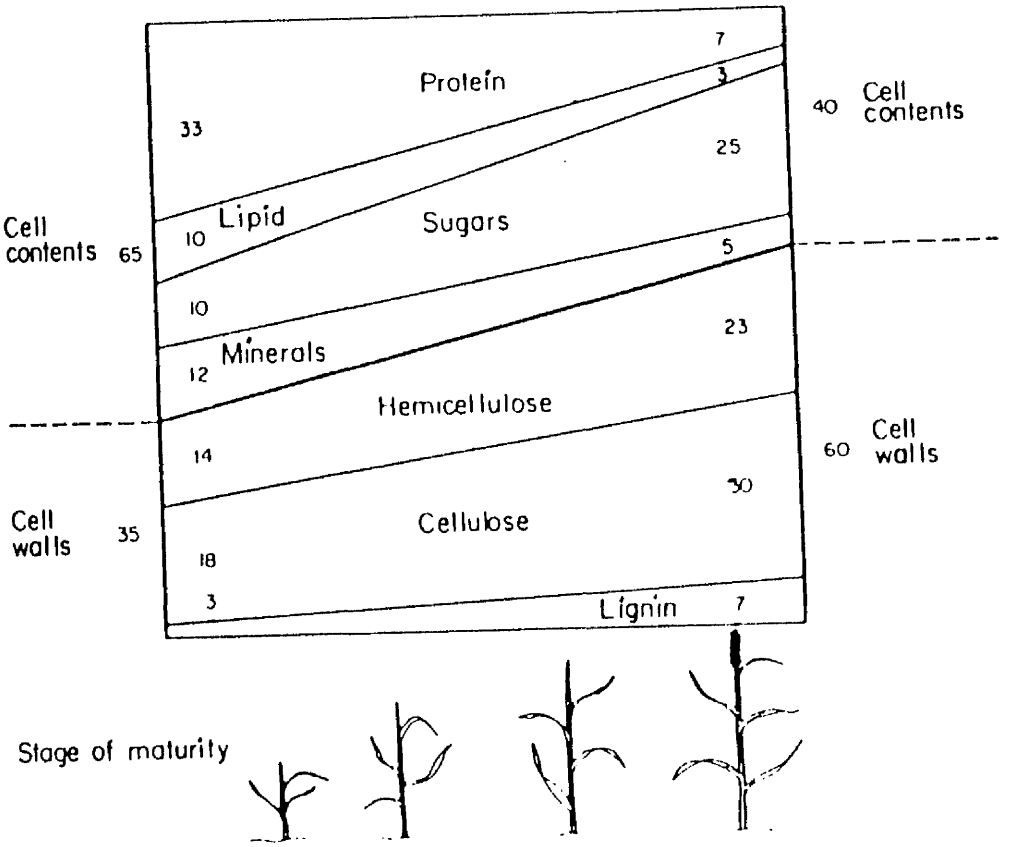
Digestibility is considered to be a useful index of a forage nutritive value. It is well documented that the digestibility of a good quality silage is similar to the digestibility of the crop from which it was made (Harris and Raymond, 1963). More recent studies by McDonald and Edwards (1976) confirmed these findings. They found that the digestibilities of 36 different silages and grasses from which they were made were 0.768 and 0.767 respectively.

Wilting generally results in a small reduction in digestibility (Harris et al, 1966; Marsh, 1979). The depression in digestibility is likely to be a reflection of changes occurring in field drying rather than during ensiling (Thomas and Thomas, 1985).

Formic acid may increase digestibility slightly (Castle, 1972; Wilson and Wilkins, 1973; Thomas and Thomas, 1985), although Waldo (1978) claimed a 7% increase in digestibility of the organic matter of silages studied. Formalin may reduce OMD by 2-4 units (Wilkins et al, 1974). This depression of digestibility by the addition of formalin may be due to the disturbance of the rumen function with the consequence that cellulose digestion is reduced.

FIGURE 1.2

Schematic Representations of the Changes in the Chemical Composition of Grasses which Accompany Advancing Maturity. All Values are in Percentages (From Osbourn, 1980).



Metabolisable energy values for silage vary widely and this is dependent on the type of fermentation during ensilage. For example, silages in which there has been extensive butyric fermentation may have much higher ME values, sometimes exceeding 13 $MJkg^{-1}DM$ (Woolford, 1984). Wilkins (1974) quoted 8 comparisons between ME values of fresh grass and ensiled grass and found that the mean value was 10.45 $MJkg^{-1}DM$ for grass and 10.41 $MJkg^{-1}DM$ for silage. However, in an experiment by Donaldson and Edwards (1976) in which lactate, wilted and formic acid-wilted silages were made from the same ryegrass herbage, they found that the ME value of the lactate silages were significantly higher than that of the fresh grass and those of the other two silages in which fermentation had been restricted. In both studies the loss of energy as methane was estimated. Because wilting restricts fermentation in the silo, it is not expected that wilting silage will change the metabolisable energy values from the original crop. This view is supported by the study of Donaldson and Edwards (1976) where they found that the metabolisable energy values of grass and wilted silages made from the same grass were 11.6 and 11.4 $MJkg^{-1}DM$ respectively.

Prediction of Silage Digestibility and Metabolisable Energy in the UK

It is well known that the measurement of digestibility and metabolisable energy of forages using animal trials is the ultimate standard of measurement. However, this method is expensive, time consuming and requires special facilities.

In the UK therefore, different advisory laboratories have used different methods to predict these two parameters. Up to now, these methods have not been compared properly. For the advisory purposes, economic consideration plays an increasingly important role for the selection of the advisory method(s), however, nutritional considerations should not be ignored. It is these aspects which form the theme of this work.

The aims and objectives of this work are as follows:

1 Test the possibility of improving the reliability of the nylon bag technique by washing the bags after or before rumen incubation with different washing reagents.

2 Examine the ability of various laboratory methods, including those used routinely by advisory services in the UK, to predict in vivo OMD of a large population of grass silages. These silages were collected from different sources around the UK.

3 Explore the possibility of establishing an improved predictive technique which could be used by all advisory laboratories in the UK.

4 Investigate the effect of environmental and other factors on in vivo digestibility - laboratory method relationship for each predictive technique.

5 Study the ability of these predictive techniques to predict the digestible energy of grass silages.

CHAPTER ONE

REVIEW OF LITERATURE

SECTION 1

Energy Evaluation

1.1.1 Introduction

When food is burned completely in a bomb calorimeter, energy is released and this is termed as the gross energy (GE) of the food. A scheme showing the manner in which the GE in herbage is partitioned and finally used for productive purposes in ruminants is shown in Figure 1.3.

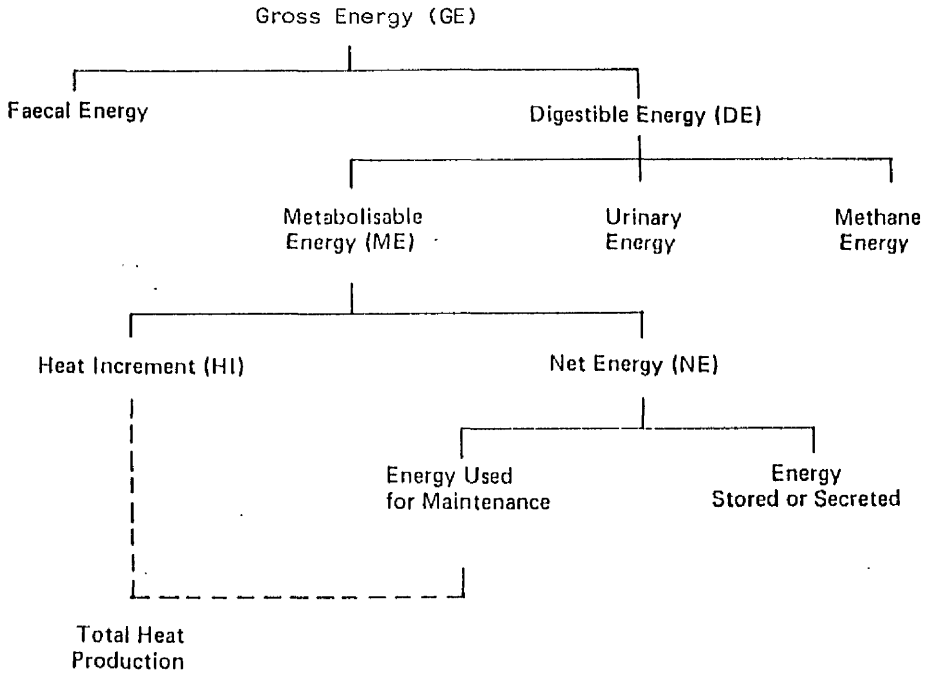
1.1.2 Historic Developments of Forage Evaluation in the UK

The Starch equivalent system of Kellner and Kohlen (1900) was the basis of expressing the energy value of ruminant feeds in the UK. This system was introduced by Wood (1917) and adopted in the UK by 1921.

After World War II, calorimetric measurements began to be made again under the direction of Kenneth Blaxter at the Hannah Dairy Research Institute (Blaxter et al, 1954). He was the first person to propose a new approach to energy systems based on the determination of ME, to describe the values of feeds and the requirements of ruminants (Blaxter, 1962). His approach was accepted by the UK Agriculture Research Council (ARC, 1965) and subsequently adopted as the official advisory method for allocating energy allowances for ruminants at a Joint Conference on "Nutrient Standards for Ruminants" held in London on 12 April 1972. Thereafter, an Energy Requirements Working Party was set up to implement the system and concluded that the new system was superior to the Starch equivalent system and recommended its adoption, but in a modified form more suitable for advisory purposes. This led to the publication of a practical manual, Technical Bulletin No 33 (MAFF, 1975), describing the derivation and use of the modified ME system in detail.

FIGURE 1.3

Partitioning of Food Energy within the Animal
(after MAFF, 1975)



A major problem which faced the advisers when it was decided to change to an ME system in 1972, was which sources of information should be used to obtain an estimate of the ME values of feedstuffs.

At that time, the Working Party preparing the Technical Bulletin 33 felt that while there was no correct estimate of ME values, factors may be used to convert the digestible proximate constituents of a food to ME values as indicated in equation No 2 in Technical Bulletin 33. These were factors proposed by workers at the Oskar Kellner Institute at Rostok, West Germany:

$$ME\ (MJ\ kg^{-1}DM) = 0.0152\ DCP + 0.0342\ DEE + 0.0128\ DCF + 0.0159\ DNFE$$

Where:

DCP = Digestible Crude Protein g/kg
DEE = Digestible Ether Extract g/kg
DCF = Digestible Crude Fibre g/kg
DNFE = Digestible Nitrogen-Free Extractives g/kg

The use of such an approach was accepted in the UK by the advisory organisations as a means of smooth and rapid change-over to be made in 1975 from a starch equivalent system to an ME system. Eventually, however, the weakness of such an approach began to be realised because it was questionable whether the ME values obtained from nineteenth century German studies would be applicable to all British foodstuffs grown lately under modern conditions. These uncertainties provided some of the reasons for the establishment of two Feed Evaluation Units at Rowett Research Institute in Scotland in 1972 and the ADAS Nutrition Chemist's Unit in England in 1976. The aim of these units was to provide feed values for the different British feeds, but more importantly, it was

asked to provide a simple way of estimating ME from readily determined parameters.

1.1.3 ME as a Measure of Nutritive Value of Grass Silage

The ME of food is measured in a trial similar to the digestibility trial but additional steps have to be determined. The urine must be collected and its GE value measured. More importantly, the determination of the losses in combustible gases, mainly methane, is required and calls for the use of an expensive and complicated apparatus, a respiration chamber, and involves much labour as well as technical skill accordingly. The measurement of methane is an essential part of the determination of the ME content of feed for ruminants.

Ideally, the ME values used by the advisers should be directly determined by the animal accounting to all of the energy losses of GE. However, such an approach is labour intensive, expensive and requires special facilities and consequently, this direct method is not likely to be a practical proposition.

The use of the table of feed composition to assign a single ME value for the ME content of silage is not feasible. Edwards (1986) has argued against such a proposition and concluded that for the comparatively small number of relatively uniform samples examined in the Rowett Research Institute Studies (Mean = 11.44 MJ kg^{-1} DM, SD = 0.787), it may be calculated that 52% of samples would differ from the mean by more than 0.5 MJ kg^{-1}DM and 20% by more than 1 MJ kg^{-1}DM.

The search for a simple laboratory method to accurately predict the ME of grass silage has been continued vigorously for many years, in particular at the two feed evaluation units mentioned previously. The studies involved a comprehensive measurement of <u>in vivo</u> ME

values of grass silages together with appropriate prediction equations using different laboratory measurements. These studies have been described in detail in the Rowett Research Institute (1975), Wainman et al (1978 and 1984), Barber et al (1984) and Givens et al (1989a).

1.1.4 The Dilemma of Estimating ME for Ration Formulation

Since the implementation of the ME system, the different advisory laboratories in the UK have used different routes in which ME values of grass silages can be predicted. Figure 1.4 shows the two optional routes that existed in the UK for calculating ME values and subsequently the use of these values for ration formulation.

Scottish Agricultural Colleges (SAC) used and are still using the first route where they predict the OMD of silage, measure ash, calculate DOMD and then apply a constant factor to calculate ME. In the past, ADAS used the first route and by 1983 they changed to use the second route (Barber et al, 1984; Givens, 1986, Givens et al, 1989a). In addition to the different methodologies of estimating ME, different laboratory methods have been used to predict ME values (Table 1.1).

Scientifically, route (II) is the preferred approach. However, two aspects have to be clarified before such a conclusion can be made:

A The Reliability of the Current ME Measurements!
B What is the Efficiency of ME Utilisation?

A The Reliability of the Current ME Measurements

Since the ME system was adopted in the UK, up to now there have been relatively small numbers of grass silages which have measured in vivo ME. The only documented in vivo ME values were the ones

FIGURE 1.4

The Two Possibilities that Exist Within the Different Advisory Organisations in the UK in Calcuating ME.

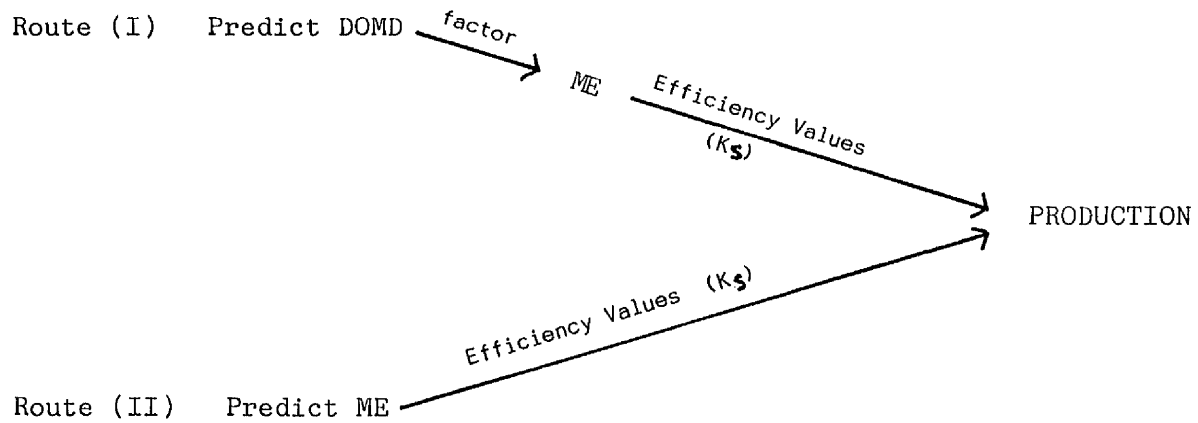


TABLE 1.1

Methods Used to Predict ME by UK Laboratories

Methods	UK Laboratories					Reference
	ADAS	WSC	NOSCA	ESCA	DANI	
IVOMD	x	x				Tilley & Terry, 1963 (ADAS); Alexander & McGowan, 1966 (WSC)
MADF	x	x		x	x	Clancy & Wilson, 1966
LIGA			x			Morrison, 1972
NCD	x					Dowman & Collins, 1982

publicised by Rowett Research Institute (RRI) reports (RRI, 1975, Wainman et al, 1978 and 1984). Despite the fact that ADAS feed evaluation unit (FEU) reported a measured in vivo ME, however, the methane production was predicted by the Blaxter and Clapperton (1965) equation due to the unavailability of a respiration chamber to measure energy loss as methane gas.

Barber et al (1984) reported prediction equations for silages based on the ADAS FEU data, these equations are listed on Table 1.2. They concluded that the relatively poor associations of the different predictions of ME must be caused by variation in calculating in vivo ME values due to other factors and the accuracy of prediction of DOMD was somewhat better and was comparable to those for fresh grass and hay. As more silages become available, these equations were updated by Givens (1986) and Givens et al (1989a).

Edwards (1986) analysed the equation for predicting ME content from MADF and NCD proposed by Givens (1986). The values of standard error of prediction (SEP) for predicted silage ME of 11.5, 10.8, 10.0 and 9.3 MJ kg^{-1}TDM by using MADF equation were 0.99, 0.97, 0.87 and 1.0 respectively. He concluded that at a predicted ME of 10 MJ kg^{-1}TDM, some 57% of estimates will differ from the true value by more than $\pm$ 0.5 MJ kg^{-1}TDM and 25% by more than $\pm$ 1 MJ kg^{-1}TDM. Similarly, at a predicted ME of 10 MJ kg^{-1}TDM using the proposed NCD equation some 60% of estimates will differ from the true value by more than $\pm$ 0.5 MJ kg^{-1}TDM and 31% by more than $\pm$ 1.0 MJ kg^{-1}TDM.

TABLE 1.2

DOMD and ME Prediction Equations for Silages (Barber et al, 1984)

		n	R^2 (%)	RSD (%)
DOMD %	= 25.04 + 0.605 NCD %	68	62.6	2.93
DOMD %	= 26.55 + 0.651 CD %	55	67.3	3.23
DOMD %	= 8.10 + 0.881 IVD %	56	76.9	2.70
DOMD %	= 98.31 - 1.04 MADF%	79	53.6	3.64
DOMD %	= 96.31 - 0.583 NDF %	77	46.3	3.97
DOMD %	= 95.20 - 0.883 ADF %	79	48.4	3.84
ME (MJ kg^{-1}DM)	= 4.35 + 0.092 NCD %	68	25.6	0.968
ME (MJ kg^{-1}DM)	= 4.51 + 0.100 CD %	55	34.6	0.969
ME (MJ kg^{-1}DM)	= 1.90 + 0.132 IVD %	56	37.5	0.939
ME (MJ kg^{-1}DM)	= 15.97 - 0.175 MADF%	79	32.4	0.944
ME (MJ kg^{-1}DM)	= 14.91 - 0.084 NDF %	77	20.3	1.03
ME (MJ kg^{-1}DM)	= 14.55 - 0.122 ADF %	79	19.2	1.03

The use of MADF to predict the ME content of 48 silages of RRI (RRI, 1975; Wainman et al, 1978 and 1984), was also commented on by Edwards (1986). The residual standard deviation (RSD) of the prediction equation was 0.76. He concluded that at a predicted value of 11.5 MJ kg^{-1}DM, 51% of estimates will differ from the true value by more than $\pm$ 0.5 MJ kg^{-1}DM and 19% by more than $\pm$ 1 MJ kg^{-1}DM.

From the foregoing discussion it is clear that the current prediction of ME using route (II) is unsatisfactory. An attempt to examine some of the problems associated with the measurement of the ME content of silage and the areas which need further research will be presented below.

1 GE of Silage

It is commonly assumed that food in general has a GE value of 18.4 MJ kg^{-1}DM (MAFF, 1975). However, it is unlikely that this common value is true for silages and many workers have commented on the apparently high GE of silages. Alderman et al (1971) reported a mean value of 45 grass silages of 20.17 MJ kg^{-1}DM. This value is 10% higher than the usual value of 18.4 MJ for herbage. McDonald and Edwards (1976) obtained GE values of 18 silages of 20.0 MJ kg^{-1}DM and 18.4 MJ kg^{-1}DM of the original grass, representing an increase during ensiling of 8.7%.

The reason for these high GE values of silages stems from the fact that ensilage is an energy concentrating process and that it comes through the replacement of low energy substrates by high energy fermentation products. Several of the products of fermentation are of higher GE value than the original substrates. For example, butyric acid and ethanol

have GE values 24.9 and 29.8 MJ kg^{-1}DM respectively, compared with glucose and fructose which have values of 15.6 and 15.7 MJ kg^{-1}DM respectively.

The GE of silages is not only high compared to the original material before ensiling, but also variable. Table 1.3 illustrates the wide range of gross energy contents of grass silages accumulated from different institutions and demonstrates the danger of applying a fixed value for all silages. For these 238 grass silages, the GE mean was 19.1 MJ kg^{-1}DM with a standard deviation of 0.71 and a range of 17.0-21.1 MJ kg^{-1}DM.

It is not surprising that the GE of silages is widely variable (see Table 1.3) considering the nature of the fermentation process that takes place inside the silo and the production of fermentation end products with variable GE contents. It is this characteristic which makes it difficult to measure GE of silages.

Sampling problems is a major contributor to the variation of GE of silages. A recent study by Norcross et al (unpublished) where she determined the GE of silage sampled by a variety of techniques, indicated that there were significant differences ($P < 0.05$) between the treatments studied. The nature and quantity of volatiles are in part responsible for the variation in the GE value of silage, therefore any loss of volatiles from the exposed silages result in a change in GE value. Other losses of energy when silage is exposed to air are through the oxidation processes of aerobic deterioration.

TABLE 1.3

Gross Energy Contents of Grass Silages
(MJ kg^{-1}DM*) [Edwards, 1986]

Source	N	Mean	SD	Range
Edinburgh School of Agriculture	60	19.2	0.67	17.8–20.7
MAFF/ADAS	126	19.0	0.80	17.0–21.1
Rowett Research Institute	48	19.2	0.49	18.1–20.2
Hannah Research Institute	4	19.0	0.93	18.1–20.0

* Dry matter determined by toluene distillation with a correction for ethanol.

Conventionally, the GE of silages is measured with an adiabatic bomb calorimeter. However, different varieties of techniques have been used with regard to the primer and currently polythene is the most common. Reproductibility of the determination of the GE of silage has been found to be difficult, therefore a trimmed mean approach was used to exclude high GE figures (Norcross et al, unpublished).

The measurement of in vivo ME in a ring test involving ADAS FEU, DANI and NOSCA indicated that, although there was good between-centre agreement with regard to in vivo digestibility, the ME values obtained varied between laboratories. The problems in the measurement of GE in each centre was stressed (Unsworth, unpublished).

These variations in GE determination of silages focused the attention on the use of the GE values as a bivariate in ME prediction equations. Givens and Brunnen (1987) and Givens et al (1989a) stressed this aspect strongly and indicated that the GE of silages accounts for 50% of the variability in ME concentration and yet it is ignored in present laboratory prediction methods. For example, the accountable variance (R^2 %) for the prediction of in vivo ME concentration for 115 silages increased from 23.9% when IVOMD was used as a single predictor to 77.4% when GE was included as a bivariate (Givens et al, 1989a). However, it is important to realise that this approach is unlikely to be adopted for advisory purposes because of the difficulties in measuring GE for silages and also the lack of a quick and easy method to measure GE for advisory purposes.

2 The Expression of the DM Base

Routinely, the ME concentration of silages is currently expressed on an oven dry matter (ODM) basis which involves drying the silage samples in an oven at 105°C for 24 hours. This method however, has been recognised for a long time to be inaccurate for fermented products because it fails to consider the loss of dry matter as volatile substances during the normal drying process.

These volatiles are part of silage dry matter and many workers have drawn attention to the importance of taking into consideration the volatiles lost on oven drying in order to assess the true dry matter content of silage (Haigh and Hopkins, 1977). Different methods have been suggested by many workers to determine the true dry matter content of silages, but a toluene distillation method developed by Dewar and McDonald (1961) has been recommended for the routine determination of dry matter content in silage.

The losses of these volatile compounds can be significant during the normal oven drying procedure. For example, oven drying at 100°C can lead to dry matter losses of up to 16%, depending on the quantity of organic acids present (Minson and Lancaster, 1963). Compared with the toluene dry matter method, the losses of the dry matter of silages can be 9.7, 6.8, 8.6 and 10.7% when the dry matter was determined by freeze drying, microwave drying and oven drying at 70 and 100°C respectively (Aerts et al, 1974).

Barber et al (1984) stressed the importance of including the weight of volatile compounds and indicated that by necessity, the ME concentration

equations must be expressed on true dry matter (TDM) [see Table 1.1]. More recently, Givens (1986) confirmed this aspect and concluded that the expression of silage ME values on an ODM basis is both mathematically and biologically incorrect and gives rise to erroneously high values. Therefore he replaced the old prediction equation which was used to predict ME on an ODM basis by a new equation based on a TDM basis. Accordingly, this equation has been used by ADAS Nutrition Chemists from mid 1985.

The new proposed ADAS FEU equations (Barber et al, 1984) were found to give a predicted ME value 7-12% higher than that predicted from equations in current use. This difference depends on the amount of volatile compounds present and also the fermentation quality of the resulting silage. These high ME values raised suspicion among advisers about the applicability of these new equations in ration formulation and evaluation. Assessment studies performed by Barber et al (1980) and Barber et al (1983), suggested that the current ME equation which is based on ODM gave no bias prediction of animal performance as compared with the new proposed equation. An over-prediction of 7-10% of milk yield has been caused by the use of the new equation. Possible explanations suggested for this discrepancy between actual and predicted yield of dairy cows caused by the new ME values include loss of silage volatiles during the normal feeding conditions on the farm which are initially included on the calculated ME value of the silage and the over-estimation of the efficiency of utilisation of ME.

At present, no routine method can be used for advisory purposes to measure the true dry matter of

silages. The method of Dewar and McDonald (1961) is lengthy and complicated and therefore, prediction equations were suggested to predict silage volatiles. At the West of Scotland College, 150 advisory silages were examined during 1982 to establish regression equations which can be used to predict silage volatiles, however no satisfactory relationship has been found for this purpose (Barber, et al, 1983). Barber et al (1984) and Givens (1986) suggested a single fixed correction factor of 2.3% and 1.9% to convert ODM to TDM. However, considering the nature of silage fermentation and the variability of the volatiles raises questions about the validity of this approach.

For practical reasons, the advisory laboratories in the UK has been forced to ignore the volatiles contained in the silages unless an accurate and routine method can be discovered to measure these important compounds.

3 Measurement of Methane Energy

To determine in vivo ME, faecal, urinary and methane energy needs to be measured. The faecal and urinary energy can be easily determined using conventional metabolism cages. On the other hand, methane energy loss measurement is more complicated and requires special equipment. Understandably, many workers tried to predict this parameter from the knowledge of the amount and type of the food eaten by the animal (Bratzler and Forbes, 1940; Swift et al, 1948; Blaxter and Clapperton, 1965).

Several workers measured methane production and found it to be remarkably similar to the figure of 8% of GE commonly assigned in general for foods, suggesting that methane production differs very little

from this common number. For example, Ekern and Sundstol (1974) found that methane production for two silages was 7.5 and 7.9% and for two hays was 8.1 and 8.3% made from the same grass. Sundstol and Ekern (1976) found methane production for fresh grasses, hays and silages made from the same grasses was 7.5, 7.0 and 6.6% respectively. The examination of methane production figures reported by RRI (RRI, 1975; Wainman et al, 1978 and 1984) revealed that the losses of energy as methane for 39 dried grasses, 26 hays and 48 silages were 7.4, 7.9 and 7.7% respectively (Edwards, 1986).

In the UK, the regression equation derived by Blaxter and Clapperton (1965) at the Hannah Research Institute is used routinely to predict methane production in cattle and sheep. It has been used extensively at the ADAS FEU so that in vivo ME values can be calculated (Givens, 1986). This equation was derived by using 48 different diets and in excess of 2500 determinations of methane production by cattle and sheep. It is defined as:

$$Cm = 3.67 + 6.2D$$

where Cm is the percentage energy lost as methane per 100 units of GE and D is the apparent digestibility of GE.

Recently, doubts have been raised about the validity of the Blaxter and Clapperton equation to predict methane production for grass silages. Edwards (1986) compared the observed energy losses as methane with those calculated by the Blaxter and Clapperton equation. He found that for the 48 silages reported by RRI (RRI, 1975; Wainman et al, 1978 and 1984) the mean difference (observed -

calculated) was -0.48% with SD of 0.711. This suggests that by using this equation, methane production is over-estimated by 0.48%. He concluded that this bias is not of major importance since it could be easily corrected and moreover, it is only 0.1 MJ of GE. The latter worker however, did not report the regression statistics obtained by the regression equation using the Blaxter and Clapperton's equation variables, therefore the same exercise was repeated here. The bias of -0.48 and the SD of 0.711 was confirmed, however no relationship can be found between methane production and the apparent digestibility of GE (R^2 = 0.0). This suggests that for silages the use of Blaxter and Clapperton's equation is not justified and may be no better than the mean determined value of 7.7%.

B What is the Efficiency of ME Utilisation?

In progressing from GE to NE, measurement becomes increasingly difficult, but in compensation more accurate estimates of the energy required for maintenance and production are achieved. Therefore, DE can be considered a first approximation to nutritive value and NE the most precise (Ulyatt, 1973). It is NE which both the nutritionist and the farmer is interested in, thus an accurate estimate of this parameter is of primary concern to them. The ratio of NE to ME contents represents the efficiency of ME utilisation denoted as k.

The methods of measuring the efficiency of ME utilisation have been described in detail by Blaxter (1962). Two types of methods are basically used, comparative slaughter and calorimetry experiments. Comparative slaughter experiments require little equipment, but are expensive in terms of animals. In calorimetry, the animals are kept in calorimeters and these call for elaborate and expensive equipment and labour input.

For silages, few calorimetric studies have been made to determine the efficiency of ME utilisation. Consequently, a means of predicting the efficiency using a suitable regression equation was used. In the UK, the equations derived by the ARC (1980) were used for this purpose. These equations are:

$$k_m = 0.35\ q_m + 0.503$$
$$k_f = 0.78\ q_m + 0.006$$
$$k_l = 0.35\ q_m + 0.42$$

where q_m is the metabolisability (ME/GE).

For advisory ration formulation purposes, k_m and k_l were assigned single values of 0.72 and 0.62 respectively, and for k_f the following relationship was given (MAFF, 1975):

$$k_f = 0.0435\ M/D$$

where M/D is the ME per kg DM.

Thomas and Thomas (1985) reported k_m values for 8 silages and these were compared with calculated values using the ARC (1980) equation (Table 1.4). These workers found that the calculated k_m values were always higher than k_m values determined calorimetrically. The mean difference between the two values were 0.04 and the two means were significantly different ($P < 0.001$).

Similarly, McDonald (1983) showed that the calculated k_f values using the ARC equation (1980) were on average, higher than the k_f values determined calorimetrically (Table 1.5). The mean difference between the two values were 0.09 and the two means were significantly different ($P < 0.05$). For k_f values, we can see from the table that the size of the difference between calculated and determined values was much greater than the k_m values. Some of the individual differences were extremely large, which causes a lot of concern.

TABLE 1.4

The Partial Efficiency of Utilisation of Metabolisable Energy for Maintenance (k_m) Determined Calorimetrically and Calculated by the Equation of Agricultural Research Council (1980) for Eight Silages (after Thomas and Thomas, 1985)

Silage	Determined k_m	Calculated k_m*
1	0.69	0.75
2	0.71	0.74
3	0.68	0.70
4	0.66	0.70
5	0.71	0.71
6	0.68	0.70
7	0.65	0.74
8	0.66	0.74
Mean	0.68	0.72
SD	0.023	0.022
SE of Difference	0.011	

*k_m = 0.35 ME/GE + 0.503

TABLE 1.5

The Partial Efficiency of Utilisation of Metabolisable Energy for Fattening (k_f) Determined Calorimetrically and Calculated by the Equation of Agricultural Research Council (1980) for Fourteen Silages (after McDonald, 1983)

Silage	Determined k_f	Calculated k_f*	Silage	Determined k_f	Calculated k_f*
1	0.21	0.44	8	0.43	0.49
2	0.51	0.56	9	0.39	0.47
3	0.42	0.42	10	0.34	0.53
4	0.41	0.45	11	0.23	0.61
5	0.49	0.43	12	0.61	0.61
6	0.54	0.51	13	0.52	0.61
7	0.40	0.53	14	0.45	0.58
Mean k_f determined	0.43				
SD	0.112				
Mean k_f calculated	0.52				
SD	0.069				
SE of the Difference	0.031				

* Calculated from $k_f = 1.32\ ME/GE - 0.318$

Several workers commented about the low k_f values sometimes obtained for silages. Thomas and Chamberlain (1982) suggested that the very low figures obtained for k_f by some experiments (see Table 1.5) could partly be explained by technical difficulties in obtaining correct estimates, but in the other experiments the low k_f values represent a true inefficiency in energy utilisation of silages. Thomas and Thomas (1985) indicated that these low efficiencies may arise because of a nutritional imbalance in silage diets, resulting from the poor utilisation of silage nitrogen in the rumen. The work of Donaldson and Edwards (1977 and 1979) supported this view where they found high rumen ammonia concentrations when the animals were fed silage diets only. Furthermore, Kelly and Thomas (1978) found that the low k_f values were only apparent when the silage was given alone, and therefore this might not be of a major concern under farm conditions where silages are given as part of a mixed diet.

Thomas and Thomas (1985) also suggested that silages might contain specific substances which could disturb the energy metabolism inside the animal. They further added that dried silage extracts can increase the basic metabolic rate of rats, due apparently to the presence of certain flavenoids (McLaren et al, 1964; Qasim and Stelzing, 1973).

Very few calorimetric determinations have been carried out to measure k_l for grass silages. Unsworth and Gordon (1985) reported a k_l value of 4 silage diets supplemented with 40% concentrate. The comparison was made between determined k_l and predicted k_l using the ARC (1980) equation (Table 1.6). The discrepancy between determined k_l and

TABLE 1.6

Efficiency of Utilisation of Metabolisable Energy of Silage Diets for Lactation (after Unsworth and Gordon, 1985)

Silage	k_1	
	Determined	Calculated*
a. Unwilted	0.56	0.65
Wilted	0.58	0.64
b. Unwilted	0.50	0.64
Wilted	0.53	0.65

* $k_1 = 0.35\ q_m + 0.42$ (ARC, 1980)

SE of Difference = 0.018

predicted k_1 was thought to be caused by the fact that the ARC (1980) equation was derived by using a non-silage diet (Unsworth and Gordon, 1985).

For advisory purposes, k_1 is assumed to be constant at 0.62 (MAFF, 1975). The work of Barber et al (1980) and Barber et al (1983) suggested that the use of this constant figure may be unjustified. These workers found that over-prediction of milk yield was detected when a new high ME value was calculated using a new prediction equation (Barber et al, 1984) based on a TDM basis. They concluded that the cause of this biased prediction of animal performance may be due to the fact that silage ME is utilised less efficiently than what it is thought to be.

1.1.5 The Use of Digestibility to Calculate ME.

It is possible to calculate ME from digestibility measurements and this approach has been widely practised by most advisory organisations in the UK. In this case, ME is calculated from the multiplication of DOMD% by a constant factor. This factor is based on the assumption that the energy value of the DOM is relatively constant between foods (Switt, 1957), together with a constant ratio of ME/DE (Blaxter, 1964).

MAFF (1975) suggested the use of a multiplication factor of 0.154 for various foodstuffs (19.0 MJ kg^{-1}DOM * 0.81). However, this factor is unlikely to hold for silages because of the presence of high energy volatile compounds. Indeed, several investigators suggested the use of higher coefficients to account for these volatiles. Thomas and Chamberlain (1982) stated the use of a multiplication factor of 0.163 (19.35 MJ kg^{-1}DOM * 0.84) for well preserved high digestibility silages. In a

comprehensive study reported by Givens et al (1989a), they calculated a factor of 0.161, however the GE of the DOM and ME/DE ratio were somewhat different than the previous study (19.9 MJ kg^{-1}DOM and 0.81 respectively). Based on the 48 RRI silages (RRI, 1975; Wainman et al, 1978 and 1984), a factor of 0.166 has been found (20.14 MJ kg^{-1}DOM * 0.826) which is higher than the factors stated by the previous workers (Givens, personal communication). We might observe that the ME/DE ratio for RRI silages is relatively higher (0.826) than the one reported by Givens et al (1989a) [0.81]. This discrepancy is possibly related to the lower methane losses measured calorimetrically for RRI silages compared to those predicted by the Blaxter and Clapperton equation (1965) for Givens et al (1989a) silages.

This latter aspect has been confirmed by a cow study performed in Northern Ireland where an ME/DE ratio was found to be 0.84 (Unsworth, personal communication).

It is clear from the previous findings that the calculated factor for grass silages is subject to considerable variation, an area which needs further study and investigation.

1.1.6 Summary

Silage is no longer considered as roughage feed which provides the animal with maintenance requirements only. The principles of making a good quality silage are now more fully understood. Considering the nature of the fermentation that takes place inside the silo, it is not surprising that silage is not an homogeneous entity. Therefore, a correct estimate of its nutritive value is of major concern to nutritionists and farmers.

The ME is the basis for allocating energy allowances for ruminants in the UK. Up to now, there has been a lack

of accurate, fully determined ME values from measurements of faecal, urinary and methane energy losses. There is doubt about the applicability of Blaxter and Clapperton's equation to predict methane production, at least for silages and may be it is no better than the mean determined figure.

Silages have been known to have higher GE values compared to the original herbage. Also due to fermentation, silages contain a variable quantity of volatile substances. Taking these two aspects into account when calculating ME intake is preferred. However, practical considerations should not be ignored, namely the difficulties of measuring the GE of silages and also finding a suitable routine method which accounts for the volatiles. As a result of these difficulties, advisers have ignored these important factors.

The recent proposed ME prediction equations have been found to give a higher ME value than that calculated from equations in current use. This results from the inclusion of the extra energy of the volatile substances. A practical assessment of the new proposed regressions showed that their use will result in an over-prediction of animal performance when compared to the current prediction equations.

The fate of the volatile substances prior to consumption by the animal and also the the lack of justification for the use of the assigned efficiency values, could explain this bias prediction of animal performance.

The current situation about the efficiency of ME utilisation is not fully understood. The use of ARC (1980) to predict the efficiency values for silages may be unjustified. This is because firstly, none of these equations are based on silage diets and secondly, the

efficiency of ME utilisation is subject to numerous variations and it is not only related simply to metabolisability (q_m). More research is needed in this area.

The above uncertainties will prevent, in part, the immediate adoption of route II as shown in Figure 1.4. The advisers will prefer an approach which is based on simple and sound practical advice than one which appears scientifically correct, but more complicated and not fully understood. Until these uncertainties are removed, route I will provide an alternative simple approach which is sound practically and more understandable. It is therefore important to find a good method for the prediction of silage OMD.

SECTION 2

PREDICTION OF THE ORGANIC MATTER DIGESTIBILITY OF GRASS SILAGE

1.2.1 Introduction

The nutritive value of forages cannot be considered as a single parameter, but it can be classified into three general components: digestibility, intake and the efficiency of utilisation of digested feed (Raymond, 1969). In this section, some related aspects of digestibility will be discussed and the methods used to predict this parameter will be reviewed.

1.2.2 Definitions

Some related definitions of digestibility which are used commonly are going to be mentioned here. These will include:

1 Dry matter digestibility (DMD) % =

$$\frac{\text{DM consumed - DM excreted}}{\text{DM consumed}} \times 100$$

2 Organic matter digestibility (OMD) % =

$$\frac{\text{OM consumed - OM excreted}}{\text{OM consumed}} \times 100$$

3 Digestible organic matter in the dry matter (DOMD) or (D) value (%)=

$$\frac{\text{OM consumed - OM excreted}}{\text{DM consumed}} \times 100$$

OR

$$\frac{\text{Digestible Organic Matter (DOM) consumed}}{\text{DM consumed}}$$

This equation is used frequently for forages, particularly silages where soil contamination can occur during crop harvesting. Since this term is expressed on the DM consumed, it is always less than OMD by an amount depending on the extent of soil and mineral content of the herbage.

The relationship between the major measures of digestibility is shown in Figure 1.5.

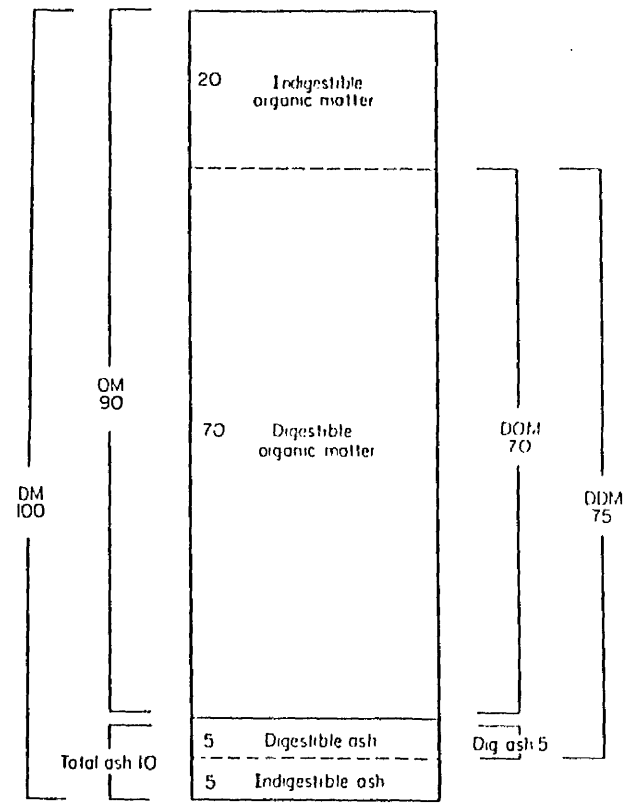
1.2.3 The Measurement of Digestibility *in vivo*

Digestibility is considered to be a simple nutritional balance where a measurement of the amount of food eaten (or a particular nutrient) and the quantity of faeces produced from that food (or the particular nutrient in the faeces) is made over a period of time. The difference between the two quantities is the amount of food (or nutrient) which has been digested by the animal and when this is expressed on the amount of food (or nutrient) consumed, this is defined as the apparent coefficient of digestibility.

To measure digestibility, the experimental ration is fed in measured quantities for a long period in order to ensure that a steady state of faecal excretion is reached, and the faeces excreted are collected over a measured interval of time. A simple trial lasts 2-3 weeks and animals are kept in individual pens to facilitate the measurement of feed intake and faeces collection. For precise measurement, a preliminary test period of at least 10 days is recommended. The work of Blaxter *et al* (1956) showed that the necessary length of this period varied with type and level of feed, but it is usual for it to extend over 7-14 days and 10 days may be used in almost all cases.

FIGURE 1.5

The Relationship Between the Major Measures of Digestibility (after Osbourn, 1980)



$$\text{DMD} = \frac{\text{Wt of DDM}}{\text{Wt of DM}} \times 100 = \frac{75}{100} \times 100 = 75\%$$

$$\text{OMD} = \frac{\text{Wt of DOM}}{\text{Wt of OM}} \times 100 = \frac{70}{90} \times 100 = 77.7\%$$

$$\text{D-Value} = \frac{\text{Wt of DOM}}{\text{Wt of DM}} \times 100 = \frac{70}{100} \times 100 = 70\%$$

1.2.4 The Importance of Digestibility as a Useful Index of Nutritive Value

Historically, digestibility trials were known as early as 1860 (Schneider and Flatt, 1975) and many feed evaluation units considered it to be an important part of their programs. This parameter is regarded as the most useful and basic index of nutritive value available at present. It is a reproducible characteristic and can be measured easily and accurately in many experimental stations around the world. However, digestibility cannot be regarded as the only criterion which describes nutritive value (Raymond, 1969).

Despite the fact that nutritive value is a product of three components, namely digestibility, intake and efficiency (see 1.2.1), most of the research has been more successful in predicting digestibility of forages than the other two components. This is because digestibility offers considerable less animal variations as compared with intake and efficiency (see Table 1.7). Intake is largely influenced by the characteristics of the animal, therefore it is not surprising that intake can be predicted with less confidence as compared with digestibility. Castle (1982) indicated that for most of the trials conducted at the Hannah Research Institute, DOMD was the major factor affecting milk production when various silages were offered ad libitum. He concluded that on average, an increase of 10g kg^{-1} in the DOMD concentration of the silage increased daily milk yield by 0.24 kg. Similar effects have been found for live weight gain (Wilkinson, 1985).

1.2.5 Factors Affecting the Digestibility Measurements

1 Species of Animal

Several investigators have commented about the use of either sheep or cattle when performing the digestibility trials (Schneider and Flatt, 1975).

Table 1.7

Approximate Relative Variation Due to Forages and Animals (Van Soest, 1982)

	Coefficient of Variation (%)	
	Forage	Animal
Digestibility	30	3
Intake	50	30
Efficiency*	50	20

* Use of digested energy for productive purposes.

Wainman (1977) and Aerts et al (1984) reported some differences in the digestive capacity between cows and sheep, however these differences may be small and it is questionable whether it bears a practical significance. Aerts et al (1984) found that there were no systematic differences in the digestive capacity of cows and sheep for 26 maize silage, 24 grass silage and 18 grass hay with an OMD of 65% or higher.

Cows appear to digest low quality rations better than sheep and on the contrary sheep appear to digest very high quality rations better than cows (Aerts et al, 1986). For medium quality rations, which form the major part of the rations for ruminants, the differences in the digestive capacity between sheep and cows are small and thus corrections may be unnecessary (Aerts et al, 1986).

Two possible reasons were given by the latter workers for the better digestion by cows. Firstly, low quality rations may have longer retention time in the rumen compared with sheep. Secondly, minerals are better recycled via cows' saliva therefore more efficient microbial activity is achieved.

Different breeds of sheep have not been found to have differences in their digestive capacity. A study by Blaxter et al (1966) at the Hannah Research Institute indicated that there were no differences in the digestive capacity between six breeds of sheep fed dried grass.

In conclusion, for dairy and beef cattle nutrition, it seems logic to use cows as the experimental animals. However, this proposition may be impractical since

digestion trials with cows are expensive and difficult to operate. Therefore, for evaluation purposes a simpler and cheaper digestion trial using sheep appears to offer an acceptable alternative.

2 Level of Feeding

For diets containing forages, it is not surprising that as level of feeding increases, the rate of passage through the gut is also increased and accordingly, digestibility falls down (Wainman, 1977). Although this aspect seems to be simple and straight forward, the effect of feeding levels on digestibility is variable and complex and various interactions can occur between feeding level and other factors such as physiological status of the animal, diet composition, animal species and duration of adaptation to the diet (Aerts et al, 1986).

Several workers commented about the feeding level effect on digestibility. Brown (1966) reported that when dairy cows were fed on grain-hay diets (2:1 ratio) each increase in feeding level of one unit of maintenance, dry matter digestibility decreased 2.02 percentage units. More recently this has been confirmed by EL Khidir and Vestergaard (1983) where they found that digestible organic matter decreased when daily intake increased as a multiple of maintenance requirements. They concluded that 70% of the decline in digestibility of organic matter was caused by a decrease in the digestibility of the cell wall constituents.

For most evaluation programs in many parts of the world, digestibility trials are commonly carried out at maintenance level of intake since this is easier and the results obtained are reproducible and not liable to be influenced by the level of feeding.

3 Associative Effects

For livestock production, it is commonly practiced that suitable concentrates are fed along with forages so that high levels of production are maintained.

In this situation, the influence of one food on the digestibility of another might occur. However, this influence should be considered together with the term "balanced ration" where all nutrients are present in quantities which do not limit the utilisation of other nutrients (Wainman, 1977). For example, the digestion of cellulose can be decreased if there is a lack of nitrogenous substances or essential minerals in the ration. In addition, the existence of large proportions of easily digested carbohydrates such as starch and molasses can depress the digestibility of the fibre, because micro-organisms attack the simpler carbohydrates first rather than attacking the fibre constituents (Schneider and Flatt, 1975).

Various experiments have been cited which suggest no associative effects between feeds, while some other experiments indicated the opposite. A study performed at RRI Feed Evaluation Unit (Wainman et al, 1976) failed to show significant associative effects when oats, barley and wheat were fed in differing ratios with silage and dried forages. This latter work has been confirmed lately by Wainman et al (1979) when maize silage and barley were fed together. However, American workers indicated the contrary where Byers et al (1976) reported negative associative effects when corn silage and corn were fed together. Wainman et al (1979) attempted to explain this discrepancy and attributed the difference between the two experiments to the physical form in which the grains were fed and in

the nature of the starch contents.

4 Individual Variations

Little work has been done on the effect of individual variations on digestibility measurements. However, in a study based on 1328 individual digestion measurements with 79 different sheep and 518 individual digestion measurements with 24 different cows, Aerts et al (1986) reported that the significant difference between individual animals appears to be small. However, they stressed the importance of using 4 animals so that reproducible and reliable digestibility measurements can be obtained. They concluded that digestibility can be determined with a standard error of 1-2 digestibility units when using 4 animals.

1.2.6 Laboratory Methods for Predicting the Organic Matter Digestibility of Grass Silages

1.2.6.1 Introduction

There is no doubt that the measurement of digestibility using animals is the ultimate standard in determining nutritive value of forages. However, for practical feed evaluation, this proposition may not be feasible since this approach is expensive, labour intensive and cannot be used on a large number of samples. As a result, simple and economical laboratory methods which can be used to predict this parameter are an absolute necessity. In the UK, the search for such method(s) has been a major concern of the different advisory organisations, in particular the two feed evaluation units established in England and Scotland. Recently, notable attempts at developing prediction equations for OMD of grass silages were reported by Aerts et al (1977), Barber et al (1984) and Givens et al (1989a).

1.2.6.2 Criteria for Selection of Laboratory Methods

The need for laboratory methods to predict in vivo digestibility of forages is well recognised. Various methods have been suggested in the literature and before any method(s) can be selected, some basic principles should be considered. These principles include:

1 The analytical procedures involved must be capable of being performed routinely on large numbers of samples with reasonable speed. Its execution should be reproducible, economical and not require a high technical skill.

2 The method(s) should have a high predictability of the standard in vivo, maximise R^2 and minimise prediction error.

3 The method(s) must be versatile, that is the equations needed for different feed types should be as few as possible.

4 The method(s) must be transferable between laboratories and should need minimal change and updating.

1.2.6.3 **Review of the Methods**

The predictive methods which are most widely used for evaluating forages may be categorised as:

I Chemical methods, which measure some chemical parameter thought to be related in some way to the digestibility of the sample;

II Biological methods, which attempt in some way to mimic the natural processes of digestion and

III A physical method (Near Infrared Reflectance, NIR), which makes more fundamental, non-destructive, measurements, related to the energies absorbed from the incident radiation by molecular groups in the sample.

1.2.6.3.1 **Chemical Methods.**

For more than 130 years, the Weende system of Henneberg and Stohmann has been used extensively to describe chemical composition of feed in many parts of the world.

This system involves the following procedures:

(1) Oven drying at 100°C for moisture determination.

(2) The dried residue is ether extracted to determine lipids.

(3) The remaining residue is refluxed 30 min with 1.25% sulphuric acid followed by 30 min with 1.25% sodium hydroxide. The insoluble organic matter residues are reported as crude fibre.

(4) Nitrogen and ash are determined in separate samples.

(5) Nitrogen-free extract is calculated as:

NFE = 100 - Ether extract - Crude Fibre - Ash - Crude Protein (N * 6.25).

This system is based on the assumption that crude fibre represents the indigestible part of the feed. For this reason, therefore, the early evaluation systems were based on regression equations relating digestibility to crude fibre (see Kivimae, 1960; Alderman et al, 1971). The latter assumption has been found to be incorrect, in particular for ruminants since these animals have the capability to digest a large part of the crude fibre.

Many attempts have been made to replace the Weende system with a more fundamental approach and the

detergent system of Van Soest (1967) is such an approach.

This system divides the forage organic matter into two main fractions: 1 - Cell contents which are soluble in neutral-detergent and 2 - Cell wall constituents which are insoluble in neutral detergent. Table 1.8 illustrates the division of forage organic matter using the detergent system and Table 1.9 shows the basic scheme of analysis.

Since the introduction of the detergent system, many workers have used its parameters to predict the nutritive value of forages. An extensive review by Barnes (1973) documented various experiments in which neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were used to predict in vivo digestibility of various forages. O'Shea et al (1972) found that ADF was a poor predictor of in vivo OMD of 31 grass silages (R^2 = 0.05). However, more recently Givens et al (1989a) reported that NDF and ADF were better predictors of in vivo OMD of 86 grass silages (R^2, SEP were -0.53, 4.5% and -0.37, 5.2% respectively) than crude fibre (R^2 = -0.33 and SEP = 5.2%).

In an attempt to improve the predictive capacity of ADF, Clancy and Wilson (1966) studied the effect of increasing acid strength and the length of boiling time from 1 to 3 hours. Two hours hydrolysis has been found to increase the R^2 of 131 herbage samples from -0.49 to -0.79 and decreased the standard deviation from $\pm$ 5.6 to $\pm$ 3.6. The use of this procedure includes oven-drying at 95°C as a preliminary step. This treatment prevents the use of acid detergent as a means of assaying for heat damaged and unavailable protein, which is one of the most valuable applications of ADF (Van Soest, 1982).

The method of Clancy and Wilson (1966) is well known as modified acid detergent fibre (MADF) and has been used

Table 1.8

Division of Forage Organic Matter by System of Analysis Using Detergents (Van Soest, 1967)

Fraction	Components	Availability
Cell contents (soluble in neutral-detergent)	Lipids Sugars, organic acids and water-soluble matter Pectin, starch Non-protein N Soluble protein	Almost completely digestible not lignified
Cell wall constituents (fibre insoluble in neutral-detergent)		
1 Soluble in acid-detergent	Hemicellulose Fibre-bound protein	Partially digestible according the degree of lignification
2 Acid-detergent fibre (ADF)	Cellulose Lignin Lignified N	

Table 1.9

Basic Scheme of Forage Analysis Using Detergents (Van Soest, 1982)

Fraction	Reagent	Treatment	Yield
Neutral-detergent residue (NDF)	Na lauryl Sulfate, EDTA pH 7.0	Boil 1 h	Plant cell wall less pectins
Acid-detergent fiber (ADF)	Cetyl trimethlyammonium bromide in 1 N H_2SO_4	Boil 1 h	Lignocellulose + insoluble mineral
Unavailable nitrogen	Acid-detergent	Kjeldahl N determination on ADF	Maillard products and lignified N
Lignin	72% H_2SO_4 treatment on ADF	3 h, 20°C	Crude Lignin
Cellulose	None	Ash residue from lignin step	Loss in weight
Silica (SiO_2)	Conc. HBr (48%) treatment of ADF ash	Treat ash dropwise 1 h, 25°C	Residue is SiO_2
Hemicellulose	None	Calculate as NDF-ADF	

extensively for many years to predict nutrient value of various forages in many laboratories in the UK. Its usage was more pronounced at the ADAS laboratories and has been considered the official method of calculating the energy value of forages (see Barber et al, 1984). However, more recently, the weaknesses of this method were evident and suspicions about its capability to predict in vivo digestibility, for silages in particular, started to arise (Givens, 1986).

Among the fibre methods, Lignin techniques were the most promising chemical methods in predicting in vivo digestibility of forages. Lignin is a completely indigestible substance known to be the main factor limiting digestibility of forages. The manner in which Lignin reduces digestibility is not fully understood, even though several theories have been proposed. Lignin is believed to link carbohydrates and make them indigestible by reducing their susceptibility to enzymatic attack. This is supported by the evidence obtained from alkali-treated straws where ester linkages between Lignin and carbohydrate are easily cleaved by alkali (Van Soest, 1982). Another theory was that Lignin accumulates at the surface of plant cells and therefore protecting the underlying polysaccharide from microbial attack (RRI, 1983).

Many workers have used Lignin to predict in vivo digestibility. For 18 grasses and legumes mixtures, Van Soest (1963) found an R^2 of 0.61 between in vivo OMD and total Lignin when the ADF fraction was extracted by 72% sulphuric acid. Aerts et al (1977) compared the ability of seven laboratory methods to predict in vivo OMD of 56 silages. He found an R^2 of 0.77 between the Van Soest and Wine (1968) Lignin method and in vivo OMD with a coefficient of variation (CV) of 5.6%.

Morrison (1972) dissolved the cell wall fraction in 25% acetyl bromide solution and measured Lignin spectrophotometrically at 280 nm. Subsequently, Morrison (1973) used his method to predict in vivo DOMD of 20 grass silages and reported an R^2 of 0.71. Recently, Givens et al (1989a) quoted an R^2 of 0.63 with an SEP of 3.7% when they used the acetyl bromide lignin method to predict the in vivo OMD of 99 grass silages. They concluded that this method performed considerably better than any other chemical methods tested in their work.

The Morrison (1972) method has been used routinely by NOSCA to predict in vivo DOMD of grass silages and subsequently to calculate the energy value for ruminants (Murray, 1986).

Despite the fact that Lignin was found to be a better predictor of in vivo digestibility of forages than any other chemical methods, it has not been used widely in practical feed evaluations. Several possible reasons can be attributed to this:

1 Various different methods of Lignin analysis have been used which suggest that there is no agreed and standard procedure to determine the Lignin content of forages. This fact has been studied by McLeod and Minson (1971) when they compared the magnitude of the error in predicting in vivo DOMD from four different methods of analysis for Lignin. They found that the error of prediction was greatly variable between methods and reported to be from $\pm$ 3.1 to $\pm$ 5.0. More recently, Givens et al (1989a) found a striking contrast between the prediction power of acetyl bromide Lignin and permanganate Lignin when these methods were used to predict in vivo OMD of 99 and 86 grass silage samples respectively.

The R^2 and SEP for each method were found to be 0.63, 3.7% and 0.22, 5.7% respectively.

2 The differences among species of plants in Lignin content are variable. The most striking difference is between grasses and legumes where the latter have relatively higher amounts of Lignin than grasses of the same digestibility (Van Soest, 1982). These species differences complicate the use of the Lignin's prediction equations where the equation for Legumes does not fit either grass data or data for mixed samples. This aspect was confirmed by Minson (1982) when he showed large differences in the relationship between Lignin content and digestibility for a variety of forage species and particularly between grasses and legumes.

3 The methods of Lignin analysis are complicated, tedious and require skillful labour to operate. It requires a very small amount of the sample which means sampling is a very important consideration, otherwise precision might be affected.

The inclusion of a Lignin analysis with other prediction methods in a multivariate relationship usually improves the predictive power of the regression equation. This is not surprising since we know the role of Lignin in forage digestibility.

Kridis et al (1987) revealed that the combination of IVOMD plus acetyl bromide lignin method in a bivariate relationship improved the prediction power of in vivo OMD of 72 grass silages from an R^2 of 0.74 and RSD of 3.4% to an R^2 of 0.80 and RSD of 3.0%. This aspect has been confirmed in a similar study by Givens et al (1989a).

1.2.6.3.2 **Biological Methods.**

For the purposes of this review, these methods will be classified into three main groups:

1 In vitro rumen liquor methods
2 Enzymic methods
3 In situ method

1 **In vitro rumen liquor methods**

As the name implies, these methods involve the use of live rumen micro-organisms to digest a known amount of forage samples anaerobically in a test tube under a controlled environment of temperature and pH for a specific period of time.

The known and unknown factors that might affect the disappearance of the incubated samples will be subjected to rumen micro-organisms. This is in contrast to the chemical methods where the analyst analysed the plant cell wall in an attempt to identify a chemical functional group(s) which might be related to digestibility. Therefore, it is not surprising that the rumen liquor method will perform better in predicting in vivo digestibility of forages than the chemical methods. Van Soest (1982) emphasised that the superiority of the in vitro method over the chemical methods relies on its ability to reflect biological events.

The use of these methods was early reported as a one-stage process. Asplund et al (1958) predicted in vivo DMD digestibility of 11 hays with an R^2 of 0.56 and RSD of 3.5% when using 24 hours incubation time. Subsequently, Tilley et al (1960) found that in vivo DMD of 20 cocksfoot samples can be predicted with an R^2 of 0.83 and RSD of 3.6%

when using 48 hours incubation.

The addition of a 48 hours second-stage with acid-pepsin improved considerably the coefficient of determination and lowered the standard error compared to results obtained from the one-stage technique. Tilley et al (1960) reported an improvement in in vivo DMD of 20 cocksfoot from an R^2 of 0.83 and RSD of 3.6% using one-stage process to R^2 of 0.96 and RSD of 2.0% using two-stage process.

This method has been universally known as in vitro two-stage technique and found to be superior as a predictor of in vivo digestibility over the chemical methods (Tilley and Terry, 1963).

The rumen micro-organisms in the first stage attempts to measure the digestible fibrous fraction of the plant cell wall while the acid-pepsin in the second stage solubilises the microbial dry matter and nitrogen resulting from the first stage. Therefore, the entire system is rather a simulation of the digestive process that occurs inside the ruminant animal. Van Soest et al (1966) replaced the acid-pepsin stage by neutral detergent which solubilises bacterial cell wall and other indigenous materials. They reported such systems as in vitro true digestibility rather than the apparent digestibility of Tilley and Terry. Even though the Van Soest system is two days shorter, it has not been found more accurate than the original method (Cottyn et al, 1986).

Several modifications to the Tilley and Terry (1963) original procedure were proposed. Alexander and McGowan (1966) noted that the centrifugation at the

end of each stage was not suitable to apply this method for routine use. Therefore, they suggested direct acidification at the end of the first stage followed by immediate filtration at the end of the second stage. By doing so, Alexander and McGowan (1966) were able to determine 250-300 samples per week without loss of accuracy.

To improve the accuracy of prediction of in vivo digestibility of silages, Alexander and McGowan (1969) proposed a scheme of analysis where fresh homogenised silage samples can be used instead of the dried samples so that volatile compounds usually found in silage can be included in the determinations. This scheme improved the prediction of in vivo OMD of 18 grass silages from an R^2 = 0.27 to 0.74 and lower RSD from 3.6% to 2.2%. The latter approach was adopted at the West of Scotland College as the routine method to evaluate silages.

The Tilley and Terry (1963) method has been cited as the best and the most accurate technique of predicting in vivo digestibility of forages over a variety of other techniques (Oh et al, 1966; McLeod and Minson, 1978; Abrams et al, 1981; Morgan and Stakelum, 1987; Coelho et al, 1988). For silages, limited work has been reported, however the work of Aerts et al (1977), Barber et al (1984) and Givens et al (1989a) were the most notable. All these researchers showed an accurate prediction of in vivo OMD of 56, 56 and 117 grass silages respectively, when using the Tilley and Terry (1963) method.

Being a biological system, the in vitro rumen liquor method is liable to many sources of error which

make this technique difficult to standardise. The most important single source of error is the rumen liquor. Several factors were found to influence the rumen liquor activity. These include the diet of the donor animal, method of collection, sampling, and processing of the rumen liquor (Barnes, 1973). The activity of rumen liquor collected from cattle or sheep has also been found to be different (Van Dyne, 1962). Other sources of variations like grinding procedures, maintaining adequate pH, sample size, temperature, adequate anaerobiosis and length of incubation time have been found to be important to obtain satisfactory results (Barnes, 1973). Alexander and McGowan (1966) included standard samples in each *in vitro* run so that between-run drifts can be corrected for.

Despite the fact that the *in vitro* rumen liquor method improved substantially the accuracy of predicting *in vivo* digestibility, this method has not been widely accepted to evaluate forages routinely. Beside the uncontrolled variations mentioned above, major difficulties of this technique are worth mentioning. These include its dependence on fistulated animals, expensive, slow and difficult to operate. More importantly, the objection to the use of fistulated animals by the animal welfare organisations is strong. These reasons will undoubtedly make the using of this method limited and it is likely to decline in the near future. These difficulties have led to a search for more simple methods which can replace the rumen liquor, and the use of the cellulase enzyme may offer an acceptable alternative.

2 Enzymic methods

These methods are empirical procedures that

solubilise forage dry matter with little attempt to define the actual chemical groups involved (a distinction from the chemical methods) as long as the results are correlated with the in vivo parameters (Barnes, 1973). They involve the use of enzymes at moderate temperature.

Commercially produced cellulase from fungi were widely used for this purpose. Jones and Hayward (1975) tested 4 different fungal cellulases in their ability to solubilise forage and cellulose paper. They found that the Trichoderma viride cellulase was the most active in solubilising both substrates. Accordingly, this source of enzyme was used subsequently by other workers (Terry et al, 1978; Adamson and Terry, 1980; DeBoever et al, 1988). It is now the most widely accepted source and has been used successfully in many laboratories around the world.

Donefer et al (1963) were the first to examine the use of cellulase preparations to predict forage digestibility. Subsequent work by Jarrige et al (1970), Jones and Hayward (1973), Hartley et al (1974) and McQueen and Van Soest (1975), all reported a high correlation between in vivo digestibility and the solubility of forage dry or organic matter with cellulase preparations.

A second stage with acid pepsin has been proposed to follow the cellulase stage in an attempt to improve the predictability of the method (Jones and Hayward, 1973). However, these authors found that this procedure did not improve the correlation with in vivo digestibility. Jones and Hayward (1975) noted that the pretreatment of forage samples with acid pepsin for 24 hours followed by 48 hours

cellulase incubation considerably improved the prediction of *in vivo* digestibility of 19 grasses. This has been confirmed subsequently by Goto and Minson (1977) and McLeod and Minson (1978). This improvement has been suggested as being due to not only the removal of protein but also the forage cell wall became more accessible to cellulase attack (Jones and Hayward, 1975).

Limited work has been reported about the use of the pepsin cellulase method to predict *in vivo* digestibility of grass silages. The work of DeBoever *et al* (1988) and Givens *et al* (1989a) were the most recent and comprehensive studies available. The latter workers reported an R^2 of 0.71 and RSD of 3.2% for 55 grass silages.

Several workers attempted to replace the acid-pepsin stage with neutral detergent (Van Soest and Wine, 1967) so that the time required could be reduced from 24 hours to 1 hour and also forage cell walls could be prepared for cellulase attack. Hartley *et al* (1974), Roughan and Holland (1977) and Dowman and Collins (1982) all reported high correlation between this method and *in vivo* digestibility of forages. Jones (1986) criticised the pretreatment with neutral detergent and concluded that while the time required to complete the analysis was greatly reduced when compared with the pretreatment with acid pepsin, it was difficult to remove the traces of detergent from the residues. He further added that the neutral detergent acts as a potent inhibitor of cellulase, therefore, a prolonged and effective washing is required.

McLeod and Minson (1982) compared the effects of the pretreatment of acid-pepsin or neutral detergent

upon the accuracy of predicting in vivo DMD of grasses and legumes by the cellulase method. They concluded that, for grass samples, the gain in reducing analytical time with neutral detergent is offset by a larger error in predicting in vivo digestibility. This larger error is confirmed by Givens et al (1989a) where he reported that the use of the pepsin-cellulase method is relatively more accurate than the neutral detergent - cellulase method for grass silages.

It can be safely suggested that the cellulase techniques may offer another alternative to the rumen fermentation techniques (Jones, 1986). The cellulase techniques have been found to be precise and reproducible and the replicates of determinations were significantly lower than the rumen liquor method (Clark and Beard, 1977). For these reasons, the pepsin-cellulase method has been proposed to be used routinely as a predictor of in vivo digestibility of hays (Adamson and Terry, 1980) and grass silages (Givens et al, 1989a). However, Adamson and Terry (1980) stressed the importance of checking the activity of the cellulase batches since they found a significant difference between batches at 0.1% level. This confirmed earlier findings by Clark and Beard (1977). Close monitoring of the activity of cellulase between batches and from different suppliers is therefore required to ensure constant enzyme activity.

Terry et al (1978) compared the accuracy of rumen liquor and pepsin-cellulase methods in predicting the in vivo DMD of 48 grasses and 25 legumes. They showed that one regression equation can be used for both grasses and legumes when using the rumen liquor method whereas the pepsin-cellulase method

failed to satisfy this criteria. They further added that the pepsin-cellulase can be of value for grasses and, within species, for legumes because of its speed, precision, accuracy and convenience when compared to the rumen liquor method. These latter findings may raise suspicion about the applicability of the pepsin-cellulase method for practical evaluation of forages of mixed species.

3 **In situ method**

The in situ method involves placing small amounts of feed inside bags made of indigestible fabric (such as silk, dacron, nylon or polyester) and suspended in the rumen of a fistulated animal for specific incubation periods. The technique measures the disappearance of feed dry matter or specific nutrients from the bags. This is in contrast with the previous technique where the incubator was the test tube.

Quinn et al (1938) were the first to suspend silk bags in the rumen of fistulated sheep and study the disappearance of feeds. McAnally (1942) suspended silk squares in fistulated sheep and studied the disappearance of wheat and oat straw. Balch and Johnson (1950) placed cotton threads in the rumen of fistulated cows and observed the rate of the thread's breakdown.

Since these initial studies, the in situ method has been used subsequently to enhance our understanding of forage degradation and rumen functions. Erwin and Elliston (1959) measured the rate of disappearance of concentrate and hays from nylon bags. The rate of digestion of the cellulose in four forages was measured by Hopson et al (1963) using dacron bags. Chenost et al (1970) characterised the

dry matter disappearance of 84 samples of leaves and stems of 9 forages using nylon bags. Shoeman et al (1972) used the in situ method to study the effect of formaldehyde on the degradation of different protein samples. Ørskov et al (1978) studied the effect of diets supplemented with fat on fibre digestion. Rook et al (1983) determined the rate and extent to which various minerals were released from polyester bags containing grass silages incubated in the rumen of cows. Silva and Ørskov (1988) used the in situ method to study the effect of five different supplements on the degradation of straw in sheep given untreated barley straw.

The in situ method was also proven to be useful in forage evaluation. Lusk et al (1962) reported an R^2 of 0.69 between 72 hours dry matter disappearance from nylon bags and in vivo DMD of 8 hays. Chenost et al (1970) found a highly significant correlation between in vivo digestibility and 48 hours rumen disappearance from various forages including silages. Aerts et al (1977) compared the ability of seven laboratory methods in predicting in vivo OMD of 56 grass silages. They concluded that the in situ technique predicted in vivo digestibility more accurately than the in vitro rumen liquor or chemical methods. The most distinguished study about the use of this method to evaluate grass silages was reported by Cottyn et al (1986) at the National Institute of Animal Nutrition in Belgium. They revealed that the nylon bag method can predict in vivo OMD of grass silages better than in vitro rumen liquor methods. For 100 grass silages, they reported an R^2 = 0.85 and CV = 3.2%.

Variability of *in situ* results were subject to considerable study and attempts to improve its reliability were suggested. The length of incubation time in the rumen could affect the results obtained. Most of the data suggests that dry matter disappearance from bags tends to increase rapidly to a point, then reaches a plateau with little increase thereafter.

Van Keuren and Heinemann (1962) found a significant increase in dry matter disappearance of various forages from the bags with each 24 hour increase in length of incubation time. In the same study, larger variations between bags were found associated with short incubation times. These variations diminished as the incubation times increased up to 72 hours. This has been confirmed by Mehrez and Ørskov (1977) who recorded a high variability between bags when rolled barley was incubated in nylon bags for 6, 9, 12, 15, 18 and 24 hours. Chenost *et al* (1970) showed that the *in vivo* OMD of various forages was better correlated with 48 hours than with 12 and 24 hours incubation time. This time has been found to be adequate for the complete digestion of forages in the rumen.

The diet of the fistulated animals can influence the rate of the dry matter disappearance from the bags. Ørskov *et al* (1980) stressed this effect and indicated that a high concentrate diet could influence the cellulolytic activity in the rumen. Lindberg (1981b) showed that the dry matter disappearance from bags containing hay samples was significantly decreased when the amount of cereals increased in the basal diet. They concluded that this decrease in dry matter disappearance was due to changes in rumen micro-organisms from fibre digesting organisms

to amylolytic and saccharolytic organisms. These findings were in agreement with previous reports (Van Keuren and Heinemann, 1962).

The bags pore size is an important factor in regulating the flow of the rumen content out of and into the bags. Lindberg and Knutsson (1981) showed that when grass hays were incubated in nylon bags, a highly significant difference in the loss of particulate dry matter between bags with 10 and 20 um pore size occurred. The use of bag pore sizes of 20 and 35 um were found to limit gas release from the bags (Uden et al, 1974). However, Lindberg and Varvikko (1982) reported that the bag pore size had little effect on dry matter disappearance if the sample was incubated long enough. An effective washing of bags between experiments is recommended so that the pores are cleared out (Ørskov et al, 1980).

The preparation of samples before incubation in the rumen could have an effect on the in situ results. Ideally, extrusa collected from the oesophagus of the animal can be used (Playne et al, 1978). However, this approach may be impractical. Alternatively, a laboratory hammermill fitted with different screen sizes has been used. However, it is recommended that one screen size is used so that consistent results can be obtained. Grinding forage samples through 20-, 40- or 60-mesh screen was found to have little effect on the dry matter disappearance (Van Keuren and Heinemann, 1962). Similar findings were reported by Lindberg (1981a) when silage samples were milled to pass through 1 and 4.5 mm screens. They concluded that fineness of grind has little effect on dry matter disappearance during longer incubation times.

However, the loss of particulate matter was more than doubled using a 1-mm screen compared to the 4.5-mm screen (Lindberg and Knutsson, 1981). Ørskov et al (1980) recommended the use of 2.5-3.0 mm screens for dry forages in the nylon bag studies. The use of fresh forage could eliminate the problem of grinding, however this is an area where further research is needed.

Rapid fibre digestion can be obtained if the bags are incubated in the ventral rumen sac rather than the dorsal sac (Balch and Johnson, 1950). Rodriguez (1968) attached different weights to nylon bags and observed a significant increase in dry matter disappearance from bags as compared with bags which did not have attachments. However, the variation between bags was higher for the attached bags. In the same study, he further found that the variation between bags was reduced when they were tied to 50 cm of string rather than to 30 cm. He suggested that the longer string allowed free movement of the bags within the rumen and thus minimised variation.

It is necessary to incubate an adequate sample size inside the bags so that enough material can remain after incubation for subsequent analysis. However, this depends on bag size. Many workers showed a reduction in dry matter disappearance as the sample size, for a given bag size, was increased (Van Keuren and Heinemann, 1962; Mehrez and Ørskov, 1977; Lindberg, 1981a). Ørskov (1980) recommended a sample size of 2 g air dry ground straw, 3 g good hay or dried grass, 5 g of concentrate and 10-15 g of fresh herbage for a bag size of 140 x 90 mm.

A major source of variation which could influence the in situ results may be the washing procedure after bags are removed from the rumen. However, few reports have been cited to study this effect (Chenost et al, 1970; McManus et al, 1972; Aerts et al, 1977; Cottyn et al, 1986; Kridis et al, 1989). Aerts et al (1977) and Cottyn et al (1986) commented about the superiority of the in situ method in predicting in vivo OMD of grass silages when bags were treated with acid-pepsin after removal from the rumen. They concluded that this pepsin treatment permits a more effective washing and leads to a better agreement between bags, probably by removing forage residues and adherent bacteria.

The results obtained from the in situ method should be treated with caution and Ørskov et al (1980) suggested three aspects which need to be considered about the use of this method. Firstly, the sample in the bag is not exposed to physical breakdowns due to mastication and rumination. Secondly, forage samples will be degraded to smaller particles until they become small enough to leave the bag through the pores. Thirdly, the in situ method measures the disappearance of the incubated sample from the bag not the complete breakdown to chemical compounds. Taking these reservations in mind, Ørskov and Mcdonald (1979) developed a technique to estimate the actual degradability within the rumen. They proposed an exponential expression of the form $p = a + b(1 - e^{-ct})$ where "p" is the actual degradability after time "t", "a" is zero hour degradation or the readily soluble materials, "b" represents the amount of sample which will be degraded within the rumen given sufficient time and "c" the rate constant for the degradation of "b". The combination of a + b gives the expression of

the potential degradability and represents the amount of sample which can be degraded within the rumen given sufficient time.

Ørskov and McDonald (1979) linked the degradation rate to an estimate of particle outflow rate from the rumen to give an expression of the effective degradability (P):

$$P = a + \frac{bc}{c + k}$$

where a, b and c are the constants obtained from the previous equation and k is the outflow rate. This expression represents the amount of sample which will actually be degraded in the rumen and its value varies according to the outflow rate.

This approach has been used with some success at the Rowett Research Institute to study protein degradability.

From the foregoing discussion, it is clear that the in situ method suffers from inherent limitations which make it very difficult to standardise, and hence, reproduce. Donaldson et al (1980) attempted to standardise this technique between Scottish Agricultural Colleges, however considerable discrepancies were reported. This has been confirmed by a subsequent ring test involving 13 laboratories (Evans and Cottrill, 1984). As with the in vitro rumen liquor method, its dependence on fistulated animals is a major problem. These limitations will undoubtedly make this method less attractive for routine use. However, for initial forage evaluation and trying to establish calibration sets, the in situ method may prove to be a powerful

technique.

1.2.6.3.3 **A Physical Method. (NIR)**

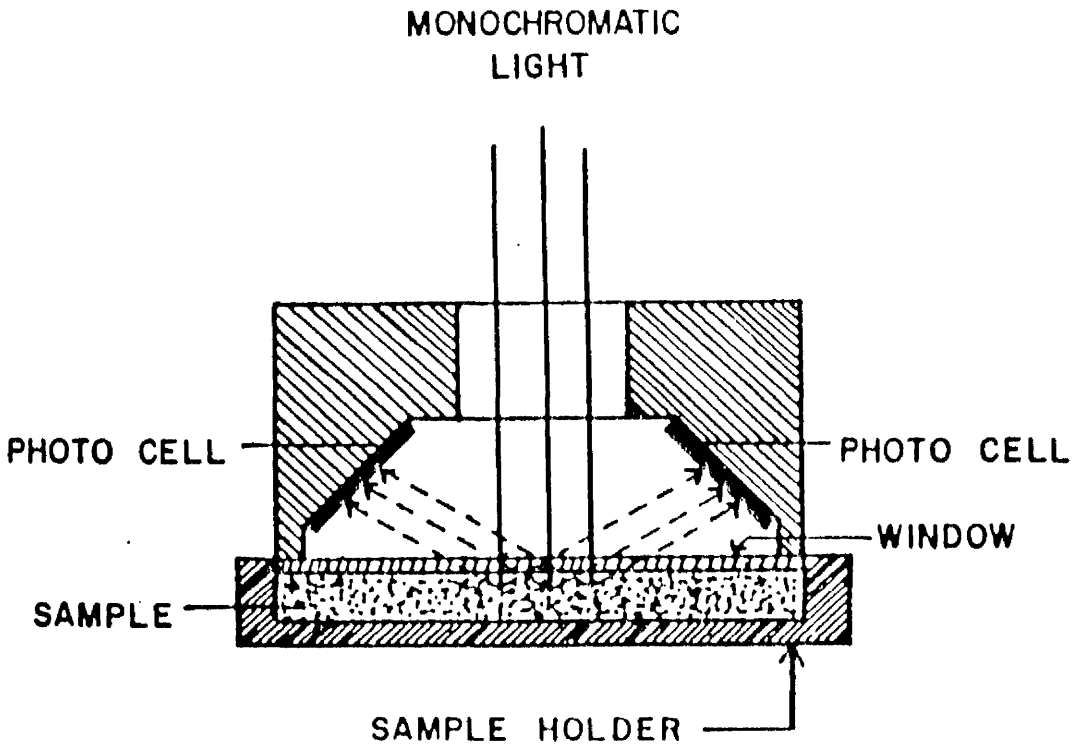
Near infrared reflectance is a non-destructive physical method capable of predicting the chemical composition of a solid sample rapidly with little sample preparation. After making the measurement, the sample can be retained intact for future or subsequent analysis. This technique is relatively new technology and has emerged in the last 10 years as a rapid method in forage testing.

The near infrared is that part of the electromagnetic spectrum which lies between the visible and infrared regions and covers the wavelength range between 800-2500 nm. For most quantitative analysis, the most useful region is from 1200-2500 nm (Norris, 1985). The properties of the hydrogen bearing functional groups like -CH, -OH and -NH (Kaye, 1954) and their hydrogen bonding environment are considered to be useful to reveal the biological availability of energy and other compositional information. Any effect which could alter bond strength such as aromaticity, polar groups or hydrogen bonding to adjacent atoms in the sample, is going to change the spectral characteristics (Murray, 1986).

When the sample is submitted to the instrument a narrow beam of light strikes the sample and the reflected radiation from the surface of the sample is collected by detectors [photocells] (see Figure 1.6). The signals from the detectors are converted to a digital signal at defined intervals and stored in a coupled computer as the logarithm of reciprocal reflectance (Log $\frac{1}{R}$). Each Log $\frac{1}{R}$ value represents a wavelength and the wavelength interval can be from 0.1 to 10 nm, with 1 to 2 nm being the most selected interval (Norris, 1985). The computer performs stepwise multiple linear regression analysis to

FIGURE 1.6

Optical Geometry for Diffuse Reflectance Measurements based on Using 0° illumination and 45° collection with Large Area Photocells (from Norris, 1985).



relate optical data to the calibration set at its disposal and then select wavelengths which best fit the component being predicted. The best selected prediction equation takes the following form:

$$\text{Predicted Composition} = B_o + B_1W_1 + B_2W_2 + B_3W_3 + \ldots B_nW_n$$

Where B_o is the regression constant, B_1 to B_n are partial regression coefficients and W_1 to W_n are reflectances or derivatives of these at wavelengths $\lambda 1$, $\lambda 2$, ... λn.

There are 3 kinds of NIR commercial instrumentation available, namely the monochromator, scanning filter instruments and discrete filter instruments (Shenk, 1986). The monochromator has the capability of scanning the entire NIR region of spectra and thus more spectral information can be collected compared to the other two types. The monochromator used in this project scans the NIR region between 1100 to 2500 nm and can generate 700 Log $\frac{1}{R}$ values (data points) at 2 nm intervals. On the other hand, the scanning filter instruments, and in particular the discrete filter instruments, have less NIR coverage and hence less data points. Therefore, it is not expected that the performance obtained from these instruments will match the performance of the monochromator. In forage analysis, it has been found that the scanning and the discrete filter instruments gave less satisfactory results when compared to the monochromator (Minson et al, 1983).

Before calculating regression equations, spectral data are subjected to mathematical transformations which were found to be useful in reducing noise due to factors unrelated to forage chemistry and the extraction of as much chemical information from the sample as possible (Westerhaus, 1985a).

Derivative techniques have been used to give a smoothing effect and sharpen the detail along the NIR spectral curve (Savitsky and Golay, 1964). Norris et al (1976) were the first to suggest that the use of this technique could improve the predictive ability of NIR when analysing forages by reducing the particle size effect which is a feature of ground forage samples. They found that the NIR performance improved when using the second derivative of Log $\frac{1}{R}$ values rather than Log $\frac{1}{R}$ values per se. They further examined the use of first and third derivatives, however no improvement was found over the second derivative. This technique has been used to transform forages spectral data with relative success by many workers (Barton and Burdick, 1981).

Recently, interest has been renewed in the use of alternative spectral data manipulation. The principal component analysis (PCA) is such a development. This technique reduces the number of variables by creating synthetic variable called "principal components" which are related to the original variable and then selecting the most significant wavelengths (Robert et al, 1986). By doing so, each principal component is independent of each other and therefore, reduces the possibility of inter-correlation between spectral data which might occur in the derivative method (Downey et al, 1987). Another feature of this approach is to permit a close investigation of each component and therefore allow chemical interpretation of the selected wavelengths (Downey et al, 1987). Robert et al (1986) compared the predictions of 87 in vivo grasses obtained by multiple linear regression of Log $\frac{1}{R}$ values and the PCA technique and found that the standard errors of calibration (SEC) and prediction (SEP) were not significantly different between the two techniques.

Calibration of NIR instruments is an important concept so that this technology can be applied successfully for

predictive purposes. The NIR technique cannot be expected to give satisfactory results beyond the range of available information at its disposal, therefore a careful calibration is an absolute requirement. This is a serious problem when it comes to a heterogeneous population, like, for example, the grass silage population in the whole of the UK. A calibration based on defined boundaries (ie: limited range in chemical, physical and botanical composition) is not expected to predict a population with undefined boundaries. Murray (1986) stressed the importance of this concept and indicated that the calibration samples should have a wide range and even distribution in composition, precise analytical data and typical properties of all future unknown samples. An adequate in vivo calibration set for grass silage should include for example, different species, harvests for different years, wilting time, stage of maturities, growing environments, methods of ensiling and different silage additives.

It is difficult to decide how many samples should be included in the calibration set, however this may depend on the heterogeneity of the population. Trying to predict animal response (for example, digestibility) may require a calibration set with large numbers of samples (Windham and Coleman, 1985). Very few studies have been reported about the number of samples recommended for calibration, however the work of Abrams et al (1987) may be the most useful. They studied the effect of increasing the number of calibration samples for various assays, including IVDMD, on the standard error of prediction. They observed that SEP was reduced as the number of samples in the calibration set was increased and concluded that a minimum of 100 calibration samples were needed to minimise SEP and bias.

Sample selection is an important factor in successful NIR calibration. Three methods of data selection were suggested, namely; structured, random and spectral characteristics (Abrams, 1985). The first method depends on the available information, like stage of maturity, which can be used to describe the sample. This information is classified and then proportional sampling from each class is selected. This will ensure that each class is equally represented in the calibration set and thus bias will be avoided. Random selection is appropriate when there is no prior knowledge about the population. The third method requires obtaining the spectral data for all the samples intended to be included in the calibration set. The spectral characteristics are then grouped into clusters and random samples selected from each cluster are used to obtain the calibration set. This approach is scientifically sound because the samples selected represent the entire spectral characteristics of the population available. However, Abrams et al (1987) found that this method did not have any advantage over random selection as a means to select samples.

It is important to validate the equations generated by the multiple linear regressions so that a proper equation(s) can be selected to predict the unknown samples. The equation(s) which has minimum SEP, minimum bias and slope closest to unity should be selected (Westerhaus, 1985b; Murray, 1986).

Near infrared reflectance techniques have been used initially to predict protein and oil in grains (Hymowitz et al, 1974) and fat, protein and moisture in fresh meat (Kruggel et al, 1981). Norris et al (1976) were the first to use NIR to predict the nutritive value of forages. They reported an R^2 of 0.90, 0.78 and SEC of 2.64 and 3.58 for predicting IVDMD and in vivo DMD respectively, when 76 forage mixtures were analysed by monochromator.

In this study, they used a multiple linear regression equation involving 9 terms. Barton and Burdick (1981) used monochromator to predict chemical and biological parameters for hay samples. They reported an SEC% and SEP% of 1.78 and 2.54 respectively for predicting in vivo DMD. Based on broad and large calibration sets, Abrams et al (1987) demonstrated the accuracy of this technique in predicting chemical and biological parameters of diverse hay samples. These results were obtained by using a monochromator and 9 terms calibration equations. Equations based on principal component analysis were successful to predict in vivo OMD of dried grasses with an R^2 of 0.95 and SEP% of 2.51 (Robert et al, 1986).

Few investigators used NIR to predict the nutritive value of grass silages. Using commercial farm silages, O'Keefe et al (1987) reported an accurate prediction of crude protein (CP) [SEP = 0.63%], but IVDMD was not predicted with sufficient accuracy (SEP = 2.96%). The low accuracy of predicting IVDMD could be due to the use of fixed filter instruments fitted with 19 filters only. Downey et al (1987) reported better accuracy when predicting IVDMD of grass silages using a monochromator (SEP = 2.7%).

The Use of NIR to Evaluate Silages in the UK

The need for economical, fast and accurate laboratory methods to predict the nutritive value of grass silage is well recognised by the advisory organisations in the UK. Since commercial NIR instruments became an accepted methodology in forage evaluation, recently ADAS, SAC and DANI adopted this technique in their silage evaluation programs as a means of rapid and, more importantly, economical method to predict nutritive value of grass silages. However, instead of direct calibration on in vivo digestibility, those laboratories have used NIR to predict MADF, LIGNIN and MADF respectively. Digestibility and ME are then calculated using appropriate regression

equations. Two fundamental errors can be noted when using this indirect approach, namely, the error resulting from NIR prediction of these chemical parameters and then the error resulting from the use of these parameters to predict digestibility. These errors are additive which ultimately, reduces the accuracy of *in vivo* digestibility prediction.

In this thesis, it is intended to investigate this problem and explore the possibility of calibrating NIR directly with a large *in vivo* digestibility data set. If this can be achieved, it would be appropriate to recommend this technique to be used by all the advisory laboratories in the UK in a more sound and fundamental way.

Conclusions

The near infrared technique is a fast and economical method and could provide some potential for silage evaluation. Provided that NIR is calibrated adequately, it might show a reliable alternative to the conventional methods. Because the parameter intended to be predicted is determined in a multivariate way, this could lead to accurate prediction greater than any traditional method. Simplicity is another attractive feature of this technique, since little sample preparation is required other than simple grinding and drying.

Given these features however, the NIR does pose some problems. The cost of the instrument are relatively high, and the calibration procedures and subsequent equation selection and testing demands a skillful and experienced personnel with access to powerful computers. The development of a calibration set is sometimes expensive, in particular with biological parameters, for example digestibility. In addition, each calibration is specific for the component to be predicted, therefore several

calibrations are required for other components. Spectral data treatments are rather complicated and need to be standardised among users. The noise level is a serious problem of NIR and the need for constant monitoring and updating of the instrument to control this noise is required. The NIR instruments are sensitive to temperature and humidity and these must be maintained at the recommended level to ensure adequate performance.

1.2.7 Summary

Reliable silage evaluation is an important program in providing satisfactory farm advice and conducting adequate research. Digestibility is an important aspect of nutritive value and its determination is essential for practical feed evaluation and ration formulation. Ideally, the use of animals to determine digestibility is the ultimate measurement, however, this approach is expensive and cannot be used on large numbers of samples. Therefore, a rapid, simple and comparatively inexpensive laboratory method to predict this parameter is an absolute requirement.

The use of chemical methods to predict <u>in vivo</u> digestibility was the first to be suggested and speed, reproducibility and cheapness were the most attractive features of these techniques. Since these methods rely on pure statistical association between digestibility and chemical entities (cellulose, lignin), it should not be expected that these techniques will accurately describe this complex biological parameter. While these methods, in particular lignin methods, are of value between species of grasses, it is found to be inaccurate for a mixture of species, a feature found in real farm situations. This demands the need of separate regression equations for each species if these methods are to be implemented. However, this might be impractical for silage evaluation.

These inaccuracies led to a search for methods which might reflect biological reality and the rumen liquor and *in situ* techniques were such a development. The prediction superiority of these methods over the chemical methods may be due to the sensitivity of the rumen micro-organisms to the determined and undetermined factors which might affect digestibility. The *in situ* method benefits from a lesser variation due to rumen liquor activity when compared with *in vitro* methods, since bags are incubated in the rumen itself (Aerts *et al*, 1977). This feature along with its simplicity, makes the *in situ* method a powerful technique for the evaluation of forages and improving our knowledge of the processes of degradation which take place inside the rumen.

However, these methods were found to be difficult to standardise. More importantly, their use demands free access to fistulated animals and in many situations this might be impractical and difficult to achieve. The pressure from animal welfare organisations against the use of surgically prepared animals is apparent. These limitations make these techniques unfavourable and their applications for routine use is likely to diminish in the future.

The use of cellulase enzyme methods were suggested as a means of replacing the rumen liquor. These methods profit from their speed, precision and convenience relative to the *in vitro* rumen liquor technique. They are found to be accurate in predicting *in vivo* digestibility of individual species, however for a mixture of species it was not sufficiently accurate and in this case, the need for separate regression equations was found to be essential. The activity of the cellulase enzyme requires constant monitoring between batches and from different suppliers so that the method's accuracy can be maintained.

With increasing economic pressures to find an accurate and reliable method for predicting in vivo digestibility, NIR might be of potential value for routine silage evaluation. Provided that this method is calibrated properly, it might offer a reliable and acceptable alternative to the conventional techniques.

CHAPTER TWO

MATERIAL AND METHODS

2.1 Silages Studied

One hundred and seventy dried samples of grass silage which had been evaluated in vivo, were collected from different sources around the UK. Table 2.1 shows the sources of the silage populations used in this work.

The distribution of in vivo OMD and chemical and biological predictors of the populations studied is shown in Table 2.2. The distribution of in vivo OMD for all silages studied is shown in Figure 2.1.

The composition of silages studied for all populations is shown in Table 2.3.

All silages were obtained from in vivo trials conducted during 1974 to 1986 at the different institutions shown in Table 2.1. The known characteristics of the silage used in this study are shown in Table 2.4. The in vivo determinations of OMD have been described in Table 2.5.

2.2 Analytical Methods

2.2.1 Dry Matter Base

Where analytical results from the centres were expressed on a toluene dry matter basis, they were converted to an oven dry matter basis according to the conversion factors described by Barber et al (1984). This is done because the toluene dry matter determinations for population 4 was not available.

A Analytical data such as proximates and fibres can be converted to an ODM basis using the following formula:

$$\text{Chemical Parameter ODM} = \frac{\text{Chemical Parameter (TDM)}}{\text{ODM/TDM}}$$

Table 2.1

Sources of Silages

Source	Population	Number	Drying Temperature (°C)	Mill Screen (mm)
ADAS Feed Evaluation Unit - Pre-1980	1	28	60	0.60
ADAS Feed Evaluation Unit - Post-1980	2	72	100	1.00
Rowett Research Institute	3	43	100	1.00
North of Scotland College of Agriculture	4	27	80	1.00

Table 2.2

Distribution of in vivo OMD and Chemical and Biological Predictors of the Populations Studied (%)

		In vivo OMD (%)	MADF (%)	LIGA $cm^{-1}Lg^{-1}$	NCOMD (%)	PCOMD (%)	NB48 OMD (%)	IVOMD (%)
Pop 1	Mean	70.3	33.3	2.58	75.5	59.4	-	68.6
n = 28	Range	59.2-78.1	25.4-43.7	1.88-3.17	59.1-84.9	51.4-70.8	-	57.6-73.7
	SD	5.2	4.6	0.340	6.9	5.6	-	4.0
Pop 2	Mean	70.5	34.1	2.56	66.5	63.5	84.3	66.0
n = 72	Range	52.8-82.8	23.0-42.4	1.77-3.83	43.9-84.7	36.8-81.2	61.5-96.8	47.5-72.9
	SD	6.6	4.0	0.402	9.2	8.6	7.0	6.0
Pop 3	Mean	72.4	37.8	3.00	61.6	60.1	86.8**	68.2
n = 43	Range	60.9-81.5	30.9-45.3	2.40-3.50	48.5-73.2	45.5-73.4	76.1-95.1	55.0-78.2
	SD	4.5	3.4	0.247	7.8	7.9	4.6	5.7
Pop 4	Mean	70.1	36.5	2.60	67.9	54.0	85.3	66.9
n = 27	Range	55.2-86.7	31.7-44.1	1.80-3.38	53.8-79.8	44.6-64.3	73.8-95.4	56.4-76.2
	SD	7.1	3.2	0.412	6.3	6.5	5.7	6.8
TOTAL	Mean	71.0	35.3	2.68	67.5*	60.5	85.0***	67.1
n = 170	Range	52.8-86.7	23.0-45.3	1.77-3.83	43.9-84.9	36.8-81.2	61.7-96.8	47.5-78.2
	SD	6.0	4.2	0.403	8.8	8.3	6.3	5.7

* NCOMD data applies to 165 samples, 5 values were missing, two from population 1, one from population 2 and two from population 4.

** NB48 data applies to 24 samples only, since 19 samples contained insufficient material.

*** NB 48 data applies to 123 samples only, since population 1 was too finely milled for this method and in the case of population 3, 19 samples contained insufficient material.

FIGURE 2.1 DISTRIBUTION OF IN VIVO OMD IN THE POPULATIONS

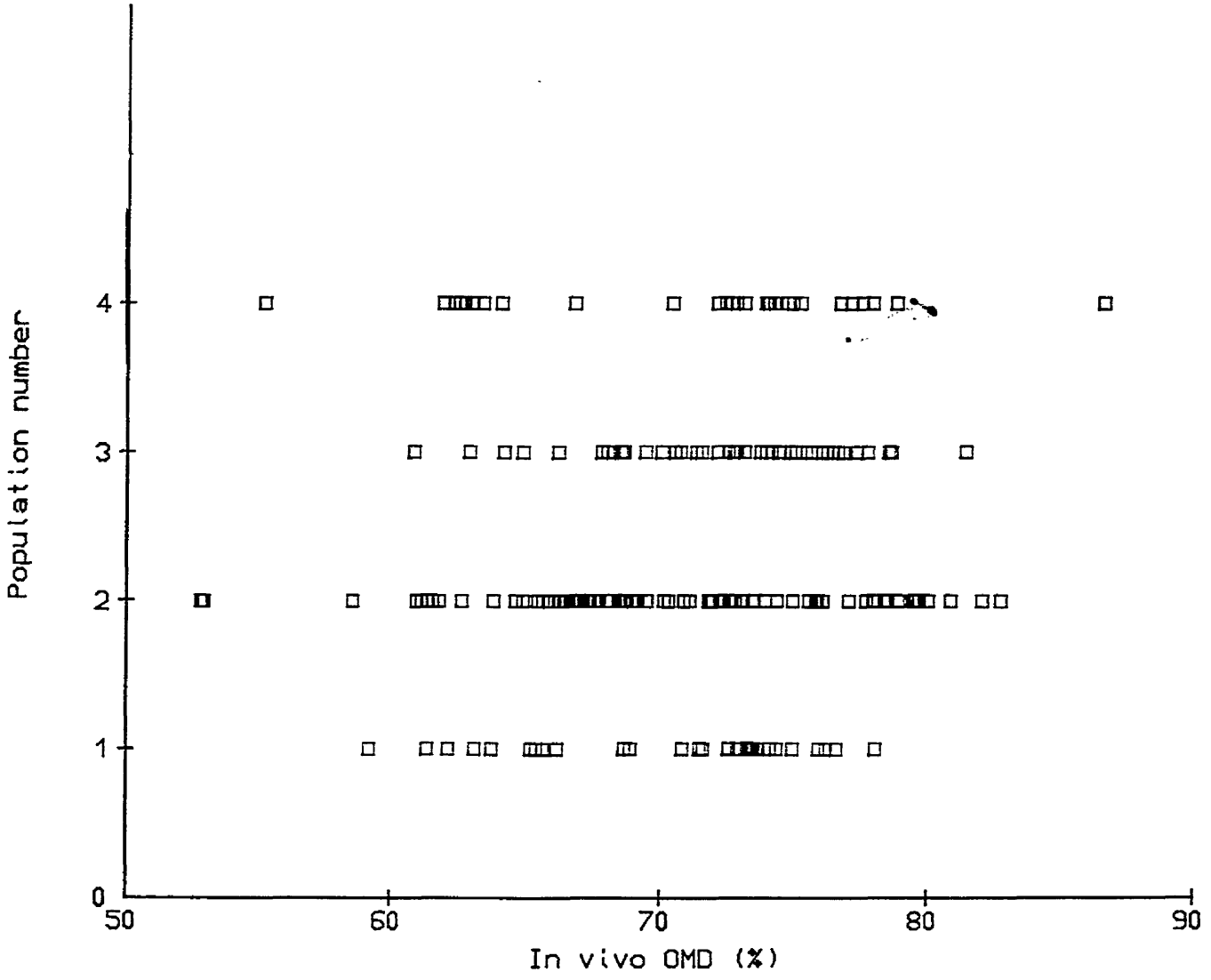


Table 2.3

Composition of Silages for All Populations Studied

Composition		Populations 1 n	1 Values	2 n	2 Values	3 n	3 Values	4 n	4 Values	Total n	Total Values
ODM (gkg^{-1})	Mean	28	295	72	275	43	215	27	226	170	255
	Range		213-461		159-580		163-358		165-361		159-580
	SD		81.2		94.1		41.7		49.7		81.0
TDM (gkg^{-1})	Mean	28	321	72	299	43	238		NDA	143	285
	Range		235-489		183-585		175-392				175-585
	SD		80.4		89.5		45.2				82.9
Volatiles as % of TDM	Mean	28	8.7	72	9.1	43	9.6		NDA	143	9.1
	Range		2.9-16.4		0.0-26.2		4.7-13.1				0.0-26.2
	SD		3.3		5.2		2.2				4.1
Ash (gkg^{-1}DM)	Mean	28	112	72	90.6	43	93	27	81.2	170	93.3
	Range		87-168		54-130		62-129		62-103		54-168
	SD		16.2		15.5		16.4		11.8		17.8
NDF (%)	Mean	6	52.7	71	57.4	43	61.3		NDA	120	58.6
	Range		42.1-64.5		42.0-85.5		45.5-73.2				42-85.5
	SD		9.9		7.9		5.8				7.6
ADF (%)	Mean	6	32.6	71	34.5	43	40.1		NDA	120	36.4
	Range		25.2-40.5		16.3-43.3		34.0-47.5				16.3-47.
	SD		6.5		4.9		3.3				5.2
pH	Mean	28	3.9	71	4.3	43	4.2	27	3.9	169	4.2
	Range		3.2-5.0		3.4-6.0		3.6-4.9		3.6-5.3		3.2-6.0
	SD		0.45		0.59		0.34		0.34		0.50
CP (gkg^{-1}DM)	Mean	28	188	72	170	43	162	27	131	170	165
	Range		115-342		93-259		108-223		93-185		93-342
	SD		50.3		40.3		24.5		25.4		40.3
Vol. N as % of TN	Mean	28	10.4	65	11.4	43	12.7	27	11.0	163	11.5
	Range		6.1-18.8		3.7-31.1		5.6-258		5.4-66.8		3.7-66.8
	SD		3.2		5.3		4.4		11.5		6.3
Lactate (gkg^{-1}DM)	Mean	28	63.7	70	69.5	43	61.3	8	66.0	149	65.8
	Range		8.9-132.6		7.9-203		17.7-104		2-150		2-203
	SD		32.5		44.1		24.2		52.6		37.5
Acetate (gkg^{-1}DM)	Mean	28	25.6	67	30.0		NDA		NDA	95	28.7
	Range		7-54.4		1.9-254						1.9-254
	SD		13.5		40.0						34.2
GE ($MJkg^{-1}$DM)	Mean	28	20.8	72	21.0	43	21.3		NDA	143	21.0
	Range		19.1-23.1		15.4-25.7		19.6-22.6				15.4-25.7
	SD		1.0		1.5		0.67				1.2
ME ($MJkg^{-1}$DM)	Mean	28	11.7**	72	12.0**	43	12.6*		NDA	143	12.1
	Range		8.1-14.3		6.1-15.3		9.5-14.7				6.1-15.3
	SD		1.5		1.7		1.04				1.5

NDA = No Data Available
* = Methane losses are measured
** = Methane losses are predicted from Blaxter and Clapperton equation (1965)
n = Number of samples for which the analytical results are available.

Table 2.4

Characterisation of the Silages Studied*

	Characteristics	Number of Silages
1	Method of Ensiling	
	- Clamp	72
	- Big Bale	19
2	Cut Number	
	- First Cut	74
	- Second Cut	31
	- Third Cut	7
3	Wilting Time (hours)	
	- $\leqslant$ 12	26
	- > 12-40	72
	- > 40	18
4	Age of Ley (years)	
	- $\leqslant$ 1	17
	- > 1-5	32
	- > 5	17
	- Permanent Pasture	16
5	Additive Application	
	- None	54
	- Add-F	40
	- Sylade + F100	26
6	Fertilisation (Kg N/ha)	
	- $\leqslant$ 100	28
	- > 100-200	45
	- > 200	9

* Not all the 170 silages have known characteristics

Table 2.5

Description of *in vivo* Trials Performed on Silages Used in this Study

Populations	Number of Sheep	Diet Fed With Grass Silage	Adaptation Period (days)	Collection Period (days)
1	4	None	10	10
2	4	None	10	10
3				
- first report	4	Oats + Barley	10	10
- second report	4	Wheat + Wheat Offals	10	10
- fourth report	4	Maize	10	10
4				
- Connolly (1984)	6	None	NS*	8
- Siddigi (1985)	5-6	None	10	8
- Helliwell (1986)	4	None	10	10

*NS = Not Stated

B Digestibility values such as <u>in vivo</u> DOMD can be converted to ODM basis using the following formula:

$$\text{DOMD (ODM Basis)\%} = \frac{[(\text{DOMD (TDM Basis)} - 100) + (100 \times \text{ODM/TDM})]}{\text{ODM/TDM}}$$

Note that where the digestibility values were on OMD, they must be converted to DOMD first using:

$$\text{DOMD\%} = \text{OMD}_{\text{fraction}} \times \text{OM \%}$$

2.2.2 **Oven Dry Matter Determination**

A known quantity of sample was dried in a forced draught oven at the temperature indicated in Table 2.1. After a constant weight was reached, the dry matter was expressed as a percentage of fresh weight.

2.2.3 **Ash Determination**

Approximately 3g of dried sample was placed in a pre-weighed dry crucible and ashed in a muffle furnace at 500°C overnight. The crucibles were cooled in a dessicator then re-weighed and the ash content calculated.

2.2.4 **Modified Acid Detergent Fibre**

The MADF content of the samples was determined according to the method of Clancy and Wilson (1966).

1 g of dried silage was transferred into a beaker or flask. 100 ml of sulphuric acid - cetyltrimethylammonium bromide solution was added to the beaker or flask and then a condenser was fitted. The content was rapidly boiled and then gently boiled for 2 hours. The digest was then filtered hot through a weighed filter crucible using gentle suction. Excessive foaming in the filtrate

was reduced by the addition of 1-2 drops of octane-2-ol. The residue was then washed with 3 portions of approximately 50 ml of almost boiling water and then with acetone. The crucible and content was then dried in an oven at 102°C overnight. The crucible was allowed to cool in a dessicator and then weighed. The increase in weight was multiplied by 1000 and the results were reported as g/kg of MADF in the sample DM.

2.2.5 **Acetyl Bromide Lignin**

The lignin content of the samples was determined according to the method of Morrison (1972).

The silage samples were dried at 80°C for 18 hours and then ground to pass through 0.8 mm screen. The dried sample (50 mg) of known ash content was heated with 20 ml distilled water at 70°C with occasional shaking. It was then filtered hot through a glass fibre filter paper (Whatman GFA 2.5 cm). The residue was washed thoroughly on the filter with hot water, 6 times with 5 ml ethanol, 4 times with 5 ml acetone and 4 times with 5 ml diethyl ether. The cell wall preparation and filter paper was transferred by tweezers to a glass stoppered test tube (Quickfit MF 24/3) and heated at 100°C for 15 minutes to remove traces of solvent.

Five ml of 25% acetyl bromide was then added to the tube and stoppered and heated for 30 minutes at 70°C. The tube and its contents were then cooled to room temperature. Five ml of the digest was taken and mixed with 20 ml of acetic acid and then stoppered and shaken. An aliquot of 1 ml added to 1.2 ml of 2.06% sodium acetate trihydrate in a 10 ml stoppered centrifuge tube, 7.5 ml of ethyl alcohol and 0.3 ml of 0.5 M hydroxyl ammonium chloride solution was also added and the tube stoppered and shaken. The tube was centrifuged

for 10 minutes at 1500 rpm and then was allowed to stand for one hour. The samples were read on a Cecil spectrophotometer using 10 mm manual flow cell at 280 nm. In every set, reagent blanks were included. Absorption value A was calculated from the equation:

$$A = \frac{ODs - ODb}{C}$$

Where:

ODs = optical density of sample

ODb = optical density of blank

C = concentration of dry OM in the final solution gl^{-1}.

2.2.6 **Pepsin-Cellulase OMD Determination**

The pepsin-cellulase OMD of the samples were determined according to the method of Jones and Hayward (1975).

The cellulase enzyme derived from fungi Trichoderma viride (BDH Ltd, Poole, Dorset, England) was used. Pepsin (1:10,000) was obtained from Zimmerman and Hoppes Ltd, Milton Keynes, England.

200 mg of the ground dried silage sample was mixed with 20 ml of acid pepsin solution (0.2% of pepsin in 0.1 M HCl) in a screw cap plastic tube. The tube was placed in a water bath at 40°C and incubated for 24 hours. The content of the tube was swirled occasionally. After incubation, the suspension was centrifuged (maximum speed for 5 minutes). By means of suction, the supernatant was removed as completely as possible leaving the residue inside the tube. Using a dispenser, 20 ml phosphate citrate buffer (pH 4.6) was then added to the tube to wash the remaining traces of the acid pepsin solution. The stopper was firmly placed and the residue was suspended by vigorous shaking. The suspension was

recentrifuged (maximum speed for 5 minutes). The supernatant was then removed by suction leaving the residue inside the tube.

Twenty ml of buffered cellulase solution (125 mg cellulase in 20 ml phosphate citrate buffer pH 4.6) was added to the tube. The content was thoroughly mixed and the tube was then placed in a water bath at 40°C and incubated for a further 48 hours. The content of the tube was swirled occasionally during this period. The residues were recovered at the end of 48 hours incubation in the presence of a filter-aid (hyflo supercel) by filtration through a fibreglass paper. The fibreglass papers and residues were dried at 100°C, cooled and weighed. They were then ignited at 480°C for 16 hours, cooled and weighed again. A parallel estimate of the total OM by ignition of 1 g of sample at 480°C for 16 hours and the inclusion of control tubes with buffer and cellulase only, allow the digestibility coefficients for the samples to be calculated:

$$\text{Pepsin-Cellulase OMD\%} = \frac{\text{Wt of OM in Sample} - \text{OM of sample residue} - \text{OM of control residue}}{\text{Wt of OM in Sample}} \times 100$$

2.2.7 **<u>In vitro</u> OMD Determination**

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

Exactly 0.5 g DM of the ground sample was weighed in duplicate directly into glass tubes. One litre of rumen liquor was obtained from each of 3 fistulated sheep and then filtered as quickly as possible through 2 layers of

muslin, swept with CO_2 and was added to four times its volume of McDougall's buffer (1948) previously saturated with CO_2 to a pH of 6.9. One ml of molar ammonium sulphate solution per 50 ml of the rumen liquor buffer mixture was added. The sample inside the tube was inoculated by adding 50 ml of the rumen liquor/buffer mixture. The tube was swept with CO_2 and then capped with rubber bungs fitted with bunsen valves. The tubes were then placed in a water bath at 38.5°C. The pH of the digests were adjusted electrometrically to 6.9 after 24 hours of incubation. The digestion was carried out for a period of 48 hours during which a gentle swirling was performed from time to time. To terminate the 48 hours stage, 1.5 ml of 6 M HCl followed by 2.5 ml of 6 M HCl were injected into each tube. Five ml of aqueous pepsin containing 0.12 g 1:10,000 pepsin (Zimmerman and Hoppes, Milton Keynes, England) were injected into each tube after electrometric adjustment of the pH to 1.2. The content in each tube was then further incubated for 48 hours at 38.5°C during which the tubes were gently swirled from time to time. At the end of the pepsin digestion stage, the residues were recovered in the presence of filter-aid (hyflo supercel) by filtration through a fibreglass paper. The fibreglass papers and residues were dried at 100°C, cooled and weighed. They were then ignited at 480°C for 16 hours, cooled and weighed again. A parallel estimate of the total OM by ignition of 1 g of sample at 480°C for 16 hours and the inclusion of control tubes with buffer and rumen liquor only, allow the digestibility coefficients for the samples to be calculated:

$$\text{IVOMD \%} = \frac{\text{Wt of OM in Sample} - \text{OM of sample residue} - \text{OM of control residue}}{\text{Wt of OM in sample}} \times 100$$

2.2.8 In situ OMD Determination

2.2.8.1 The Preparation of Hay Samples.

One sample of timothy hay was used. The hay was first chopped twice to a length of 5-6 cm in a "GHL" straw chopper. Then it was mixed through a "Hobart" mixer slicer attachment 3 times until it reached a length of < 1 cm.

2.2.8.2 The Preparation of Grass Silage Samples.

The milled grass silage samples were sieved through a 45 um sieve to remove fine particles. Approximately 5.5 g sample was weighed into each bag. The bags were incubated in batches of five at one time and then replicated in 3 sheep. Four bags represent 4 different silage samples and the fifth one was an internal standard (alfalfa nuts milled through 1 mm screen). This standard allowed adjustment for variation between rumens (between sheep and with time).

2.2.8.3 Animals and Their Diets.

Four mature 8 year old Suffolk wethers were used. They were housed indoors in loose pens and each fitted with a 55 mm rumen cannula with a screw top. Once the top was removed a bung of the correct diameter could be inserted to which were attached the 5 bags for incubation. Each sheep was fed a maintenance ration of 800 gd^{-1} of meadow hay and 200 gd^{-1} of Ewbol concentrate in two meals offered at 9.00 am and 5.00 pm. The composition of the diet fed to the sheep is shown in Table 2.6. Fresh water was given ad libitum.

2.2.8.4 Polyester Bag Technique.

The bags were made of synthetic polyester fibre (Sericol Group Ltd, London) with pore size of 40-50 um. They are measured 10 x 21 cm with a round base to prevent the sample building up in the corners (Figure 2.2). Approximately 5.5 g air-dry samples were put in

Table 2.6

Composition of the Diet Fed to the Sheep

I Composition of Hay

DM (gkg^{-1})	820
OM (gkg^{-1} DM)	930
CP (gkg^{-1} DM)	82
In vitro D-value (%)	53.2
ME ($MJkg^{-1}$ DM)	8.2

II - Composition of Concentrates (302 Ewbol Pencils)*

				Vitamin IU kg^{-1}			Minerals	
Protein (%)	Fibre (%)	Oil (%)	Ash (%)	A	D_3	E	Selenium ($mgkg^{-1}$)	Magnesium (gkg^{-1})
14	13.5	3.0	9.8	5000	2000	7.5	0.2	12.0

* BOCM, UK.

Figure 2.2

Five Polyester Bags Ready to be Inserted into a Fistulated Sheep

pre-dried bags. The bags were sewn with 2 ply 50 denier polyester thread, 10/70 ball point needle, lock stitch and approximately 10 stitches per cm. The neck of the bag was twisted tightly then bound with approximately 115 cm length of strong nylon string, leaving loose length of 50 cm after a knot had been tied. Then the strings were threaded through a 17 cm piece of polythene tubing and then through a holed rubber bung. Further knots were tied to ensure the strings did not slip back through the hole. The ends of the string were melted over a bunsen flame to prevent fraying. For the washing experiment (Chapter 3), four bags were tied into each bung. The bags were pushed into the sheep's rumen through the cannula so that the bung was sealing it. The polythene tubing prevents tangling of string and spreads the bags out in the rumen.

2.2.8.5 **Analytical Methods of the Bags Residue after Incubation.**

The incubated material still in the bag was oven-dried at 60°C for 48 hours and this was then stored in a dessicator until analysed. Dry matter disappearance was determined by weight difference. Organic matter disappearance was calculated after ashing the undigested residue at 500°C overnight. Dry matter and OM of the original sample was determined by oven-drying at 100°C and ashing at 500°C respectively overnight.

2.2.8.6 **Washing the Bags.**

After incubation, the bags were then washed in a domestic washing machine (Zanussi Z915T) with hot detergent wash (Kridis et al, 1989). Each wash involved a batch of 25 bags so that each bag could be washed evenly with the detergent.

2.2.8.7 **Total Nitrogen Determination of Hay Samples and Residues after Incubation.**

The total-N content in hay and the dried residues after incubation was determined in triplicate by the Kjeldahl method according to the procedure described by Egan et al (1981).

Reagents.

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

a) 40 g selenium oxide in 100 ml distilled water
b) 2 l concentrated H_2SO_4

2 Buffer - 5 g NaOH + 3.74 g anhydrous Na_2HPO_4 + 31.8 g Na_3PO_4 . $12H_2O$ + 10 cm^3 sodium hypochlorite (10-14% av Cl) in 2 litres distilled water.

3 Caustic phenol - 2.4 g NaOH + 0.1 g sodium-nitroprusside + 20g phenol in 1.6 litres distilled water.

4 Ammonia standards - ranging from 0.05-0.25$g1^{-1}$ ammonia nitrogen were made from a stock solution containing 4.7168g $(NH_4)_2SO_4$ in 1 litre 10% H_2SO_4 (1 g $l^{-1}N$).

The different ammonia-N concentrations were made by appropriate dilution in 10% H_2SO_4.

Procedure.

a) Digestion

Approximately 0.1-0.15g of the sample was weighed into a 75 ml digestion tube. Using a dispenser, 8 ml of the digestion mixture was added to each tube. Three 1 ml volumes of hydrogen peroxide (100 vol)

and one piece of sintered glass were then added. The tubes were heated to 35°C for 2 hours in a block digester (Tecator Ltd, Bristol, England). After cooling, the digest was diluted with 50 ml distilled water and then the volume was made up to exactly 75 ml with distilled water. The digest was thoroughly mixed and then left to stand at room temperature for 1 hour.

b) Analysis for Ammonia-N

Ammonia 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. Twenty ml of buffer solution and 8.0 ml of caustic phenol were then added to each tube. The tubes were swirled gently and allowed to stand at room temperature for 1 hour for colour development.

Using a spectrophotometer model SP8-500 (Pye-Unicam Ltd, Cambridge, England), absorption was measured at 585 nm. A regression equation was developed between blanks and standards and absorbance from which the N content of unknown samples was calculated. From these measurements, N disappearance from bags was calculated.

2.2.9 **Near Infrared Reflectance Spectroscopy (NIR)**

All silages were scanned using a Pacific Scientific Co. Neotec 6100 scanning monochromater linked to a Digital Equipment Corp. LSI 11/03 mini-computer. Calibrations were obtained by running the PSU/USDA software of Shenk et al (1981).

The samples were packed into a sample holder which holds the sample between a clear window made of quartz

and a pressure pad to ensure a good physical contact between the sample and the window. The sample was submitted to the instrument and then it was illuminated by a narrow beam of light through the window and the reflected radiation was collected by lead-sulphide detectors equally spaced around the incident beam (see Figure 1.6).

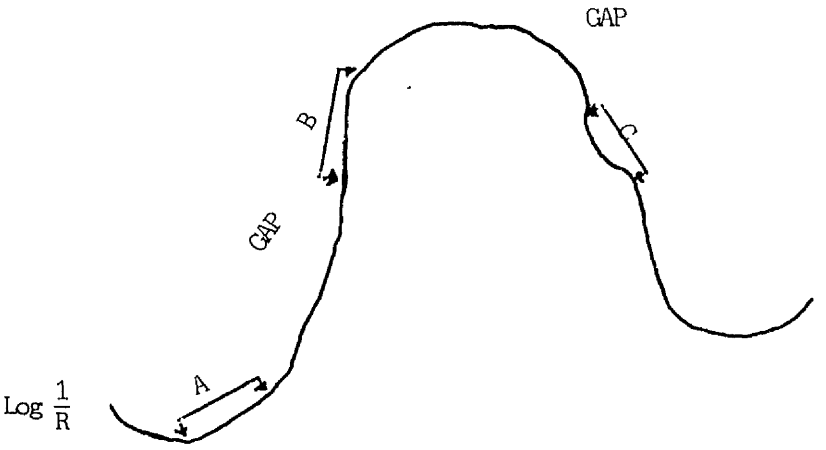
The signals from the detectors were converted to digital signals and stored into the coupled computer as the logarithm of reciprocal reflectance (Log $\frac{1}{R}$). Each sample scan is the mean of 64 individual NIR scans conducted in 55 seconds. The stored sample spectrum consisted of 700 data points (Log $\frac{1}{R}$ values) taken at 2 nm intervals between 1100 and 2500 nm. After each scanned sample, the quartz glass was wiped thoroughly with clean soft tissue to remove adhering forage particles resulting from the previous scan.

The computer then performed stepwise multiple linear regression analysis to relate optical data to the calibration set at its disposal and then select wavelengths which best fit the component being predicted.

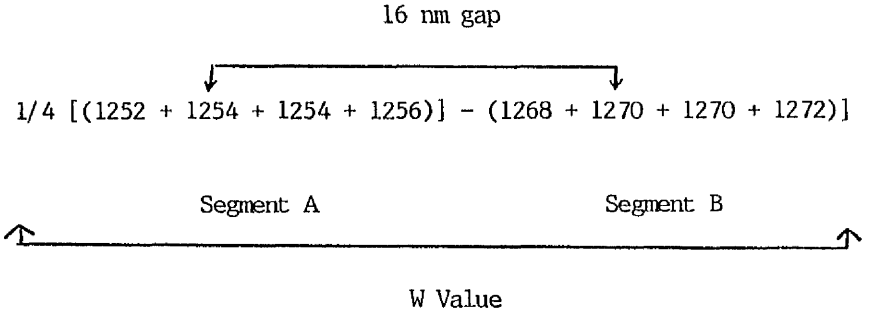
2.2.9.1 **Mathematical Transformations**

Before calculating regression equations, spectral data are subjected to mathematical transformations. The derivative technique described by Norris et al (1976) was used in this study.

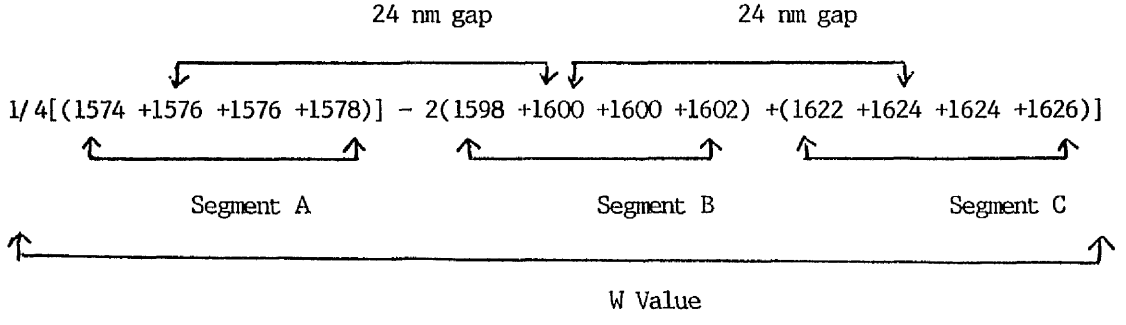
Derivative or difference spectra used in this work were either first or second derivative. A first difference spectrum is the difference between two segments of the spectra (A - B)



while a second difference spectrum is computed from (A - B) - (B - C) which is the same as A - 2B + C. For example, a derivative segment of 1262 nm using a 1, 16, 4, 4 math treatment is not a single wavelength but it is a Log $\frac{1}{R}$ segment (W value) computed as:



Similarly, a derivative segment of 1600 nm using a 2, 24, 4, 4 math treatment is a Log $\frac{1}{R}$ segment computed as:



Therefore, 1600 nm segment includes the change in Log $\frac{1}{R}$ values from 1574 to 1626 nm. A 2, 24, 4, 4 math treatment means that a segment involved was computed by a second derivative (A - 2B + C) with 24 nm gap and a first and second running smooth of 4 nm.

2.2.9.2 **Equation Output**

After the computer performed a stepwise multiple linear regression ,the equation produced took the form shown in Table 2.7.

The expressions presented in Table 2.7 can be explained as:

- B(O) is the regression constant.

- B(1) - B(5) are partial regression coefficients.

- F ratios are variance ratios which indicate how much the given term contributed to the equation.

- Wavelengths 1262 - 1230 are derivative wavelength segments at Log $\frac{1}{R}$.

- Math treatment was described above.

- Standard error is denoted as the standard error of calibration (SEC) which indicates the errors associated with regression calibration data on optical information. When testing an independent calibration equation against unknown samples and then comparing the observed values against the predicted values, SEC becomes SEP (Standard Error of Prediction).

- R^2 (ADJ) is the coefficient of determination adjusted for degrees of freedom.

TABLE 2.7

An Example of 5 Term Equation Produced by Stepwise Multiple Linear Regression

The Mean = 71.090
The Standard Deviation = 6.300
N = 122

Standard Error = 2.896
R-Squared (ADJ) = 0.789

	Coefficient	F	Wavelength		Math Treatment		
B(0) =	67.510						
B(1) =	-5938.944	37.76	1262	1	16	4	4
B(2) =	4410.001	183.22	1662	2	24	4	4
B(3) =	917.587	68.18	2266	2	24	4	4
B(4) =	-1108.577	16.44	1646	2	24	4	4
B(5) =	-4583.659	40.06	1230	2	24	4	4

Sample	Actual	Pred	Res	"T"	"H"
73	82.09	80.44	1.65	0.64	0.206*
74	61.38	65.61	-4.23	-1.77	0.323*
83	78.56	76.21	2.35	0.90	0.184*

The lower portion of the equation shows unusual observations found in the calibration set:

- 'T' statistics indicates goodness of fit between calibration data and optical data for a particular sample.

- 'H' statistics indicates how much the spectral information of a given sample differs from the spectra of the other samples used in the calibration set.

2.2.10 **Neutral Detergent Cellulase OMD Determination**

The neutral detergent cellulase OMD of the samples was determined according to the method of Dowman and Collins (1982).

The cellulase enzyme derived from fungi Trichoderma viride (BDH Ltd, Poole, Dorset, England) was used. Pepsin (1:10,000) was obtained from Zimmerman and Hoppes Ltd, Milton Keynes, England.

Neutral detergent solution: 93g of disodium ethylene diamine tetraacetate dihydrate (EDTA) and 34 g of sodium borate ($Na_2B_4O_7 10H_2O$) were dissolved in distilled water by gentle heating. To this solution 150g of sodium lauryl sulphate and 50 ml of 2-ethoxy ethanol (ethylene glycol monoethyl ether) were added; 22.8 g of disodium hydrogen sulphate was dissolved in distilled water separately and the two solutions mixed and diluted to 5 litres. A check was made to ensure that the pH was in the range 6.9-7.1.

0.5g of sample ground through 1 mm screen was digested for 1 hour with 50 ml of neutral detergent solution, 0.25g of sodium sulphite plus 1 ml of antifoam. The digest was filtered through a porosity 1 sintered glass crucible and

washed thoroughly with boiling distilled water. The residue was transferred while still damp into a polyethylene weighing bottle and excess water removed with a filter stick. The residue was incubated with 30 ml of buffered cellulase (20g litre^{-1} buffer) for 24 hours at 40°C. During this period the digests were shaken twice. Finally, the residue was filtered through a porosity 2 sintered glass crucible, washed with hot distilled water and then acetone. The contents were dried and weighed. The residue was ignited at 520°C for 4 hours, cooled and reweighed so that the organic matter in the residue could be determined. Independently, the OM in a separate sample was determined by igniting at 520°C for 4 hours.

Neutral Detergent Cellulase OMD% =

$$\frac{\text{Wt of OM in Sample - OM of Sample Residue}}{\text{Wt of OM in Sample}} \times 100$$

2.3 Laboratory Methods Comparison

In order to perform a proper comparison between the prediction techniques described previously, it was felt that 3 requirements needed to be satisfied, these include:

1 The predictive technique needs to be tested against in vivo calibration set having the following characteristics:

i) It should contain at least 100 samples. Abrams et al (1987) recommended that for NIR calibration, a minimum of 100 samples were needed to minimise SEP and bias.

ii) The sample must have been evaluated using a standardised in vivo procedure.

iii) The calibration should include a wide range of sample composition so as to fully represent the range found in practice.

iv) The samples must be fully characterised in terms of origin, plant species, preparation and chemical composition.

2 The in vivo samples need to be the same and also equal in number for each test.

3 To avoid between-laboratory differences, each method must be performed at a single laboratory. In each case, the analysis should be determined by a laboratory which makes routine use of the particular method.

The laboratory methods and laboratories involved in the work reported in this study are listed in Table 2.8.

All 170 silages from four populations were subjected to the predictive methods shown in Table 2.8. To compare the performance of these predictive methods against the population of silages obtained for this study, the calibration and validation samples were selected as described in Table 2.9.

The criterion that was used to select the calibration samples from the total population was that a complete set of predictive methods should exist for each of the chosen samples. This ensured that comparison of the techniques was made on a fair and equal basis. In practice, application of this selection criterion meant that 122 samples were chosen for calibration purposes. This number was found to be sufficient, in particular with NIR calibration. In an NIR study, Abrams et al (1987) recommended that a minimum of 100 calibration samples

Table 2.8

Analytical Methods Performed by Contributing Laboratories

Method	Laboratory
MADF (Clancy and Wilson, 1966)	ADAS, Wolverhampton
LIGA (Morrison, 1972)	North of Scotland College of Agriculture
NCOMD (Dowman and Collins, 1982)	ADAS, Wolverhampton
PCOMD (Jones and Hayward, 1975)	West of Scotland College
IVOMD (Alexander and McGowan, 1966)	West of Scotland College
NB48 (Kridis et al, 1989)	West of Scotland College
NIR Spectroscopy (Norris et al, 1976)	North of Scotland College of Agriculture

Table 2.9

Calibration and Validation Samples Used in This Study with Their *in vivo* OMD Distribution

Population	Calibration Samples				Validation Samples			
	No of Samples Used	Mean	Range	SD	No of Samples Used	Mean	Range	SD
Population 1	None	-	-	-	28	70.3	59.2-78.1	5.2
Population 2	72	70.5	52.8-82.8	6.6	None	-	-	-
Population 3	23	72.9	63.0-78.7	4.1	20	71.8	60.9-81.5	5.1
Population 4	27	70.1	55.2-86.7	7.1	None	-	-	-
Total	122	71.1	52.8-86.7	6.3	48	70.9	59.2-81.5	5.2

were needed to minimise SEP and bias.

The remaining 48 samples were used for validation and consisted of samples for which no in situ measurements could be made. This is because the samples in population 1 (No 28) were too finely milled for this method and in the case of population 3, 19 samples contained insufficient material and one sample was being a red clover silage.

2.4 Statistical Analysis

The relationship between in vivo OMD and each predictive technique was calculated using regression techniques. MINITAB Statistical Package (Ryan et al, 1985) was used for this purpose.

Analysis of variance was used to compare the regression lines of the different populations and then detect significant differences between populations in intercept and/or slope. For this purpose, GENSTAT statistical package (1983) was used to calculate 3 statistical models (see Figure 2.3).

The approach was to test significant differences between these 3 models. The test was performed as follows:

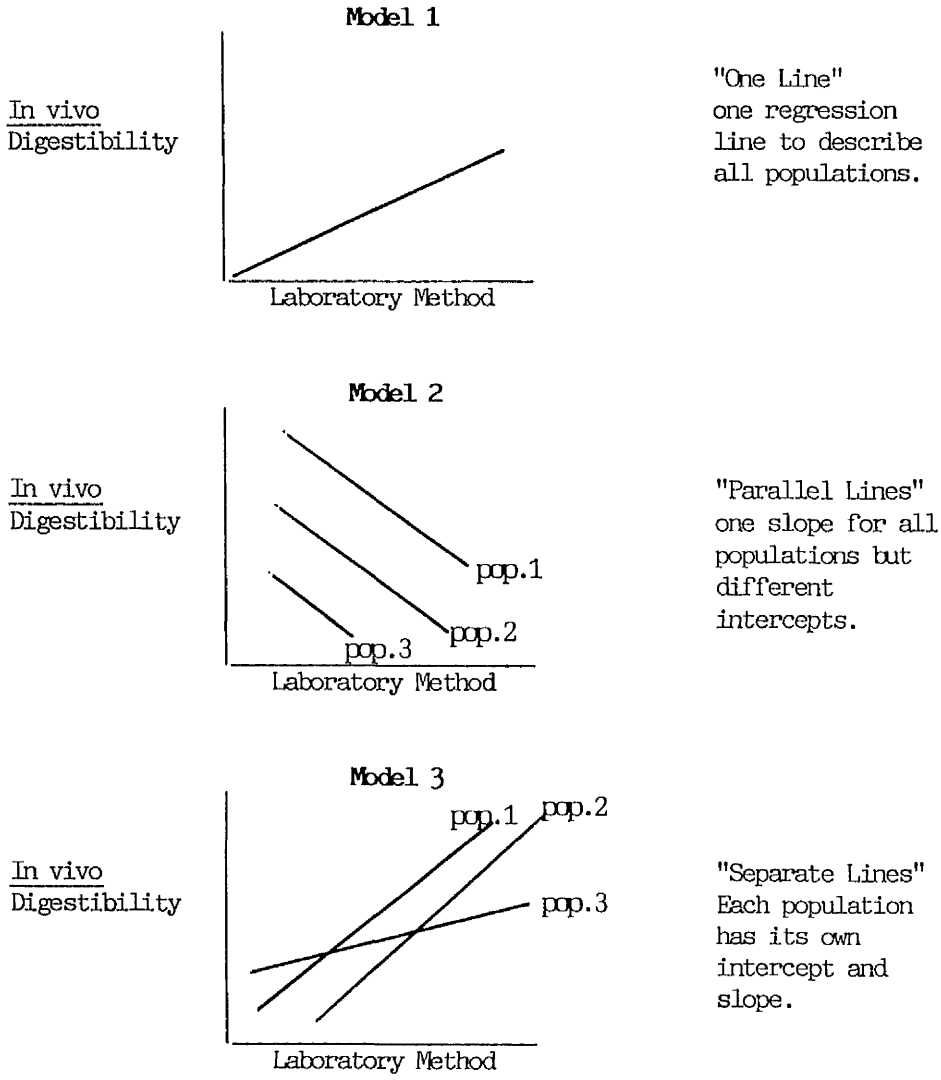
I Test Model 1 VS Model 3 ⟶ NS ⟶ one regression line to describe all data. F is calculated as:

$$F = \frac{\text{ESS for Model 1 - ESS for Model 3}}{\text{EDF for Model 1 - EDF for Model 3}} \div \text{EMS for Model 3}$$

where ESS is Error Sum of Squares, EDF is Error Degrees of Freedom and EMS is Error Mean square.

Figure 2.3

The Statistical Models Used to Detect Significant Differences Between Populations



If the test is significant at $P < 0.05$ then:

II Test Model 1 VS Model 2 ⟶ Significant ⟶ Regression equations differ in intercepts. F is calculated as:

$$F = \frac{\text{ESS for Model 1 - ESS for Model 2}}{\text{EDF for Model 1 - EDF for Model 2}} \div \text{EMS for Model 3}$$

III Test Model 2 VS Model 3 ⟶ Significant ⟶ Regression equations differ in slopes. F is calculated as:

$$F = \frac{\text{ESS for Model 2 - ESS for Model 3}}{\text{EDF for Model 2 - EDF for Model 3}} \div \text{EMS for Model 3}$$

If test II and III are significant then regression equations are different in both intercepts and slopes.

For NIR, the Shenk et al (1981) software performed stepwise multiple linear regression analysis which relates optical data to the variable being predicted. In this study, the software was allowed to select equations containing 1-9 wavelengths using all the data from all 122 samples in the calibration set. The derivatised Log $\frac{1}{R}$ segments (W values) from each population found in the calibration set were then calculated using the central wavelengths and math treatments selected by the software. It was then possible to relate the W values specific for each population to the in vivo calibration data and then produce regression equations for each population. The regression equations for each population were then compared by analysis of variance. The following example illustrates how the calculations were performed in the

case of a 5-term equation:-

1 A stepwise multiple linear regression of the 122 calibration samples was carried out and gave the 5-term equation shown in Table 2.7.

2 The 5 W values for each sample in this population were calculated from the Log $\frac{1}{R}$ values using the math treatment appropriate to each of the 5 selected wavelengths. Details of this calculation are shown in 2.2.9.1. The computer program used for the calculation is shown in Appendix 1.

3 For each population within the 122 calibration set, multiple linear regressions were carried out between the 5 W values obtained for each sample and the <u>in vivo</u> data.

4 Differences between slopes and/or intercepts of the regression equations produced in this way for each population were assessed by analysis of variance.

CHAPTER THREE

THE EFFECT OF DIFFERENT WASHING PROCEDURES ON THE LOSSES OF OM AND N FROM SAMPLES OF HAY INCUBATED IN POLYESTER BAGS WITHIN THE RUMEN OF SHEEP.

3.1 Introduction

It has been known that a limitation of the in situ technique is poor between-bag reproducibility. A major source of variation may be inconsistent post incubation washing. Aerts et al (1977) and Cottyn et al (1986) showed that additional washing in acid-pepsin reduced between-bag variability and improved the prediction of forage digestibility, probably by removing forage residues and adherent bacteria. For protein, bacterial contamination of bag residues by microbial matter may cause an underestimation of protein degradability by the bag method (Mathers and Aitchison, 1981).

Recently, domestic washing machines have been used to wash the bags after incubation (Ørskov et al, 1988). In this chapter, it is intended to investigate the possibility of combining machine wash with a range of different washing reagents to further improve the reliability and repeatability of the in situ results.

3.2 Methods

The use of chopped hay samples would be a good choice for this type of experiment. Compared to ground silage samples, chopped hay samples have the benefit of stability with time and additionally, the need to remove fine materials out of the samples will be avoided. This will ensure that the polyester bag reproducibility test will not be affected by the amount of fines present in the samples.

The preparation of hay samples, animals and their diets, polyester bag technique and the analytical methods are described in 2.2.8.1, 2.2.8.3, 2.2.8.4 and 2.2.8.5 respectively.

3.2.1 Treatments and Design

The effect of different washing techniques on the

disappearance of organic matter and nitrogen was studied. This was conducted in three consecutive periods and each period utilised a different washing reagent (see Table 3.1). The bags in period 1 received a wash with neutral detergent solution (ND) (Van Soest and Wine, 1967), in period 2 they received a wash with acid-pepsin (AP) (Tilley and Terry, 1963) and in period 3 they received a wash with commercial washing powder (WP) (non-biological Persil brand).

The different washes were applied before- (NDI, API and WPI); after- (IND, IAP and IWP) or before and after incubation (I) in the sheep (NDIND, APIAP and WPIWP). All bags received a final cold water wash in the washing machine, control bags received only this (see Table 3.1).

Each treatment was replicated in 4 sheep and incubated for 3, 8, 24 and 72 hours making a total of 192 bags.

3.2.2 **Zero Time Determination**

This procedure was set to determine the disappearance without incubation in the rumen and using the three different washing reagents (see Table 3.2).

3.2.3 **Multiple Washing Experiment**

This experiment was performed to test the effect of multiple washing on the losses of hay DM from bags.

Approximately 5.5 g of a hay sample were weighed into each bag. From one to four washes in the automatic washing machine were tested. Four bags in each wash were used and either the cold or hot cycle was was utilised. After washing, the bags were oven-dried at 60°C for 48 hours and the DM loss from the bags was calculated by difference.

Table 3.1

Treatments and Design [a]

Washing Reagent	Period	Treat. Code	Washing Procedure
None	1	A	Control. Post-incubation cold water machine washing.
Neutral		NDI	Pre-incubation treatment with neutral detergent.
Detergent		NDIND	Pre- and Post-incubation treatment with neutral detergent.
		IND	Post-incubation treatment with neutral detergent.
None	2	A	Control. Post-incubation cold water machine washing.
Acid		API	Pre-incubation treatment with acid pepsin.
Pepsin		APIAP	Pre- and Post-incubation treatment with acid pepsin.
		IAP	Post-incubation treatment with acid pepsin.
None	3	A	Control. Post-incubation cold water machine washing.
Washing		WPI	Pre-incubation treatment with washing powder.
Powder		WPIWP	Pre- and Post-incubation treatment with washing powder.
		IWP	Post-incubation treatment with washing powder.

a Refer to Appendix 2 for further details about each treatment.

Table 3.2

Zero Time Determination[a]

	Washing Procedure
5 Bags	Regular Machine washing with cold water (control)
5 Bags	Treatment with neutral detergent + machine washing
5 Bags	Treatment with neutral detergent + machine washing + treatment with neutral detergent and machine washing
5 Bags	Treatment with acid-pepsin + machine washing
5 Bags	Treatment with acid-pepsin + machine washing + treatment with acid pepsin and machine washing
5 Bags	Treatment with washing powder + machine washing
5 Bags	Treatment with washing powder + machine washing + treatment with washing powder and machine washing

a For machine washing see A in Appendix 2
For neutral detergent treatment see NDI in Appendix 2
For acid pepsin treatment see API in Appendix 2
For washing powder treatment see WPI in Appendix 2

3.2.4 Fitting the Mathematical Model

The fitted OM disappearance values for all washing treatments were obtained using the Ørskov and McDonald (1979) exponential equation which calculates the "line of best fit" through the disappearance values:

$$p = a + b\,(1 - e^{-ct})$$

where a, b and c are constants fitted by an iterative least squares procedure. "p" is the actual disappearance after time "t", "a" is zero hour degradation or the readily soluble materials, "b" is the amount of sample which will have disappeared within the rumen given sufficient time and "c" is the rate constant for the degradation of "b".

Ørskov and McDonald (1979) linked the degradation rate to an estimate of the solid particle outflow rate from the rumen to give the "effective degradability" P.

$$P = a + \frac{bc}{c + k}$$

where a, b and c are the constants obtained for the previous equation and k is the fractional outflow rate from the rumen per hour. P represents the amount of sample which will actually be degraded in the rumen and its value varies according to the outflow rate.

3.3 Results

3.3.1 Organic Matter Disappearance from Bags

The percentage disappearance of OM from the bags is shown in Table 3.3.

Table 3.3

Organic Matter Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Different Washing Techniques. Mean of 4 Sheep, one Observation per Sheep.

A Neutral Detergent Wash.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	11.7	18.7	32.0	58.0	71.0
NDI	21.9	28.4	40.5	62.8	75.6
NDINDI	23.7	28.9	43.2	63.6	78.3
IND	21.9	27.1	40.0	60.1	74.2
SED	-	0.837	1.695	1.933	0.872

B Acid-Pepsin Wash.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	11.7	16.9	31.1	58.9	73.9
API	25.0	30.6	44.1	66.3	79.7
APIAP	27.7	32.3	47.3	67.5	81.2
IAP	25.0	27.2	39.8	60.4	76.8
SED	-	0.837	1.695	1.933	0.872

C Washing Powder.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	11.7	16.9	29.7	54.9	75.4
WPI	28.2	32.8	41.3	61.1	80.9
WPIWP	35.4	37.0	46.7	66.5	82.4
IWP	28.2	30.0	41.7	63.3	78.3
SED	-	0.837	1.695	1.933	0.872

All treatments significantly ($P < 0.001$) increased the OM disappearance at 3 and 8 hours of incubation compared with the control. With the exception of IND and IAP treatments, the OM disappearance was significantly ($P < 0.05$) increased by all treatments compared to the control at 24 hours of incubation. At 72 hours of incubation, all treatments increased OM disappearance significantly ($P < 0.05$) above the control level.

There is a general pattern in that the OM disappearance was greater from bags which received before incubation washing (NDI, API and WPI) compared to bags which received after incubation washing (IND, IAP and IWP) at all incubation times. An exception to this was the WPI and IWP treatments at 8 and 24 hours of incubation. Before and after incubation washing (NDIND, APIAP and WPIAP) had the greatest effect upon OM disappearance at all incubation times.

The exponential equation (Ørskov and McDonald, 1979) (see 3.2.4) was used to obtain the fitted OM disappearance values for all washing treatments. These disappearances have been shown graphically in Figures 3.1, 3.2 and 3.3 for neutral detergent, acid-pepsin and washing powder treatments respectively. These figures demonstrate that while the 3 washing treatments increased OM disappearance from bags above the control level, the forms of the degradation curves were unaltered.

The dynamics of ruminal activity have already been recognised and feed entered into rumen could either continue to disappear or pass out at any time (Ørskov and McDonald, 1979). In order to allow for the effect of rumen retention time and passage rate on the feed and thus simulating normal rumen conditions, the data in Table 3.4 are the effective degradability at selected outflow rates after subjection of data in Table 3.3 to the Ørskov

FIGURE 3.1

Organic Matter Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Netural Detergent Reagent.

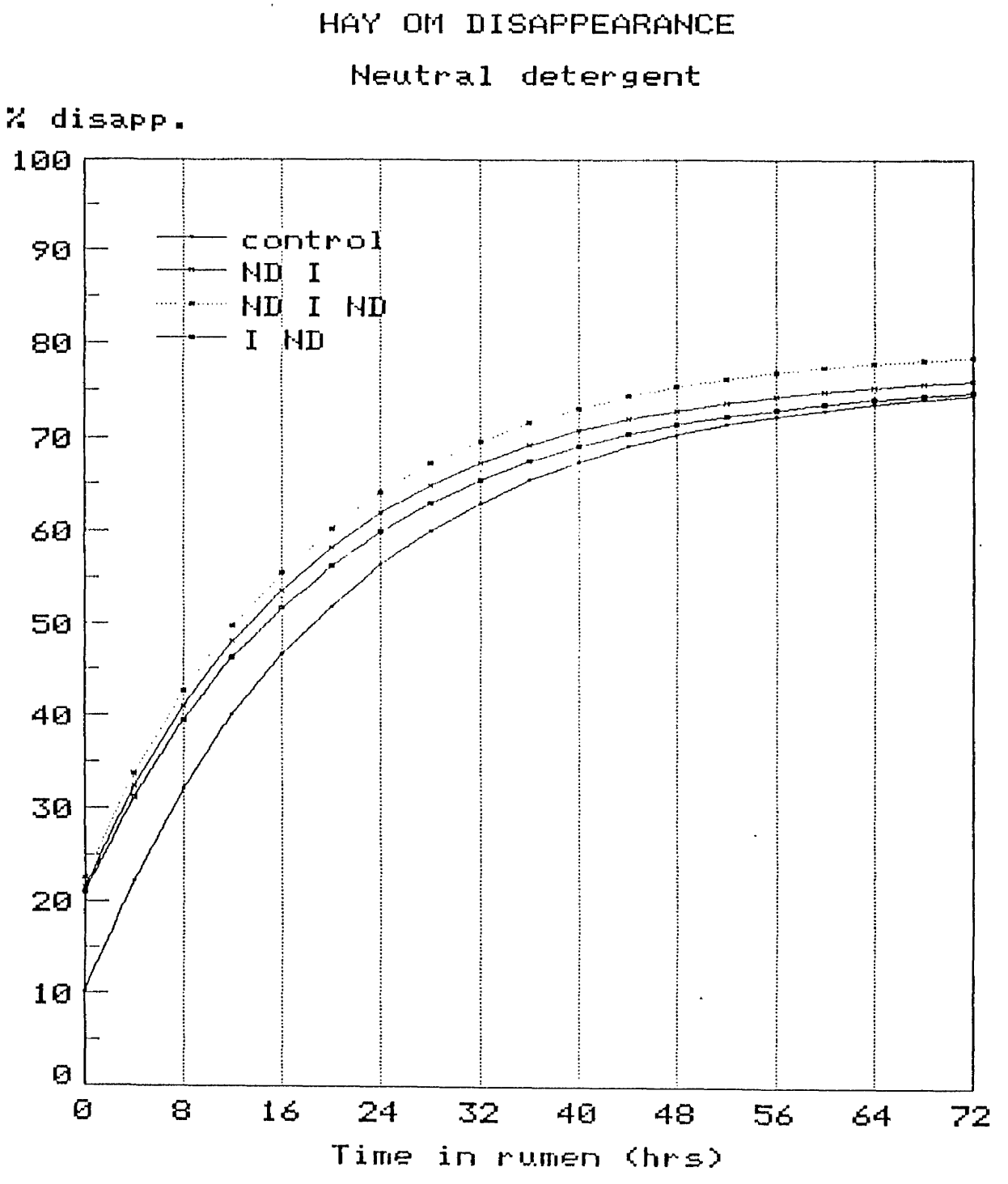


FIGURE 3.2

Organic Matter Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Acid-Pepsin Reagent.

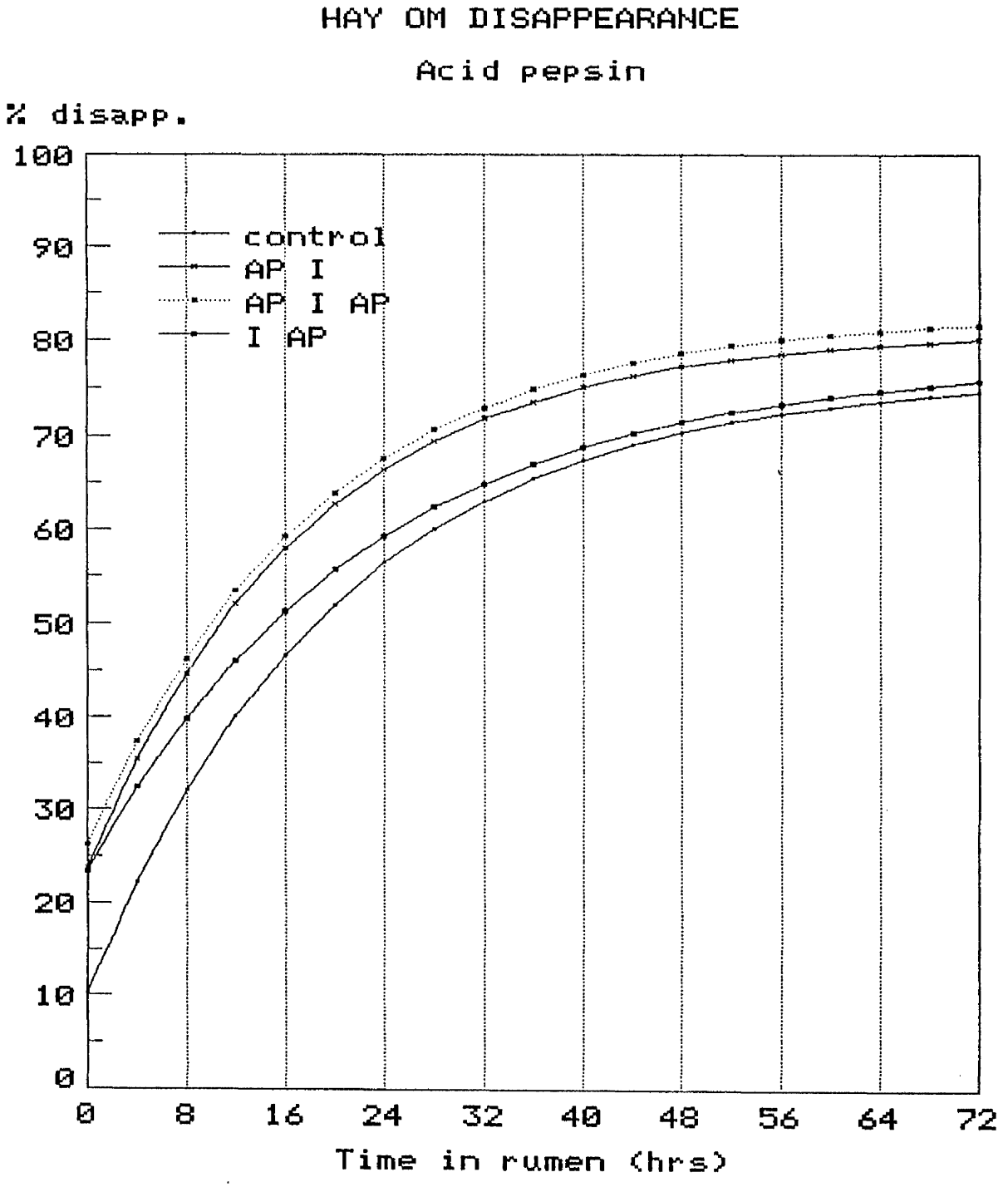


FIGURE 3.3

Organic Matter Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Washing Powder Reagent.

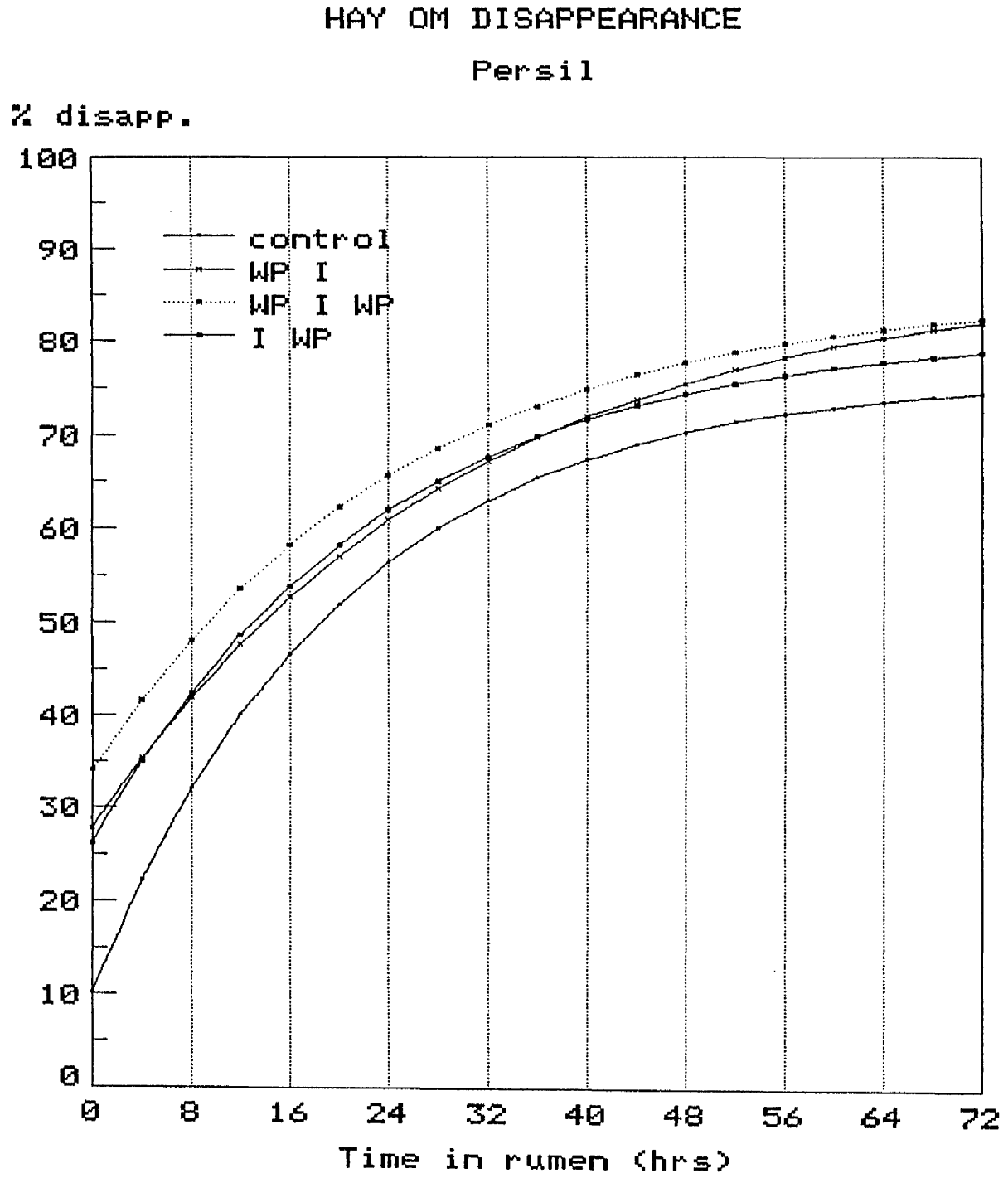


Table 3.4

Effective Degradability (%) of Hay Organic Matter Disappearance from Polyester Bags Incubated in the Rumen of Sheep and Treated with Different Washing Techniques at Selected Outflow Rates. Mean of 4 Sheep, one Observation per Sheep.

A **Neutral Detergent Wash.**

Effective Degradabilities (%)

Treatments	ED 2%	ED 4%	ED 8%
Control	56.8	47.0	36.0
NDI	61.8	53.0	43.3
NDINDD	64.3	55.5	45.3
IND	60.5	52.0	42.3
SED	0.837	1.046	1.09

B **Acid-Pepsin Wash.**

Effective Degradabilities (%)

Treatments	ED 2%	ED 4%	ED 8%
Control	57.8	47.3	35.8
API	66.0	57.3	47.5
APIAP	67.8	58.8	49.3
IAP	61.0	52.0	42.8
SED	0.837	1.046	1.09

C **Washing Powder.**

Effective Degradabilities (%)

Treatments	ED 2%	ED 4%	ED 8%
Control	57.0	45.5	34.0
WPI	65.0	54.5	45.3
WPIWP	68.0	59.5	51.0
IWP	64.0	55.0	45.5
SED	0.837	1.046	1.09

and McDonald (1979) model (see 3.2.4). The effective degradability of the OM followed the same general pattern as with the OM disappearance.

The coefficient of variation (CV%) of OM disappearance is shown in Table 3.5. Figure 3.4 shows the histogram representation of CV of OM disappearance at 24 hours of incubation. All different washing techniques decreased CV% below the control level at 24 hours of incubation with the exception of IND treatment. The coefficient of variation was further decreased at 72 hours of incubation with the exception of bags receiving neutral detergent and acid pepsin treatments (Table 3.5).

Analysis of variance on the data for organic matter disappearance (%) and effective degradability are given in Appendices 3 and 4 respectively. The individual OM disappearances (%) and the effective degradabilities (%) for all washing treatments are shown in Appendix 5.

3.3.2 **Nitrogen Disappearance from Bag**

The percentage disappearance of Nitrogen from bags is shown in Table 3.6.

All treatments (except NDI, 24 hours) significantly ($P < 0.001$) increased nitrogen disappearance at all incubation times compared with the control. NDI treatment at 24 hours of incubation time was significant at $P < 0.05$.

The effect of either before or after incubation washing on nitrogen disappearance from bags has been reversed compared with organic matter disappearance. At all incubation times, the bags which received an after incubation washing (IND, IAP and IWP) clearly have a greater nitrogen loss compared with bags which received before incubation washing (NDI, API and WPI). At 3

Table 3.5

Coefficient of Variation (%) of Organic Matter Disappearance of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Different Washing Techniques.

A Neutral Detergent Wash.

Incubation Times (Hours)

Treatments	3	8	24	72
Control	3.7	11.5	4.8	2.9
NDI	8.2	2.2	4.3	4.7
NDIND	2.7	3.2	1.8	3.5
IND	3.8	3.2	7.6	4.4

B Acid-Pepsin Wash.

Incubation Times (Hours)

Treatments	3	8	24	72
Control	7.9	8.4	10.8	0.91
API	10.1	3.5	2.2	2.3
APIAP	3.9	1.3	2.0	1.8
IAP	4.5	6.9	5.2	1.2

C Washing Powder.

Incubation Times (Hours)

Treatments	3	8	24	72
Control	2.8	5.8	7.3	2.8
WPI	1.8	3.8	4.3	1.5
WPIWP	2.3	5.8	2.4	1.9
IWP	2.8	10.3	4.0	1.4

FIGURE 3.4

Coefficient of Variation (%) of Organic Matter Disappearance of Hay from Polyester Bags Treated with Different Washing Techniques after 24 hours of Incubation.

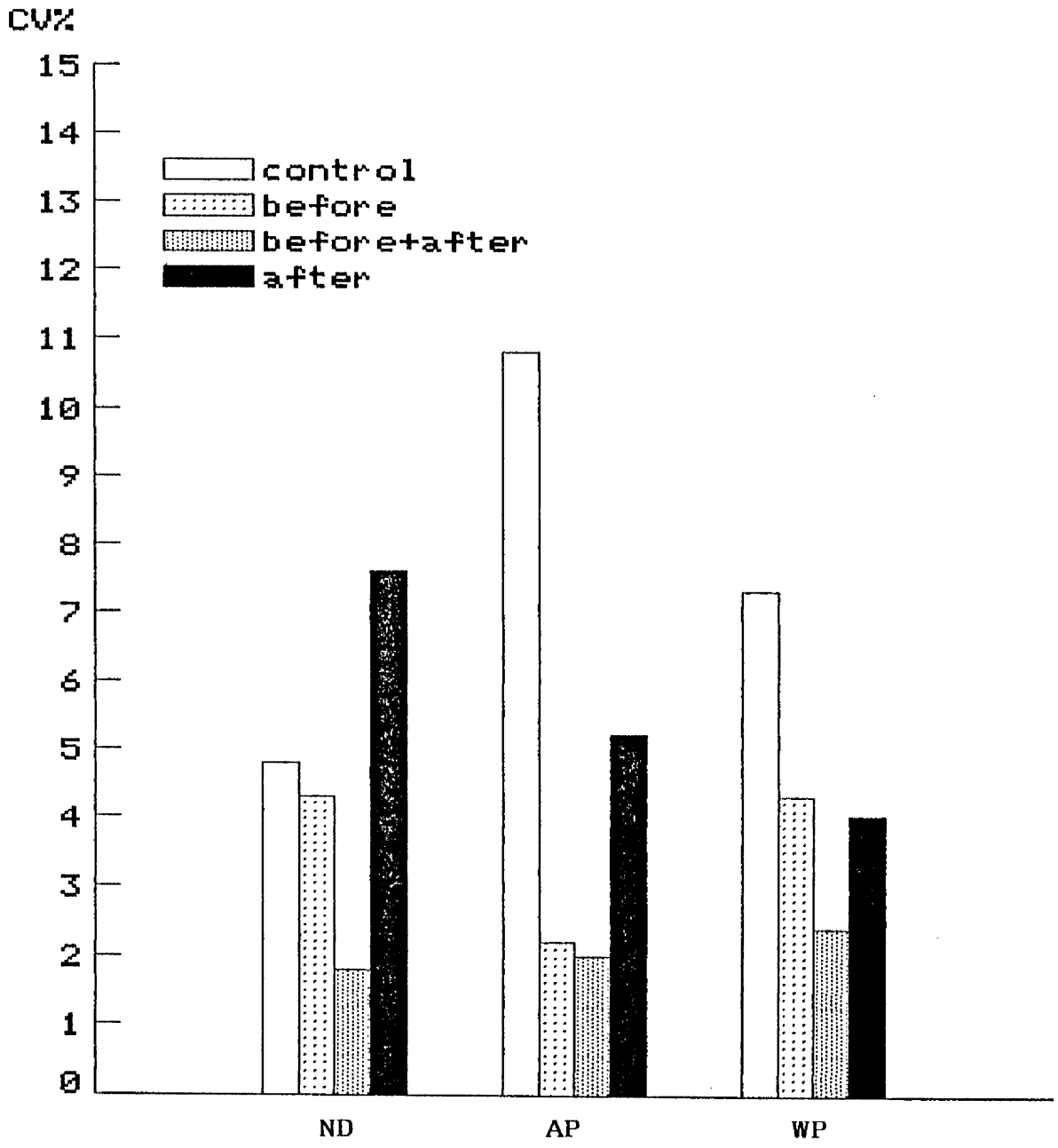


Table 3.6

Nitrogen Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Different Washing Techniques. Mean of 4 Sheep, one Observation per Sheep.

A Neutral Detergent Wash.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	16.4	27.5	41.9	64.8	79.3
NDI	37.1	46.7	58.3	68.6	84.3
NDIND	44.9	56.9	73.0	80.9	91.9
IND	37.1	48.4	65.4	79.9	89.3
SED	-	1.916	2.561	1.629	0.622

B Acid-Pepsin Wash.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	16.4	24.0	39.9	64.5	79.0
API	77.9	76.4	75.0	72.7	83.4
APIAP	83.9	82.0	88.6	88.0	89.7
IAP	77.9	79.2	81.9	86.6	88.1
SED	-	1.916	2.561	1.629	0.622

C Washing Powder.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	16.4	23.0	35.7	63.2	83.7
WPI	46.2	54.9	67.0	78.5	88.7
WPIWP	63.1	70.0	77.8	90.6	95.3
IWP	46.2	55.7	70.8	87.5	93.3
SED	-	1.916	2.561	1.629	0.622

hours of incubation the differences between before or after incubation washing were not significant. However, at 8 hours of incubation the differences were significant at $P < 0.05$ with the exception of WPI and IWP treatments. At 24 and 72 hours of incubation, these differences were highly significant ($P < 0.001$). The before and after incubation wash (NDIND, APIAP and WPIWP) has the greatest effect upon nitrogen disappearance at all incubation times.

The graphical representation of nitrogen disappearance from bags is shown in figures 3.5, 3.6 and 3.7 for neutral detergent, acid-pepsin and washing powder treatments respectively. To demonstrate the effect of washing reagents upon the removal of nitrogen from bags, Table 3.7 shows the concentration of nitrogen in bag residues. Figure 3.8 shows the histogram representation of nitrogen concentration in bag residues after 72 hours of incubation. At 24 and 72 hours of incubation, the nitrogen concentration in bags residues were significantly ($P < 0.001$) reduced by after incubation washing (IND, IAP and IWP) compared to either control bags or before incubation washing (NDI, API and WPI).

Analysis of variance on the data for nitrogen disappearance (%) and nitrogen concentration on bag residues are given in Appendices 6 and 7 respectively. The individual N disappearance (%) and the nitrogen concentration in bag residues for all washing treatments are shown in Appendix 8.

3.3.3 **Multiple Washing Experiment**

The percentage DM loss of hay samples from bags is shown in Table 3.8 and Figure 3.9.

There is highly significant ($P < 0.001$) DM loss from bags which received two washes as compared to one wash.

FIGURE 3.5

Nitrogen Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Neutral Detergent Reagent.

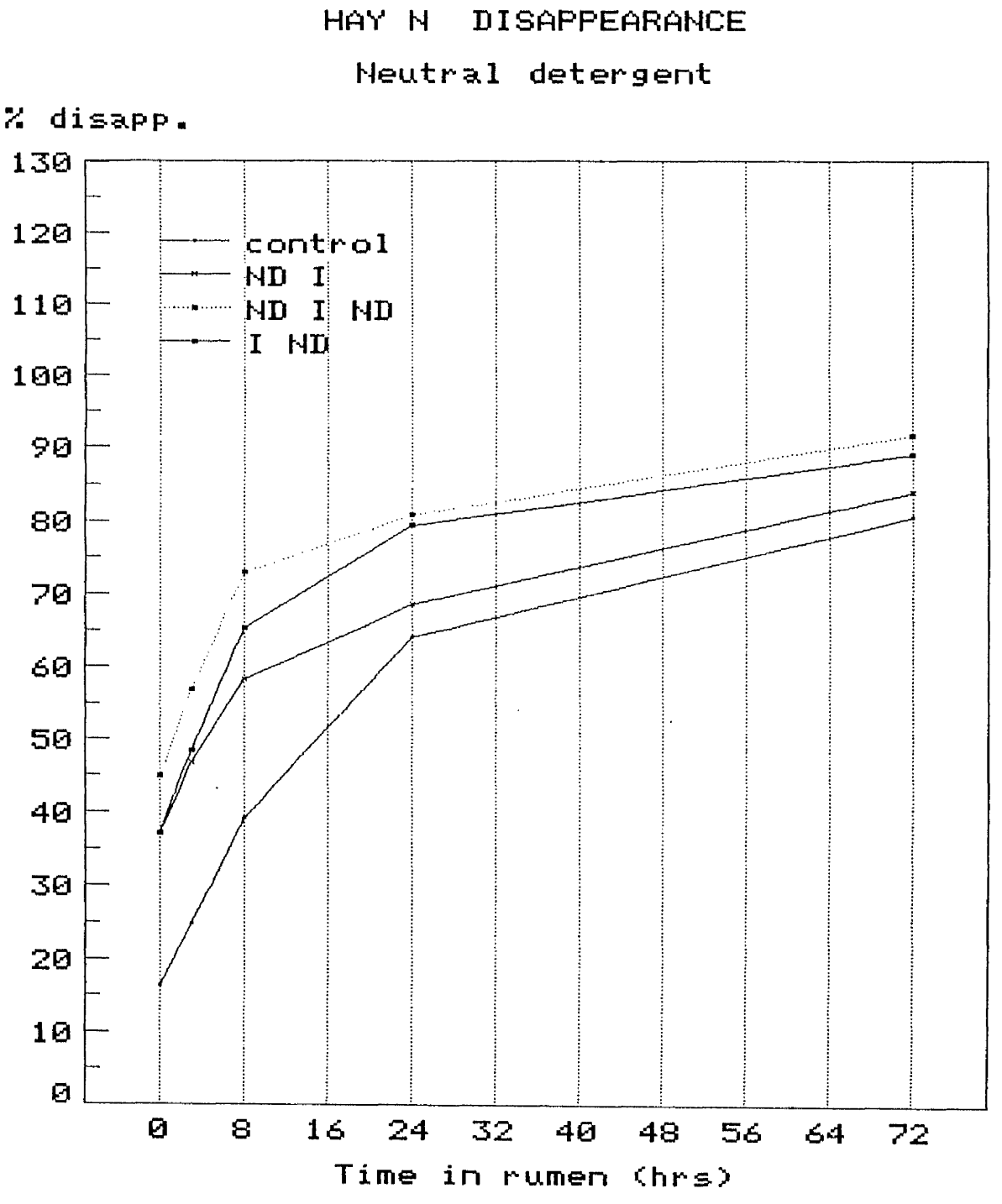


FIGURE 3.6

Nitrogen Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Acid-Pepsin Reagent.

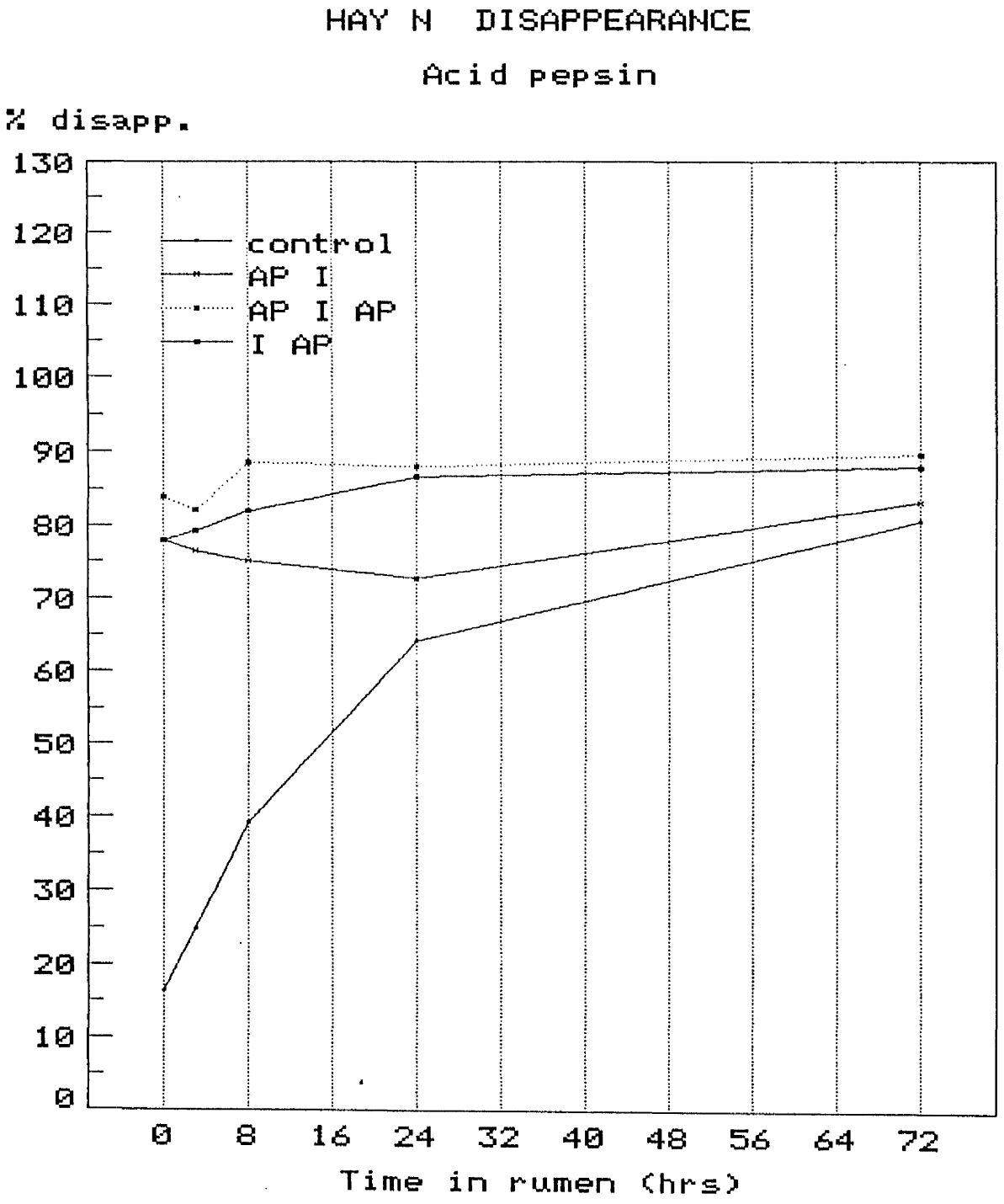


FIGURE 3.7

Nitrogen Disappearance (%) of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Washing Powder Reagent.

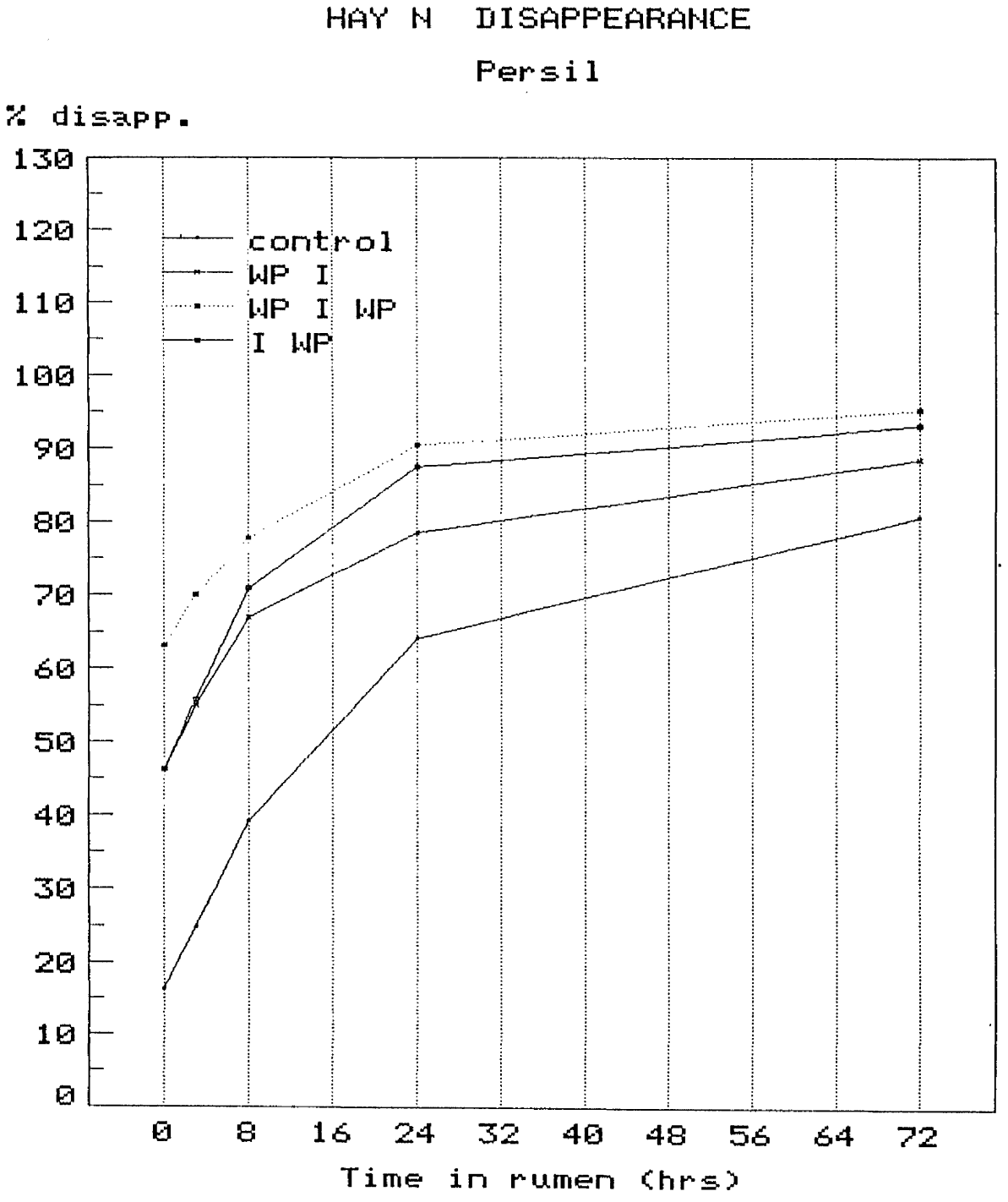


Table 3.7

Nitrogen Concentration (gkg^{-1}DM) in Bag Residues.

A Neutral Detergent Wash.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	9.8	9.3	8.9	8.7	7.3
NDI	8.4	7.9	7.5	9.0	6.8
NDIND	7.6	6.6	5.1	5.6	3.9
IND	8.4	7.5	6.1	5.5	4.3
SED	-	0.292	0.437	0.408	0.252

B Acid-Pepsin Wash.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	9.8	9.5	9.1	8.9	8.2
API	3.1	3.6	4.7	8.3	8.3
APIAP	2.3	2.8	2.3	3.8	5.7
IAP	3.1	3.0	3.2	3.5	5.3
SED	-	0.292	0.437	0.408	0.252

C Washing Powder.

Incubation Times (Hours)

Treatments	0	3	8	24	72
Control	9.8	9.7	9.6	8.4	6.8
WPI	7.9	7.1	6.0	5.9	6.3
WPIWP	6.0	5.1	4.5	3.0	2.8
IWP	7.9	6.7	5.3	3.6	3.3
SED	-	0.292	0.437	0.408	0.252

FIGURE 3.8

Nitrogen Concentration ($gkg^{-1}DM$) in Bag Residue after 72 hours of Incubation.

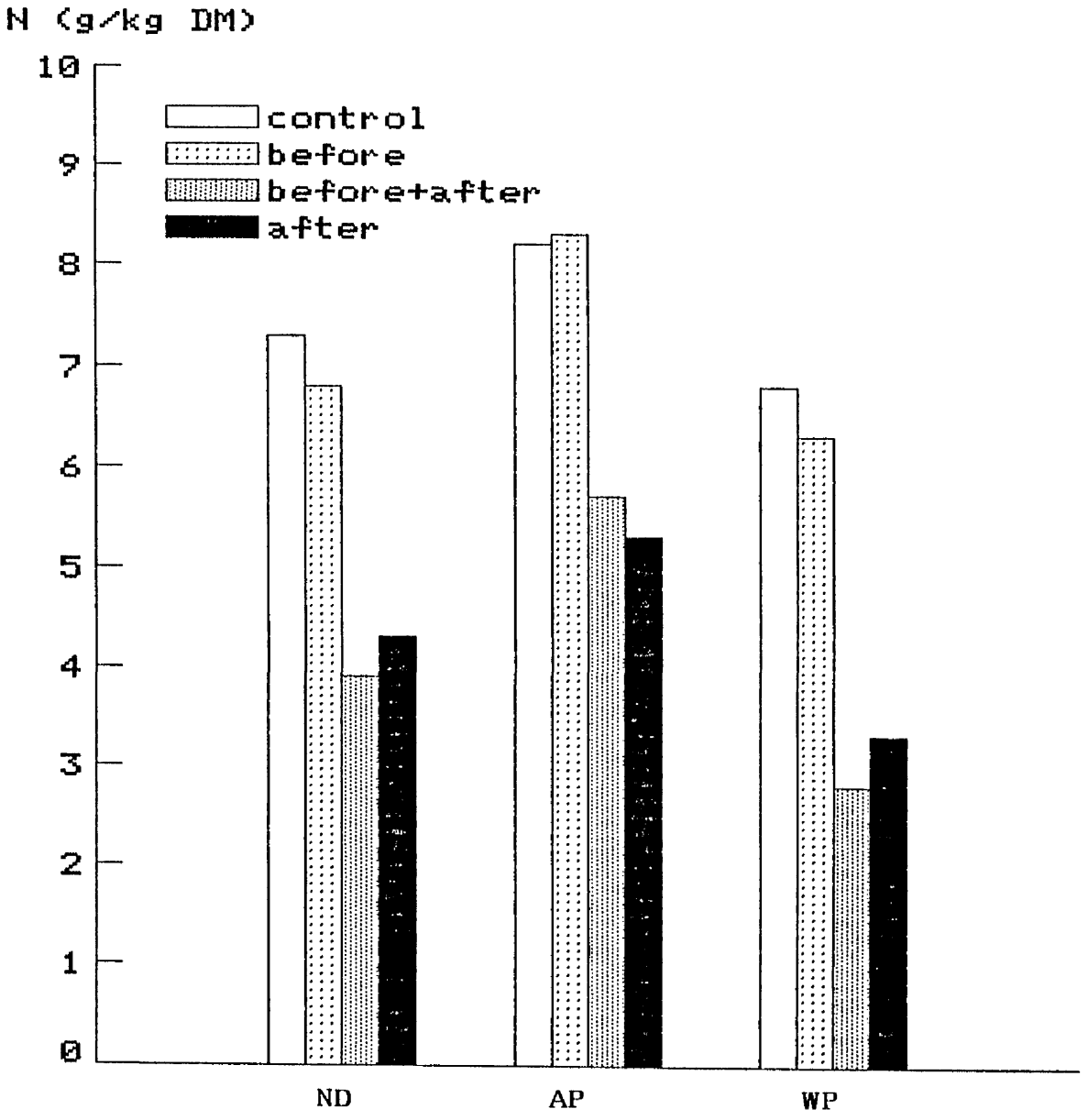


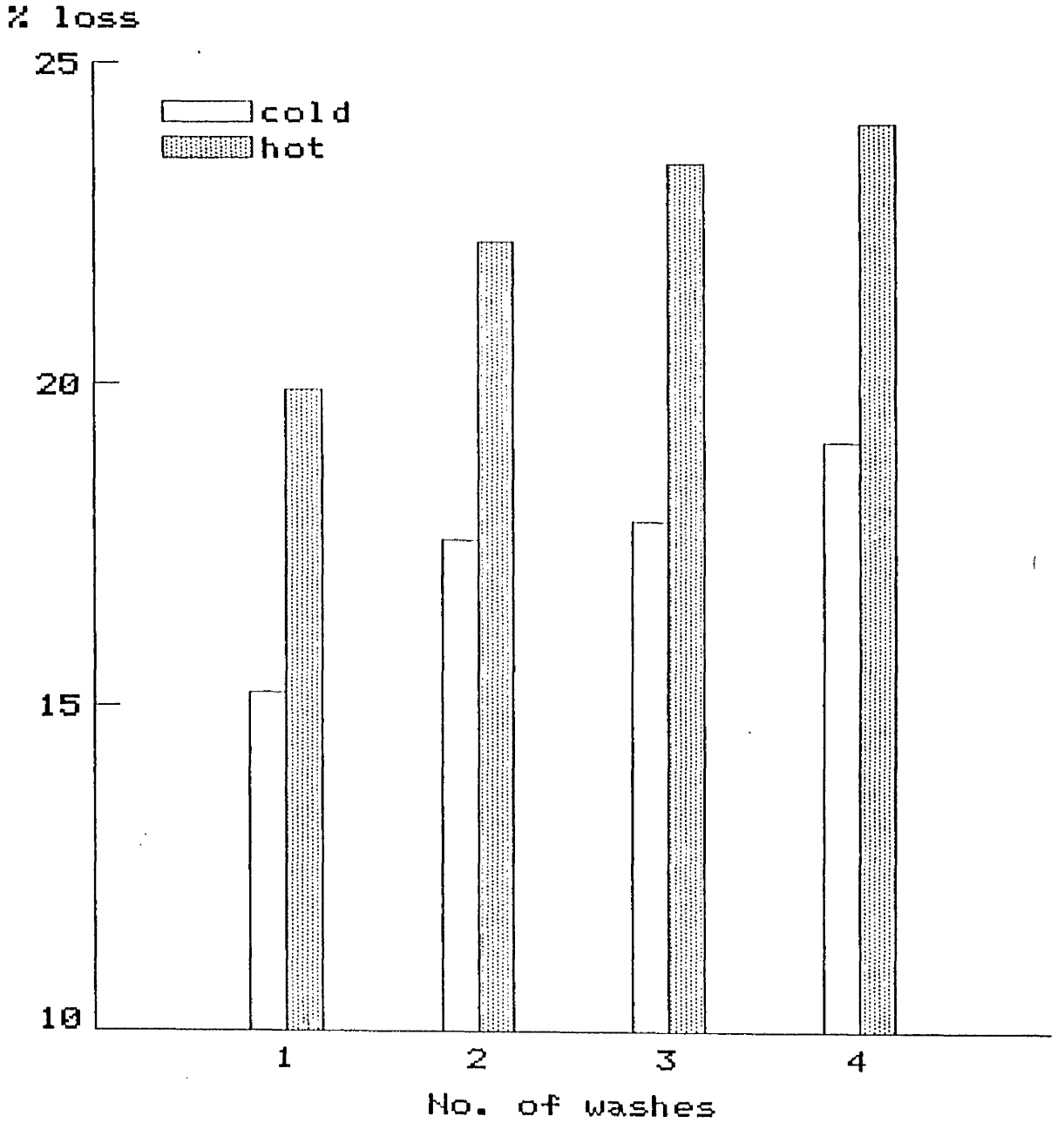
Table 3.8

The Effect of Multiple Washing on the Losses of DM (%) from Hay Samples Incubated in Polyester Bags.
Means of 4 Observations

Number of Washes	DM Loss (%)	
	Cold Wash	Hot Wash
1	15.2	19.9
2	17.6	22.2
3	17.9	23.4
4	19.1	24.0
SED^{+}_{-}	0.28	0.374

FIGURE 3.9

The Effect of Multiple Washing on the DM Loss (%) from Hay Samples Incubated in Polyester Bags.



Thereafter, the losses were gradual indicating that much of the loss occurred in the first and second washes.

3.4 Discussion

In recent years, the in situ method has been used extensively as a measure of the rate of degradation of feedstuffs in the rumen. Many factors inherent in this method have been recognised which may affect the rate of degradation. An important factor which has not been studied carefully is the washing procedure after bags are removed from the rumen. The different washing techniques described in this chapter were intended to investigate the following:

1 What is the effect of these washing techniques in reducing between-bag variability?

2 What is the ultimate consequence of these washing procedures on altering the form of the degradation curves?

3 For protein degradation measurements, can these procedures be regarded as a useful addition to the bag technique in removing adhering bacteria from bag contents after incubation in the rumen?

3.4.1 Organic Matter Disappearance from Bags

The results presented in Table 3.3 and Figures 3.1, 3.2 and 3.3 clearly indicate that the three washing reagents increased OM disappearance from bags. This is in close agreement with the findings reported by Chenost et al (1970) and Playne et al (1978). They showed that subjecting the bags after removal from rumen to a second stage digestion with acid-pepsin identical to that used by Tilley and Terry (1963) increased DM disappearance.

When the exponential equation of Ørskov and McDonald (1979) was applied to the disappearance of OM from the bags, the forms of the degradation curves were unaltered which suggest that the results are as biologically meaningful as those obtained with a cold water wash only (control) (see figures 3.1, 3.2 and 3.3).

The bags which received before incubation washing (NDI, API and WPI) were noticed to have greater OM disappearance compared to bags which received after incubation washing (IND, IAP and IWP) (see Table 3.3). This observation may be explained by firstly, the washing reagent, either ND, AP or WP, may modify the cell wall and make it more accessible to microbial attack during the subsequent rumen incubation. This observation has been reported previously by Jones and Hayward (1975) and Roughan and Holland (1977) where they indicated that the dry matter solubility was increased when a herbage treated with the cellulase enzyme is preceded with acid-pepsin or neutral detergent respectively.

Secondly, the before incubation bags received an extra machine wash when compared to the after incubation bags which might increase the loss of particulate matter from the bags as described by Playne et al (1978) and Lindberg and Knutsson (1981). This has been confirmed by the multiple washing experiment (see Table 3.8 and Figure 3.9) where there was significant ($P < 0.001$) difference in the DM loss between bags washed once or twice in the washing machine.

When allowing the factors of rumen dynamics to be considered by calculating the effective degradability of OM disappearance (see Table 3.4), all washing treatments increased the effective degradability of hay in the rumen in a pattern similar to that of OM disappearance (Table 3.3).

The precision of the in situ technique was found to be improved by the washing reagents. At 24 hours of incubation, all treatments decreased the coefficient of variation below the cold water wash only (control) (see Table 3.5 and Figure 3.4) with the exception of the IND treatment. The reason that the ND treatments didn't improve the between-bag variability at 72 hours of incubation is possibly due to the experimental and technical difficulties associated with washing the bags in the neutral detergent solution. The percentage reduction in CV at 24 hours of incubation was 41, 67 and 45% for WPI, WPIWP and IWP treatments respectively compared to the control. The detergent washing may remove contaminating residues both from the bag material and contents which enhance between-bag reproducibility.

The reduction of variability between bags has been observed previously by Playne et al (1978) when acid-pepsin was used to wash the bags after rumen incubation. They reported that the CV for the 48 and 72 hours of incubation had been reduced from 19.7 to 11.7% after the acid-pepsin treatment. Our coefficient was much lower than they reported possibly due to the use of the washing machine which gives more uniformly effective washing than the regular tap water wash.

3.4.2 **Nitrogen Disappearance from Bags**

The results presented in Table 3.6 and Figures 3.5, 3.6 and 3.7 show that nitrogen disappearance was greatly increased by the washing reagents. Neutral detergent and washing powder treatments did not however, alter the form of the degradation curve, but for bags receiving the acid-pepsin treatment, nitrogen disappearance was unaffected by the period of incubation and so no curve was fitted. This is because of the enzymatic nature of pepsin which solubilises protein readily at zero incubation (see Figure 3.6). This suggests that for protein

degradation measurements, the use of acid-pepsin as a washing agent is inappropriate. Nitrogen disappearance from bags receiving after incubation washing (IND, IAP and IWP) is increased compared to bags receiving before incubation washing (NDI, API and WPI) and the longer the incubation the greater the effect was (see Table 3.6). This greater nitrogen loss may suggest that the detergent washing may remove adhering bacterial nitrogen which is bound to hay samples after rumen incubation. This conclusion might be supported by the lower nitrogen concentration in bags residues for the bags receiving after incubation washing as compared with bags receiving before incubation washing (see Table 3.7 and Figure 3.8).

The contamination of bag residues by microbial matter has been observed in previous reports. Mathers and Aitchison (1981) reported that feedstuff residues may be significantly contaminated by micro-organisms and this contamination increased linearly with time in the rumen. This contamination has been supported by electron microscopical studies which showed that rumen bacteria colonise and adhere to plant particles during fermentation (Akin, 1979). These observations could explain our case here where nitrogen loss was greater and nitrogen concentrations in bag residues were lower for bags receiving after incubation washing as compared with bags receiving before incubation washing.

3.5 Conclusion

The use of detergent washing appears to be a useful addition to the in situ technique. For organic matter, post-incubation detergent washing reduces between-bag variability without altering the form of the degradation curve. This suggests that this modification to the in situ technique can be potentially applied with relative confidence to measure forage OM disappearance. For protein degradation measurements, post-incubation detergent

washing might remove contaminating bacteria which could otherwise lead to underestimation of protein degradability. However, further work is required to measure the precise effect of detergent washing on removing this contamination.

Washing the bags after rumen incubation with domestic washing powder in the washing machine is both cheap and convenient.

CHAPTER FOUR

PREDICTION OF ORGANIC MATTER DIGESTIBILITY OF GRASS SILAGES

4.1 Introduction

The rationing system for ruminants used in the UK requires a knowledge of the ME content of the ration. To date there have been insufficient in vivo measurements of ME (see 1.1.3) for the development of satisfactory prediction equations and the UK advisory services have therefore adopted the practice of developing regression equations for the prediction of OMD or DOMD. From these predicted values, it is possible to convert DOMD to ME using a constant factor (see 1.1.4).

To date, UK advisory laboratories have relied on a limited number of populations of silages for developing equations to predict in vivo OMD and DOMD. In this chapter, it is intended to examine the population of silages obtained from different sources around the UK [see Table 2.1] (for which in vivo digestibility data were available) using a range of laboratory methods and then to explore the possibility of establishing an improved predictive technique which could be used by all of the advisory laboratories in the UK.

4.2 Results

The predictive methods used in this study may be categorised as:

I Chemical Methods
II Biological Methods
III A Physical Method (NIR)

4.2.1 Chemical Methods

The results of using MADF and LIGA to predict in vivo OMD are shown in Table 4.1 and figures 4.1 and 4.2 respectively.

Table 4.1

Regression Statistics for the Prediction of *in vivo* OMD of Grass Silages

Pop. No.	n		Predictors						
			MADF	LIGA	NCCMD	PCCMD	NB48 OMD	IVOMD	NIR 8-term
2	72	R^2	0.54	0.73	0.62	0.79	0.70	0.75	0.87
		RSD%	4.5	3.4	4.1	3.0	3.6	3.3	2.4
3	23	R^2	0.24	0.20	0.28	0.53	0.32	0.48	0.59
		RSD%	3.5	3.6	3.4	2.8	3.3	2.9	2.6
4	27	R^2	0.49	0.89	0.57	0.82	0.77	0.83	0.91
		RSD%	5.0	2.3	4.8	3.0	3.4	2.9	2.2
TOTAL	122	R^2	0.34	0.52	0.54[a]	0.55	0.68	0.74	0.85
		RSD%	5.1	4.4	4.3	4.2	3.6	3.2	2.5
More than one line[b]			***	***	**	***	NS	NS	NS
Differences in intercepts			***	***	**	***			
Differences in slopes			NS	NS	NS	***			

[a] = three values were missing, one from population 2 and two from population 4.

[b] = shows whether the regression lines for individual silage populations are significantly different.

R^2 = adjusted for degrees of freedom.

*** = significant at $P < 0.001$
** = significant at $P < 0.01$
NS = not significant at $P = 0.05$.

FIGURE 4.1 RELATIONSHIP BETWEEN MADF AND IN VIVO OMD

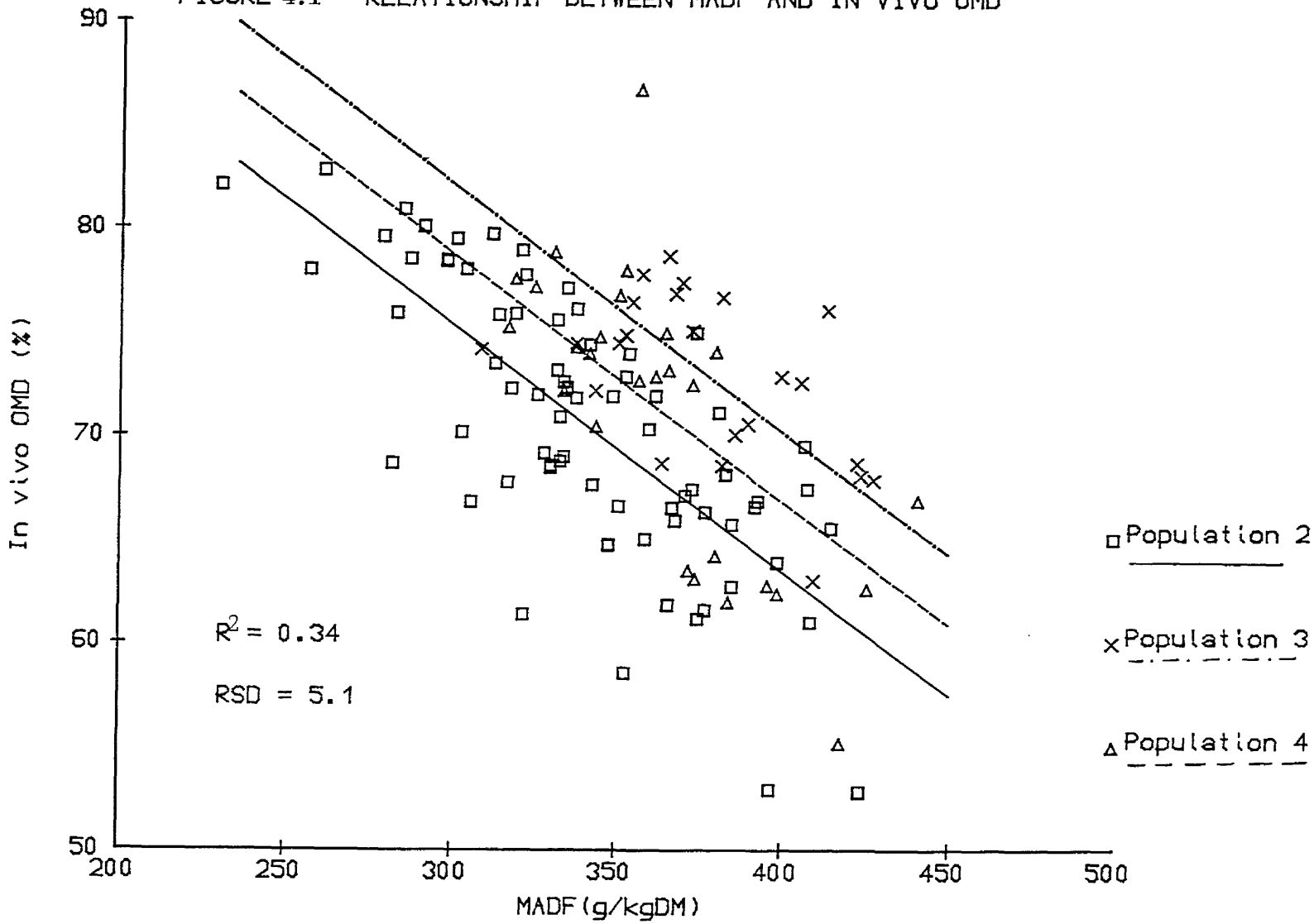
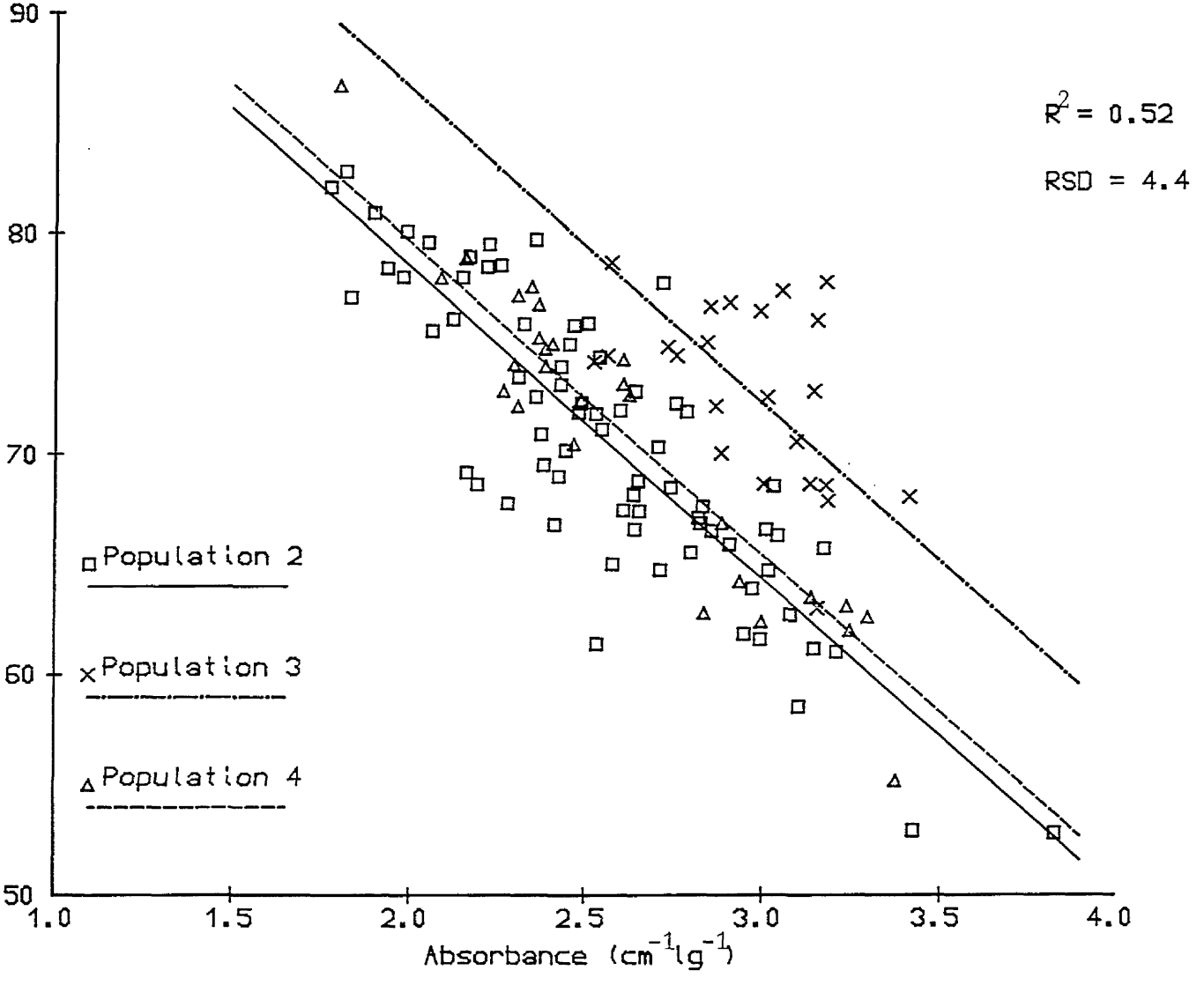


FIGURE 4.2 RELATIONSHIP BETWEEN LIGA AND IN VIVO OMD



The R^2 and RSD% for MADF and LIGA for the overall relationship (N = 122) were 0.34, 5.1 and 0.52, 4.4 respectively. LIGA gave regression for populations 2 and 4 with R^2 = 0.73 and 0.89 respectively, whereas the R^2 for population 3 was rather poor (R^2 = 0.20) [Table 4.1].

For both the MADF and LIGA methods, the regression lines obtained for the three populations of silages differ significantly (P < 0.001) in intercepts (see Table 4.1 and Figures 4.1 and 4.2). Each population regression line intercept differed significantly from the other (see Table 4.2) for MADF relationships. However, for LIGA relationships, it was population 2 and 3 and 3 and 4 for which the intercepts differed significantly.

4.2.2 **Biological Methods.**

The results of using NCOMD, PCOMD, NB48 OMD and IVOMD to predict in vivo OMD are shown in Table 4.1 and Figures 4.3, 4.4, 4.5 and 4.6 respectively.

The R^2 and RSD% for NCOMD and PCOMD for the overall relationship were 0.54 and 4.3; 0.55 and 4.2 respectively. For PCOMD, the R^2 and RSD% for populations 2, 3 and 4 were 0.79, 3.0; 0.53, 2.8 and 0.82, 3.0 respectively whereas for NCOMD, it was 0.62, 4.1; 0.28, 3.4 and 0.57, 4.8 respectively (Table 4.1).

The regression lines obtained for the three populations of silages differed significantly (P < 0.001) in both intercepts and slopes for PCOMD and only intercepts for NCOMD (P < 0.01) [see Table 4.1 and Figures 4.3 and 4.4].

For PCOMD, each population regression line intercept and slope differed significantly from each other. However, for NCOMD the intercept differences were between population 2 and 4 only (see Table 4.2).

Table 4.2

Regression Coefficients and Significance of Between-Population Differences in MADF, LIGA, NCOMD and PCOMD Regression Equations for the Prediction of *in vivo* OMD for Silages

MADF

Co-efficients	Regression Coefficients			F-Test		
	Pop 2	Pop 3	Pop 4	2 v 3	2 v 4	3 v 4
One Slope	-0.12					
Different Intercepts	111.1	118.0	114.4	***	***	*

LIGA

Co-efficients	Regression Coefficients			F-Test		
	Pop 2	Pop 3	Pop 4	2 v 3	2 v 4	3 v 4
One Slope	-14.158					
Different Intercepts	106.8	114.9	107.9	***	NS	***

NCOMD

Co-efficients	Regression Coefficients			F-Test		
	Pop 2	Pop 3	Pop 4	2 v 3	2 v 4	3 v 4
One Slope	0.58					
Different Intercepts	31.9	33.2	35.2	NS	**	NS

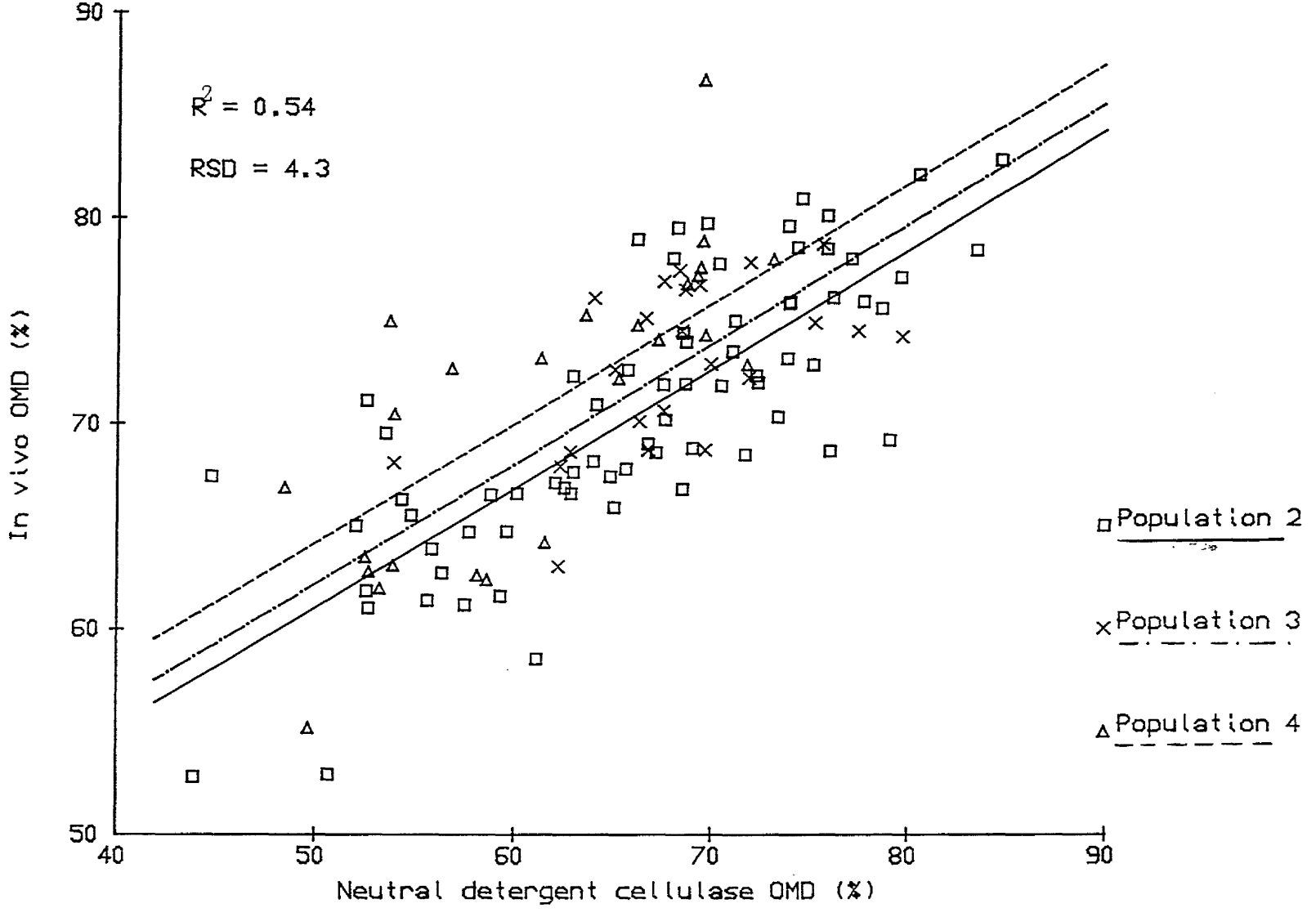
PCOMD

Co-efficients	Regression Coefficients			F-Test		
	Pop 2	Pop 3	Pop 4	2 v 3	2 v 4	3 v 4
Different Slopes	0.68	0.43	0.99	*	**	***
Different Intercepts	27.4	47.0	17.6	***	***	***

*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$

* = Significant at $P < 0.05$ NS = Not Significant at $P = 0.05$

FIGURE 4.3 RELATIONSHIP BETWEEN NCOMD AND IN VIVO OMD



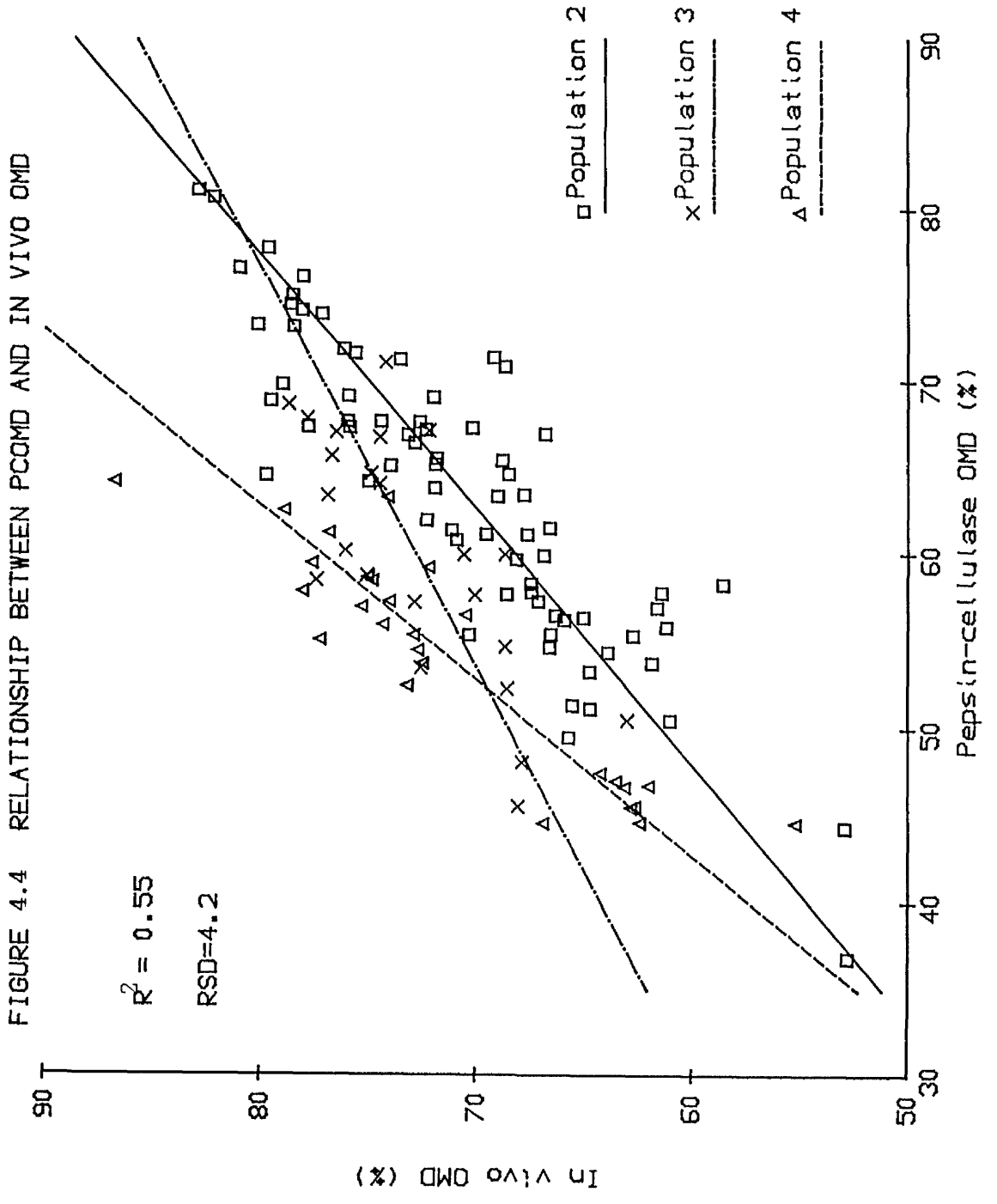
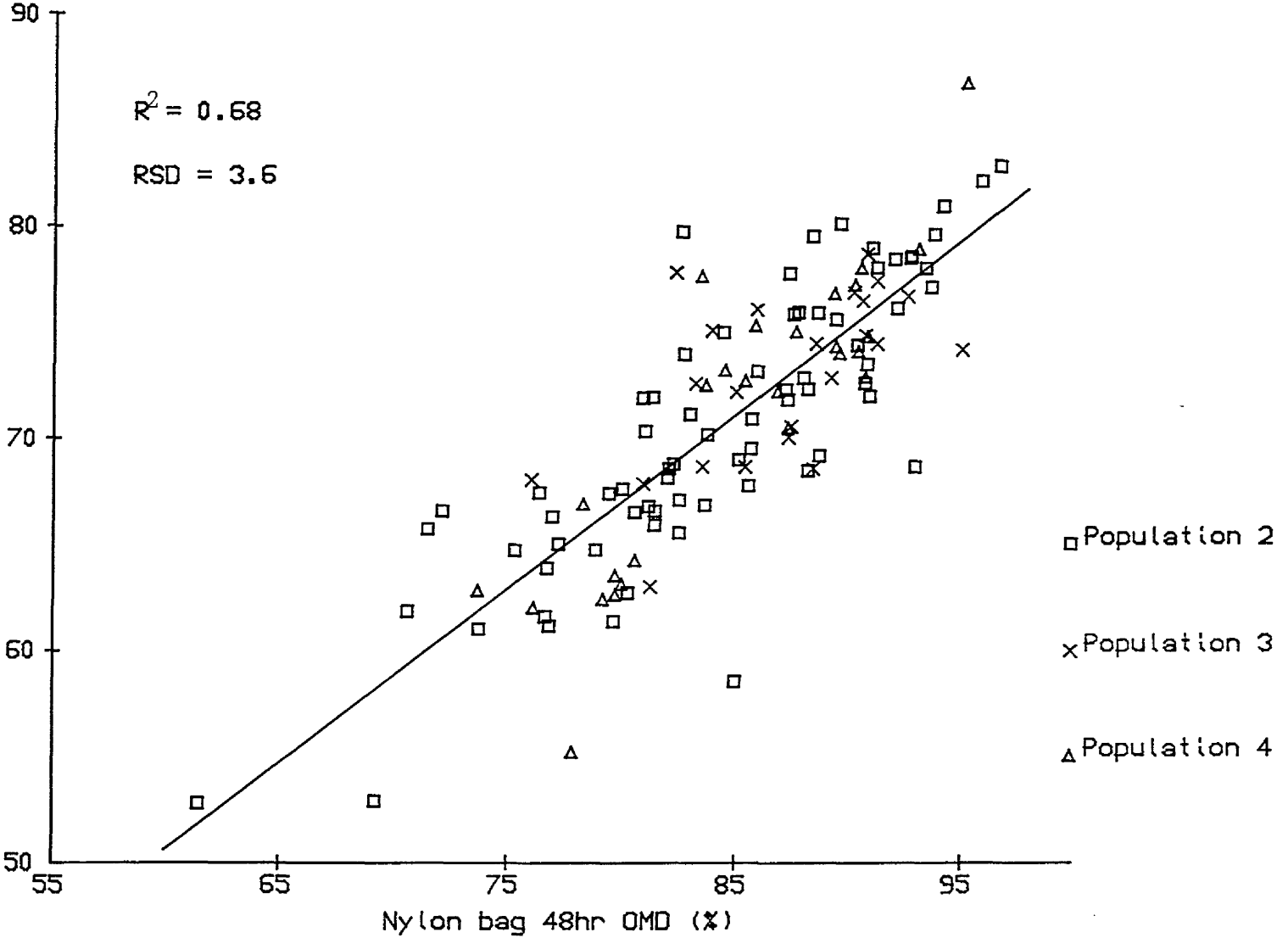


FIGURE 4.4 RELATIONSHIP BETWEEN PCOMD AND IN VIVO OMD
R^2 = 0.55
RSD=4.2
Population 2
Population 3
Population 4
In vivo OMD (%)
Pepsin-cellulase OMD (%)
90
80
70
60
50
30
40
50
60
70
80
90

FIGURE 4.5 RELATIONSHIP BETWEEN NB48 OMD AND IN VIVO OMD



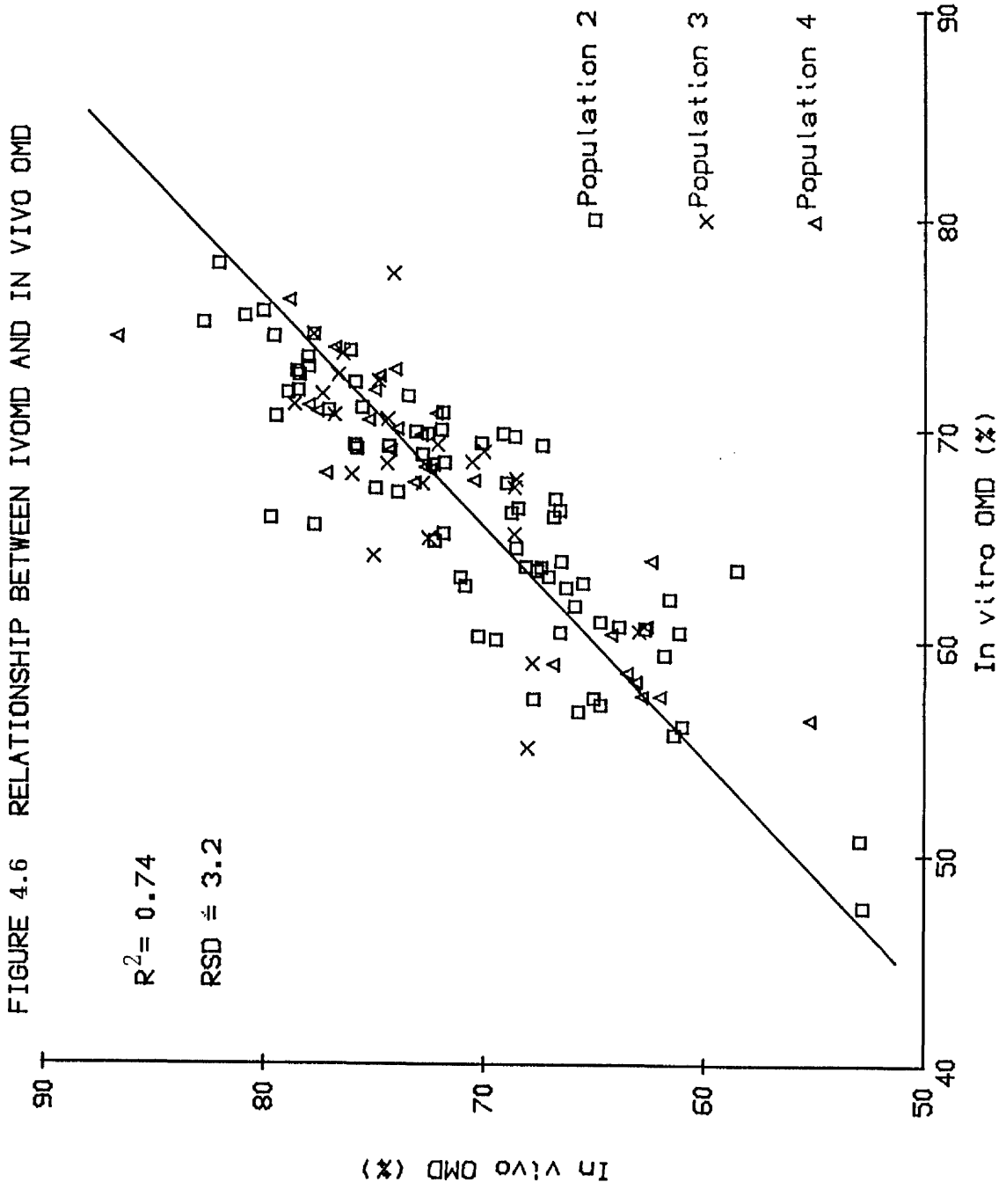


FIGURE 4.6 RELATIONSHIP BETWEEN IVOMD AND IN VIVO OMD
R^2= 0.74
RSD = 3.2
Population 2
Population 3
Population 4
In vivo OMD (%)
In vitro OMD (%)
90
80
70
60
50
40
50
60
70
80
90

For both the NB48 OMD and IVOMD methods, the R^2 and RSD% for the overall relationship were 0.68, 3.6 and 0.74 and 3.2 respectively (Table 4.1).

In contrast with the methods mentioned previously, both the NB48 OMD and IVOMD methods can describe the three populations of silages by a single regression line (Table 4.1 and Figures 4.5 and 4.6). Table 4.3 shows the regression coefficients of the regression equations in predicting the in vivo OMD by the NB48 OMD and IVOMD methods as indicated by a single regression line for all populations.

Analysis of variance of the regression equations for the 3 populations used in the calibration set for chemical and biological methods is shown in Appendix 9. Analysis of variance of the individual population regression equations to indicate which population line is different from the other for the MADF, LIGA, NCOMD and PCOMD methods is shown in Appendix 11. The individual values for in vivo OMD, chemical and biological parameters for the 122 calibration silages used in this study is shown in Appendix 12.

4.2.3 The Use of Bivariate Relationships to Predict in vivo OMD.

The chemical and biological predictors studied in this thesis have been combined in a bivariate relationship to study whether there was an improvement in predicting in vivo OMD over the univariate relationship for the 122 calibration silages. Table 4.4 shows that the combination of predictors in bivariate relationships can improve the prediction of in vivo OMD over the univariate relationship shown in Table 4.1. All possible combinations can improve the prediction over the univariate relationship significantly, with the exception of the bivariate relationships of MADF with IVOMD, PCOMD, NCOMD

Table 4.3

Regression Coefficients for the Prediction of *in vivo* OMD by NB48 OMD and IVOMD

Method	Regression Coefficients	
	Intercept	Slope
NB48 OMD	1.44	0.82
IVOMD	9.96	0.92

Table 4.4

Bivariate Regression Statistics for the Prediction of *in vivo* OMD of 122 Silages

Predictors	R^2	RSD%	Significance of the Improvement[a]
IVOMD + NB48 OMD	0.76	3.1	***
IVOMD + PCOMD	0.75	3.2	*
IVOMD + NCOMD	0.75	3.2	**
IVOMD + MADF	0.74	3.2	NS
IVOMD + LIGA	0.77	3.0	***
NB48 OMD + PCOMD	0.71	3.4	***
NB48 OMD + NCOMD	0.71	3.4	***
NB48 OMD + MADF	0.69	3.5	*
NB48 OMD + LIGA	0.72	3.4	***
PCOMD + NCOMD	0.61	3.9	***
PCOMD + MADF	0.55	4.2	NS
PCOMD + LIGA	0.61	3.9	***
NCOMD + MADF	0.55	4.2	NS
NCOMD + LIGA	0.65	3.8	***
LIGA + MADF	0.52	4.4	NS

a = To test whether there was a significant improvement over the univariate relationship.
R^2 = Adjusted for degrees of freedom.
NS = Not Significant at P = 0.05.
* = Significant at P < 0.05.
** = Significant at P < 0.01.
*** = Significant at P < 0.001.

and LIGA.

The analysis of variance test is shown in Appendix 13.

4.2.4 A Physical Method (NIR)

Figure 4.7 shows the mean NIR spectra of all silages investigated in this study. The calibration regression statistics obtained by NIR spectroscopy and the significance of between-population differences in NIR regression equations for the prediction of in vivo OMD are shown in Table 4.5. As the number of terms included in the regression increased, the R^2 value increased and the SEC decreased. The R^2 and SEC % for the NIR 8-term equation were 0.85 and 2.5 respectively.

Analysis of variance showed that at least six terms must be included in the calibration equation to avoid significant between-population differences in regression equations (see Table 4.5). Figure 4.8 shows the relationship between the OMD predicted by an 8-term multivariate regression equation and in vivo OMD for the calibration set.

The wavelengths selected by stepwise multiple linear regression to produce calibration regression equations for the prediction of in vivo OMD are shown in Table 4.6.

Figure 4.9 shows a graph of the correlation coefficients between NIR optical densities and in vivo OMD of the 122 calibration silages at each wavelength. The minimum and maximum spectral peaks indicate wavelengths where the correlation is strongest.

Analysis of variance of the regression equations for the 3 populations used in the calibration set for the NIR regression equations is shown in Appendix 10. An example of 700 Log $\frac{1}{R}$ values (data points) for one silage sample only is shown in Appendix 14. The NIR regression

FIGURE 4.7

Average NIR Spectrum of All Silages Studied in this Work.

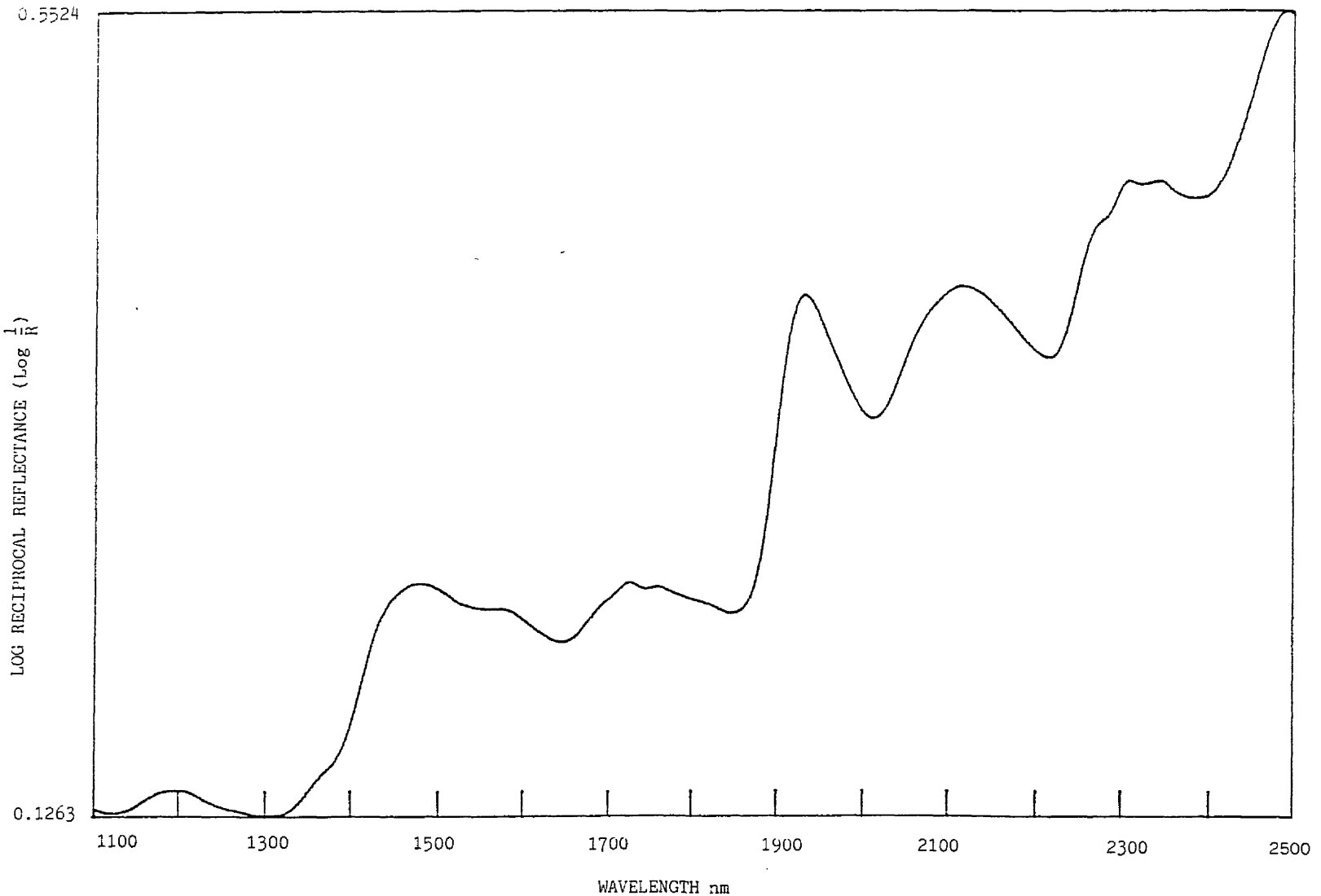


Table 4.5

Overall Population Statistics and Significance of Between-Population Differences in NIR Regression Equations for the Prediction of Silage OMD for Silages Included in the Calibration Set

No of Terms	R^2	SEC %	More Than One Line	Differences in Intercept	Differences In Slope
1	0.52	4.4	***	***	NS
2	0.64	3.8	***	***	NS
3	0.71	3.4	***	***	*
4	0.76	3.1	***	***	NS
5	0.79	2.9	***	***	NS
6	0.82	2.7	NS	-	-
7	0.83	2.6	NS	-	-
8	0.85	2.5	NS	-	-
9	0.85	2.4	NS	-	-

R^2 adjusted for degrees of freedom.

*** = Significant at $P < 0.001$
* = Significant at $P < 0.05$
NS = Not Significant at $P = 0.05$

FIGURE 4.8 RELATIONSHIP BETWEEN OMD PREDICTED BY AN 8-TERM NIR EQUATION AND IN VIVO OMD

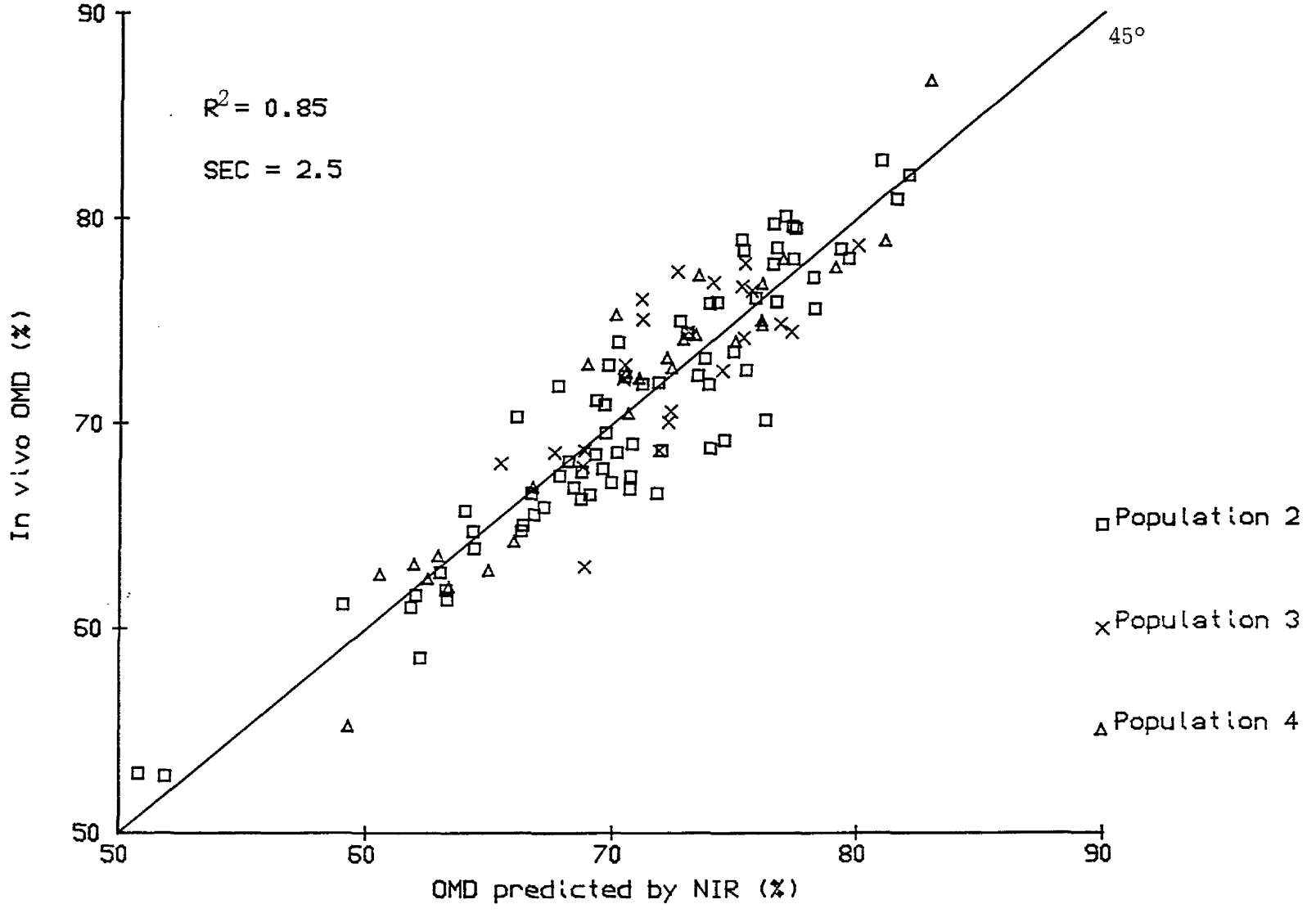


Table 4.6

Wavelength Selection by Stepwise Multiple Linear Regression for the Prediction of in vivo OMD of Silages by NIR.

No of Terms	Wavelength Selection by SMLR (λ)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1	2290"								
2	1662"	1262'							
3	1662"	1262'	1910"						
4	1662"	1274'	1850"	2254'					
4	1662"	2266"	1274'	1230"					
5	1662"	2266"	1230"	1262'	1646"				
6	1662"	2266"	1274'	1230"	1646"	1150"			
7	1662"	2266"	1230"	1274'	1646"	2426"	2354"		
8	2266"	1662"	1230"	1646"	1274'	2426"	1842'	1150"	
9	2266"	1662"	1230"	1646"	1274'	2426"	1150"	1842'	*1738"

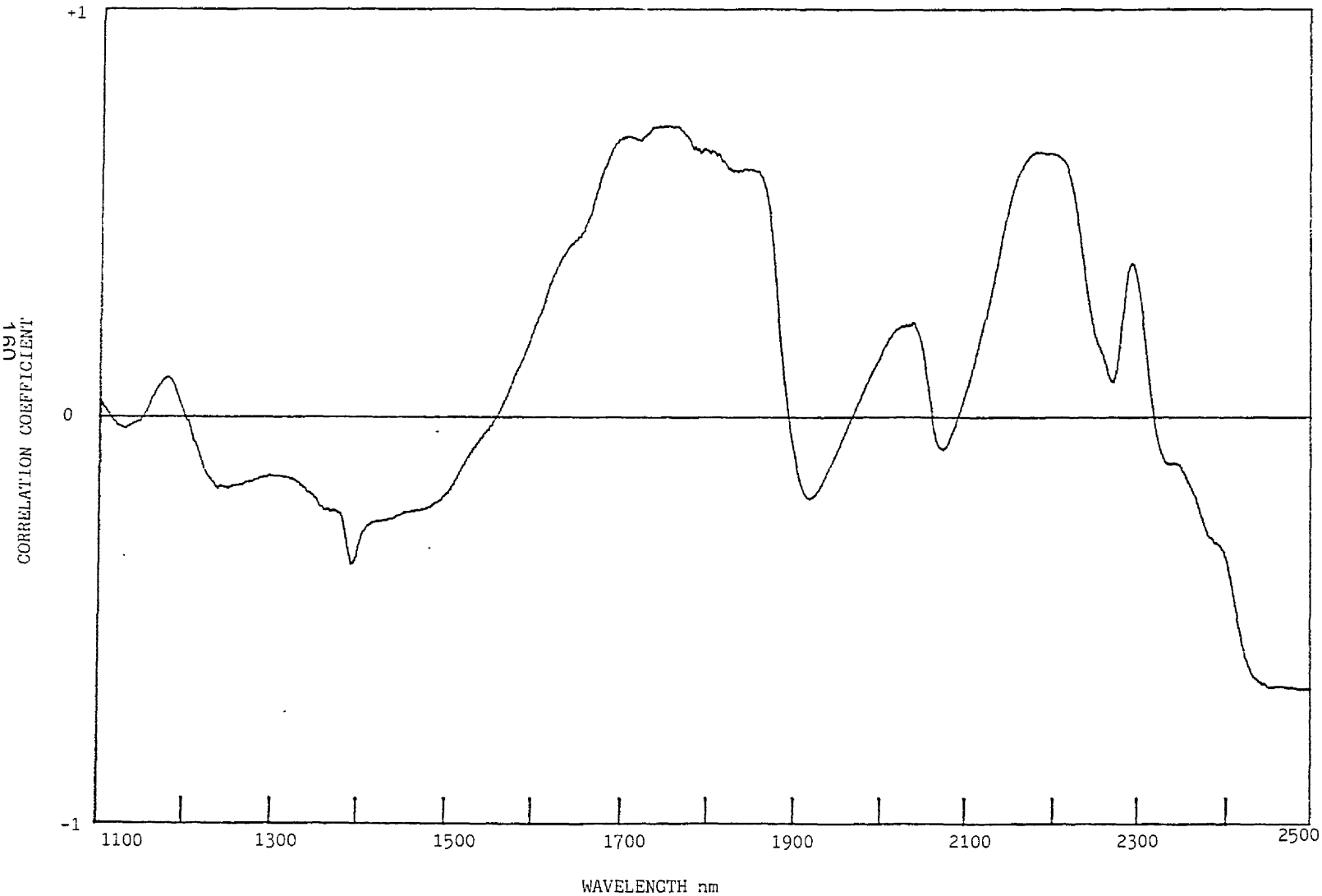
' = 1st derivative using 1, 16, 4, 4 math treatments.
" = 2nd derivative using 2, 24, 4, 4 math treatments.

Wavelengths are ordered according to its contribution to each added term.

* Wavelength which has variance ratio < 10.

FIGURE 4.9

Correlation Coefficient Between NIR Spectra of 122 Calibration Silages and In vivo OMD.



equations produced by stepwise multiple linear regression for the prediction of in vivo OMD of 122 calibration silages are shown in Appendix 15.

4.2.5 Validation of the Calibration Equations Produced by the Traditional Methods and NIR

Validation procedure is an important step to test the predictive power of any prediction equation. In this study, 48 silages were reserved for this purpose (see Table 2.9). Table 4.7 shows the results of the validation procedure for all methods tested in this study except the NB48 OMD method where data was not available (see 2.3). For the NIR calibration equations, this table shows that the SEP decreases and the R^2 increases progressively as up to six terms are included in the calibration equation. However, for the mean bias and the slope of the regression between measured and predicted in vivo OMD, there is no clear pattern in relation to the number of terms. Murray (1986) states that the criteria of selecting an NIR calibration equation for predictive purposes should be based on the following validation statistics (in vivo OMD against predicted OMD): SEP (minimum), bias (minimum) and slope (closest to unity). In addition, Westerhaus (1985c) suggests that no explanatory wavelength segment should have an F statistic of less than 10. In our study the calibration equation based on an 8-term equation best meets these criteria. Figure 4.10 shows the relationship between measured in vivo OMD of 48 validation silages and that predicted using the 8-term NIR calibration equation. The results of the NIR 8-term calibration equation produced by the stepwise multiple linear regression are shown in Table 4.8.

Of the traditional methods, the IVOMD method was the best, giving the highest R^2 and lowest SEP (see Table 4.7). The validation of the IVOMD equation with the 48 validation silages is shown graphically in Figure 4.11.

TABLE 4.7

Validation Statistics for the Prediction of *in vivo* OMD of 48 Silages not Included in the Calibration Set

Method	n	R^{2a}	SEP[b]	Bias[c]	Slope[d]
NIR	1	0.33	4.2	+0.22	0.81
	2	0.46	3.8	-0.05	0.79
	3	0.52	3.5	-0.29	0.91
	4	0.61	3.3	-0.81	0.86
	5	0.68	3.1	-0.14	0.78
	6	0.76	2.7	-1.04	0.89
	7	0.71	3.0	-0.03	0.78
	8	0.76	2.6	-0.79	0.93
	9	0.73	2.8	-0.70	0.88
MADF		0.20	5.1	-0.59	0.52
LIGA		0.14	5.3	1.18	0.48
NCOMD[e]		0.29	5.7	-3.40	0.65
PCOMD		0.40	4.7	-2.33	0.71
IVOMD		0.64	3.6	-1.85	0.89

a = R^2 adjusted for degrees of freedom.
b = SEP standard error of prediction not corrected for bias.
c = Mean bias between measured and predicted OMD.
d = Slope of regression between measured and predicted OMD.
e = NCOMD contains two missing values.
n = Number of terms in the NIR calibration equation.

FIGURE 4.10 VALIDATION OF AN 8-TERM NIR CALIBRATION EQUATION TO PREDICT IN VIVO OMD

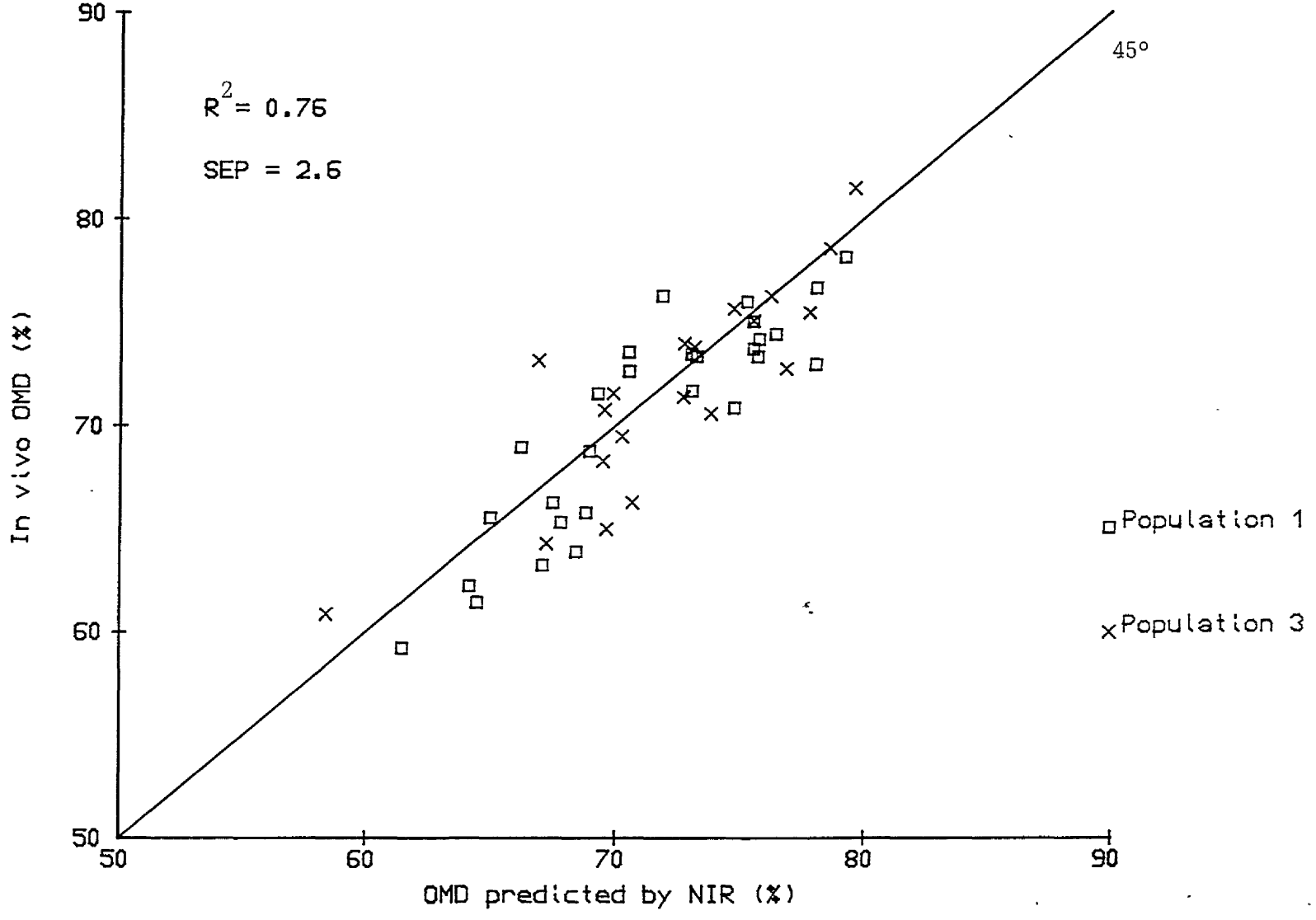


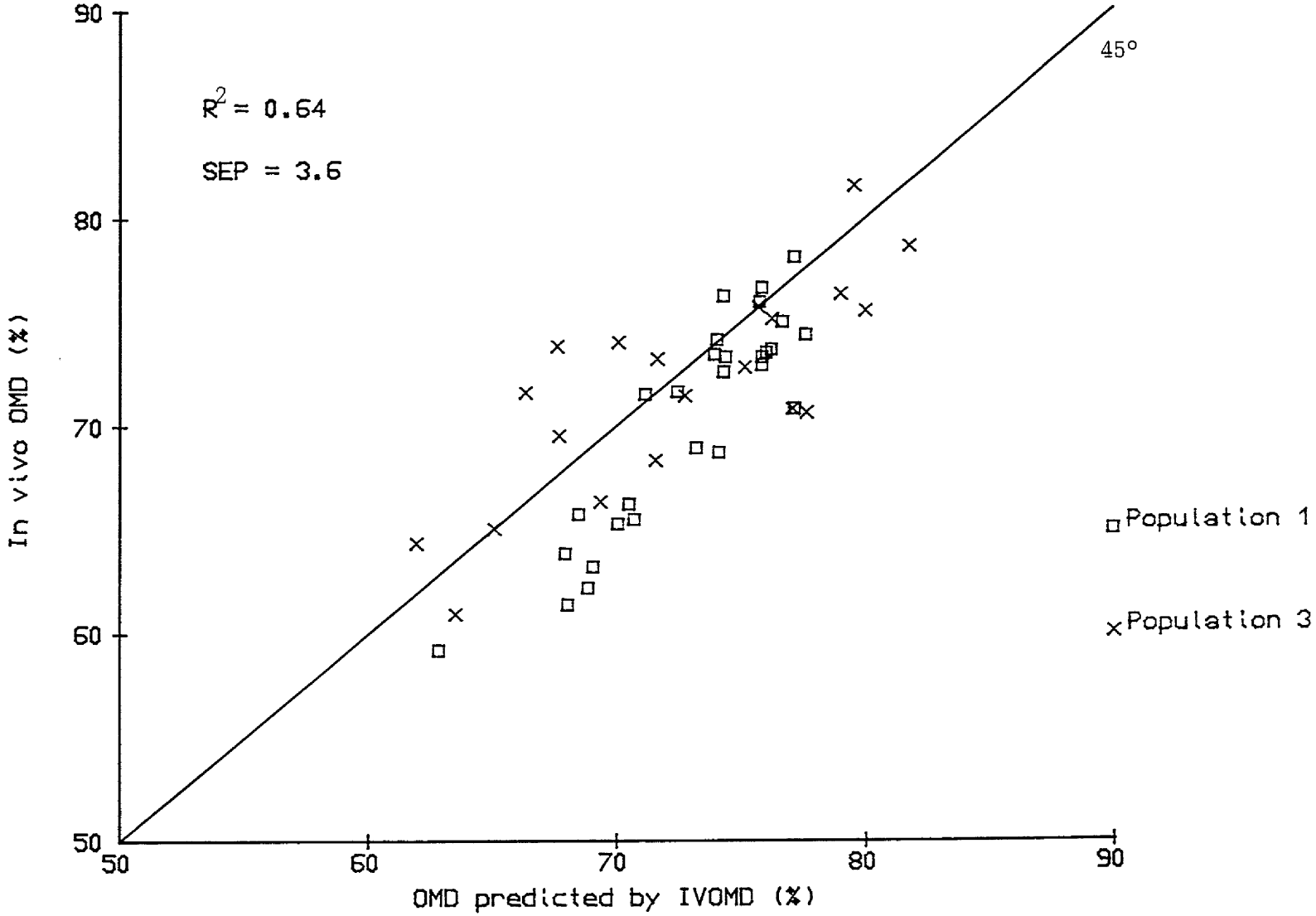
Table 4.8

NIR 8-term Equation Produced by
Stepwise Multiple Linear Regression

	Coefficient	F	Wavelength	Math Treatment
B(0) =	63.973			
B(1) =	-4311.132	15.47	1842	1 16 4 4
B(2) =	-6062.110	35.83	1274	1 16 4 4
B(3) =	3244.727	93.18	1662	2 24 4 4
B(4) =	1027.880	104.43	2266	2 24 4 4
B(5) =	-1716.427	43.90	1646	2 24 4 4
B(6) =	-4355.047	46.93	1230	2 24 4 4
B(7) =	1921.474	23.83	2426	2 24 4 4
B(8) =	3245.068	14.57	1150	2 24 4 4

SEC % = 2.5
R-squared (ADJ) = 0.85

FIGURE 4.11 VALIDATION OF IVOMD CALIBRATION EQUATION TO PREDICT IN VIVO OMD



The individual values for in vivo OMD, chemical and biological parameters for the 48 validation silages used in this study is shown in Appendix 16.

4.2.6 Validation of NIR 8-term Equation with Irish Silages.

Forty grass silages which had been evaluated in vivo at the Department of Agriculture for Northern Ireland were obtained as a second validation test of the selected NIR 8-term calibration equation. This validation will be considered as another rigorous exercise to test the performance of the NIR calibration equation on another set of UK silages obtained from a different source other than the sources studied in present work. The validation test is shown in Figure 4.12. The bias and slope between in vivo OMD measured and the OMD predicted were 1.74 and 0.99 respectively.

4.2.7 Calibration of NIR with IVOMD.

In the present work, it was felt necessary to investigate the possibility of calibrating the NIR with the IVOMD method. The same 122 calibration silages and the 48 validation silages were used in this case. The results of the calibration and validation statistics are shown in Tables 4.9 and 4.10 respectively. Based on the validation statistics, the NIR 5-term calibration equation may be the best equation to predict the IVOMD of 122 calibration silages. The R^2 and SEC % for the calibration equation were 0.83 and 2.4 respectively. The R^2, SEP %, bias and slope for the validation equation were 0.71, 2.8, 0.74 and 0.89 respectively. The relationship between measured IVOMD and that predicted by the 5-term equation for the calibration set is shown in Figure 4.13. Figure 4.14 shows the relationship between the measured IVOMD of the 48 validation silages and that predicted using the 5-term NIR calibration equation.

FIGURE 4.12 VALIDATION OF AN 8-TERM IN VIVO NIR CALIBRATION EQUATION TO PREDICT IN VIVO OMD OF 40 IRISH SILAGES.

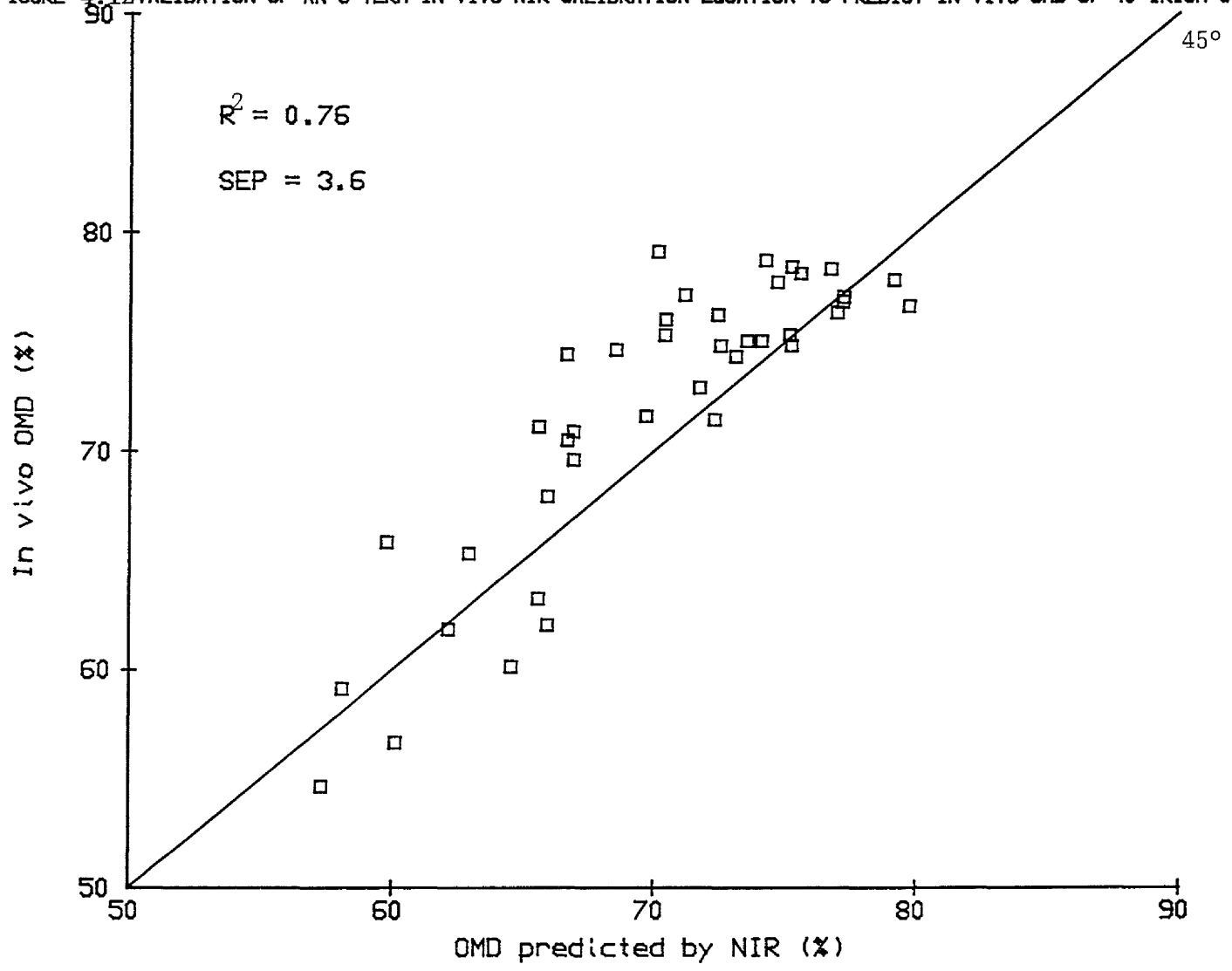


Table 4.9

Calibration Statistics Produced by Stepwise Multiple Linear Regression for the Prediction of IVOMD by NIR (n = 122)

No of Terms	R^2	SEC %
1	0.50	4.2
2	0.66	3.5
3	0.77	2.8
4	0.80	2.6
5	0.83	2.4
6	0.84	2.3
7	0.86	2.2
8	0.87	2.1
9	0.88	2.1

R^2 adjusted for degrees of freedom.

Table 4.10

Validation Statistics for the Prediction of IVOMD of 48 Silages not Included in the Calibration Set

No of Terms	R^{2a}	SEP %[b]	BIAS[c]	Slope[d]
1	0.14	5.3	2.0	0.57
2	0.54	4.3	2.7	0.99
3	0.65	2.9	0.18	0.97
4	0.55	3.4	0.48	0.86
5	0.71	2.8	0.74	0.89
6	0.65	3.0	0.24	0.89
7	0.64	3.2	-0.84	0.85
8	0.65	3.2	-0.87	0.83
9	0.65	3.4	-1.21	0.80

a = R^2 adjusted for degrees of freedom.
b = SEP standard error of prediction not corrected for bias.
c = Mean bias between measured and predicted IVOMD.
d = Slope of regression between measured and predicted IVOMD.

FIGURE 4.13 RELATIONSHIP BETWEEN IVOMD PREDICTED BY 5-TERM NIR EQUATION AND IVOMD

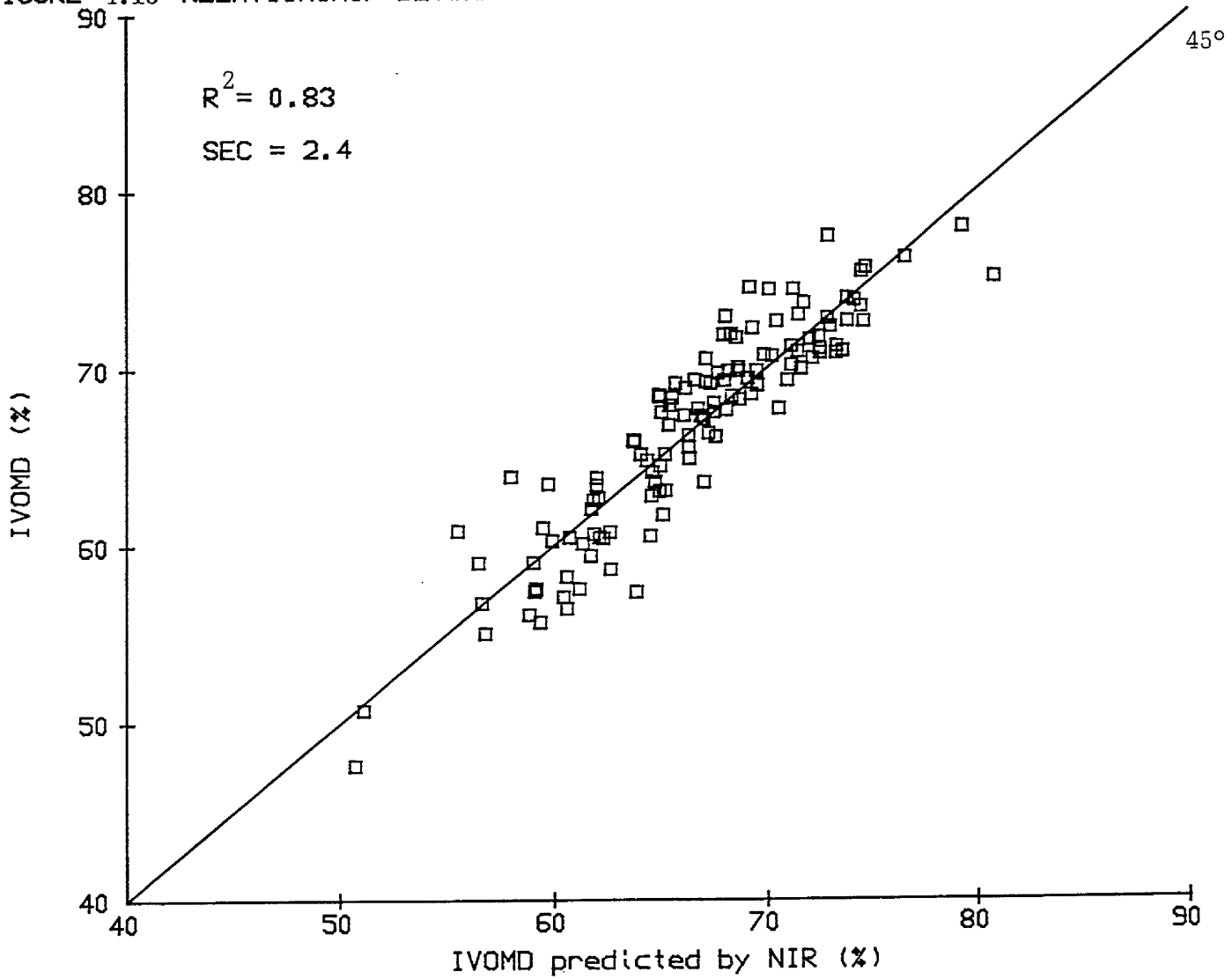
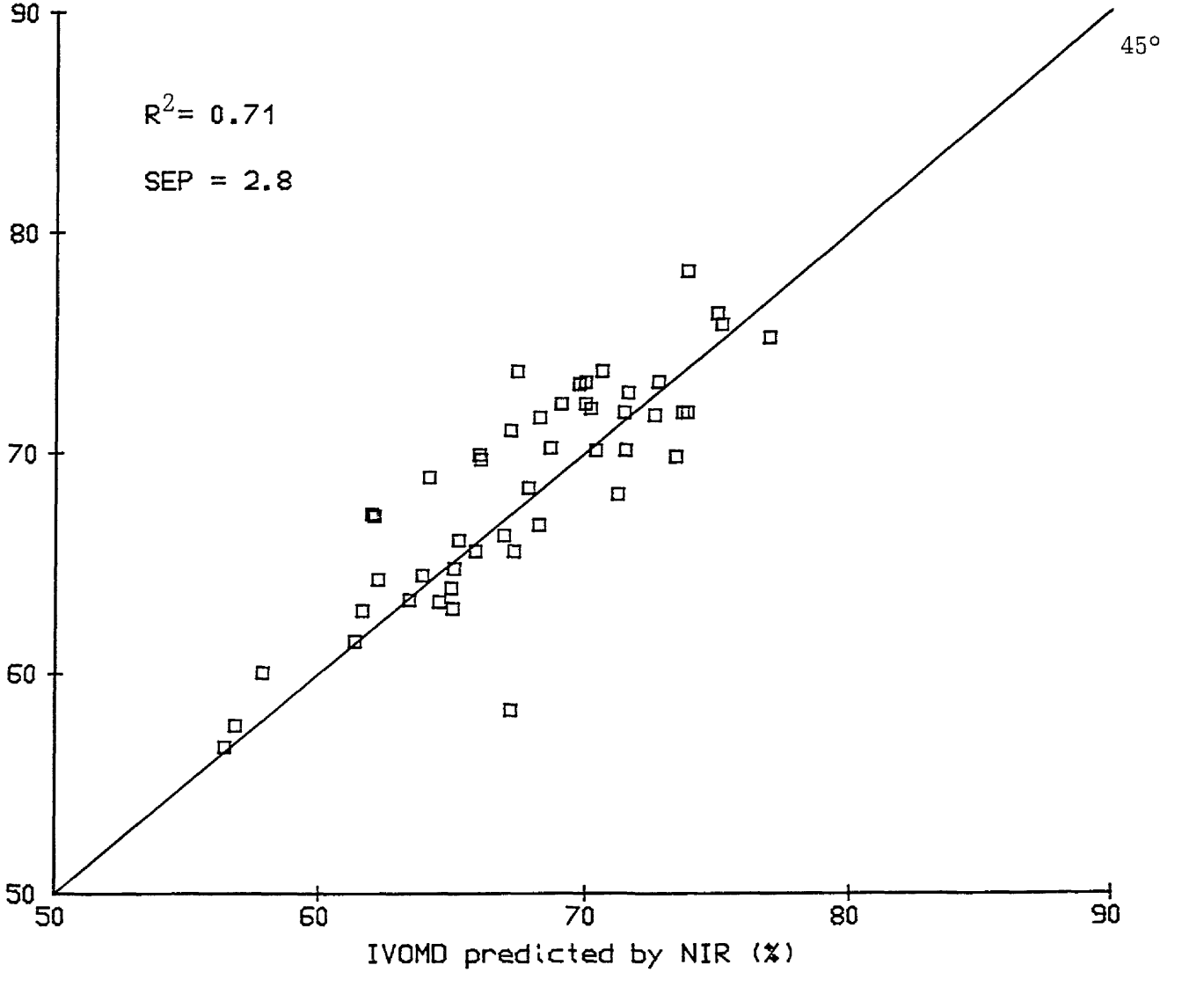


FIGURE 4.14 VALIDATION OF 5-TERM NIR CALIBRATION EQUATION TO PREDICT IVOMD



The NIR regression equations produced by stepwise multiple linear regression for the prediction of IVOMD of the 122 calibration silages are shown in Appendix 17.

4.3 Discussion

The production of well fermented grass is an important step towards making a good quality silage. pH and volatile N as a percentage of total N are two main indicators of the quality of preservation. The mean pH and volatile N as a percentage of total N for all silages studied in this thesis were 4.2 and 11.5% respectively (see Table 2.3). The achievement of a pH value of 4.2 for unwilted silage was found to be essential for successful preservation (McDonald and Whittenbury, 1973). A recent publication by ADAS (ADAS Paper No 3148, 1988) indicated that a volatile N as a percentage of total N ranging from 8-13% will yield a satisfactory fermentation quality. All silages studied in present work were found to be well preserved as indicated by these parameters.

Murray (1986) stressed that a calibration set with a wide range of analytical data is the most important criterion for successful NIR calibration. The calibration silages studied in this thesis have DOMD values spanning a range of 47.5 to 81.2%, a mean of 64.7% and standard deviation of 5.7%. These figures were found to be compatible with farmers silages analysed in the 1988/89 silage season by the Scottish Agricultural Colleges Advisory Service (see Table 4.11). This suggests that the DOMD values for the calibration silages used in this work were wide enough to cover the range which is likely to be encountered in practice.

4.3.1 Chemical Methods

The use of MADF to predict <u>in vivo</u> OMD of grass silages gave an overall R^2 and RSD% of 0.34 and 5.1 respectively

Table 4.11

The Distribution of DOMD Values for SAC Advisory Service Silages* and the Calibration Silages used in this Study

	SAC Silages** n = 2125	Calibration Silages n = 122
Mean	64.10	64.7
Min	46.90	47.5
Max	76.80	81.2
SD	4.45	5.7

* The DOMD% values are predicted.

** SAC Silages figures were compiled by Collin Jessiman of North of Scotland College of Agriculture for the 1988/89 season.

(Table 4.1 and Figure 4.1). Wilkins (1981) reported that the correlation between MADF and *in vivo* digestibility was not as close with silages as with other classes of forages. Alderman *et al* (1971) quoted an R^2 of 0.28 for the relationship of *in vivo* DOMD and MADF for 45 grass silages. However, better prediction was reported by Barber *et al* (1984) when they quoted an R^2 and RSD% of 0.48 and 3.4 for 80 silages. A further improved prediction was reported by Givens *et al* (1989a) when they quoted an R^2 and SEP% of 0.57 and 4.1 respectively when predicting *in vivo* OMD of 124 grass silages.

Analysis of variance of the population regression lines obtained in the present study revealed that the relationship between *in vivo* OMD and MADF yield significant ($P < 0.001$) differences in the regression equations and these differences were caused by a significant difference in intercepts ($P < 0.001$) [Table 4.1 and Figure 4.1]. Moreover, each population regression line intercept is significantly different from the other (Table 4.2). These differences were obtained even though the MADF values used were all obtained from one laboratory. Thus, the difference between intercepts is not explained by between-laboratory differences in the measurement of MADF.

These results confirm the observation by Barber *et al* (1984) that the MADF relationship with *in vivo* DOMD for population 3 differs substantially from that of population 2. Barber *et al* (1984) suggested these differences might be caused by geographical differences in the relationship between the measurements made using MADF and *in vivo* digestibility.

Lignin is considered to be a major factor limiting forage digestibility. For the prediction of 122 *in vivo* grass silages, LIGA method gave an R^2 and RSD% of 0.52 and

4.4% respectively (Table 4.1 and Figure 4.2). Morrison (1973) found an R^2 of 0.71 for the prediction of *in vivo* DOMD by the LIGA method for 20 grass silages. A more comprehensive study by Givens *et al* (1989a) quoted an R^2 and SEP% of 0.63 and 3.7% respectively. While these results may suggest an improvement in prediction over the results reported in this work however, our calibration samples are more heterogenous than the samples used by these workers.

As with MADF, the LIGA method relationship with *in vivo* OMD gave regression lines which differ significantly in intercepts ($P < 0.001$) [Table 4.1 and Figure 4.2]. For populations 2 and 4, LIGA predicted *in vivo* OMD with an R^2 and RSD% of 0.73, 3.4% and 0.89, 2.3% respectively, but the regression line obtained for population 3 differed significantly in intercept from the others (Table 4.2) and its precision was rather poor (R^2 = 0.20, RSD% = 3.6). These differences might be attributed to the presence of unknown proportion of clover silages in population 3. Minson (1982) commented about the large differences in the regression equations obtained for grasses and legumes when relating *in vivo* digestibility to the lignin in the food. Sullivan (1959) reported that separate regression equations were needed for grasses and legumes when the acid insoluble lignin method was used to predict *in vivo* DMD.

4.3.2 **Biological Methods**

The use of NCOMD to predict *in vivo* digestibility gave an R^2 and RSD% of 0.54 and 4.3 respectively (Table 4.1 and Figure 4.3). Several workers predicted *in vivo* DOMD by the NCD method. Downman and Collins (1982) reported an R^2 and RSD% of 0.71 and 1.9% respectively for 16 grass silages. For 68 grass silages, Barber *et al* (1984) quoted an R^2 and RSD% of 0.63 and 2.9% respectively. For 85 grass silages, Givens *et al* (1989a) reported an R^2 and

SEP% of 0.65 and 3.9% respectively. As has been said with the LIGA method, the improvement in prediction may be attributed to the homogenity of samples used by those workers compared with the samples used in this study.

The PCOMD method gave an R^2 and RSD% of 0.55 and 4.2% (Table 4.1 and Figure 4.4). This precision of prediction is similar to that found by the NCOMD method. However for PCOMD, the individual regressions for populations 2, 3 and 4 gave an R^2 and RSD% of 0.79, 3.0%; 0.53, 2.8% and 0.82, 3.0% respectively, whereas for NCOMD it was 0.62, 4.1%; 0.28, 3.4% and 0.57, 4.8% respectively (Table 4.1). This suggests that the PCOMD method would be adequate for predictive purposes compared to NCOMD. However, the regression equations obtained differ significantly ($P < 0.001$) in both intercepts and slopes (Table 4.1 and Figure 4.4) and each population line is significantly different from the other (Table 4.2). Terry et al (1978) obtained a similar result when they compared the precision of the rumen liquor and pepsin-cellulase methods in predicting in vivo DMD of 48 grasses and 25 legumes. They showed that one regression equation can be used for both grasses and legumes when using the rumen liquor method, whereas the pepsin-cellulase method failed to satisfy this criteria.

The in situ method is an old technique and has been recommended recently to evaluate feedstuffs (Ørskov et al, 1980). However, a major limitation of this technique is the variations between bag results which make it very difficult to standardise (see Chapter 1, in situ method). In this work, an improved bag washing technique was developed and adopted (Chapter 3) and an internal standard was included with each bag incubated inside the rumen so that variations between rumens (between sheep and with time) could be adjusted (see 2.2.8.2). Table 4.12 shows the coefficient of variation and the regression

Table 4.12

Coefficient of Variation and Regression Statistics for NB48 OMD Uncorrected and Corrected for the Internal Standard

Parameters	CV%	Regression Statistics	
		R^2	RSD%
NB48 OMD Uncorrected	3.6	0.68	3.57
NB48 OMD Corrected	2.9	0.67	3.61

statistics obtained for both NB48 OMD uncorrected and corrected for the internal standard. This table indicates that while the CV% decreased (3.6 for uncorrected; 2.9 for corrected), the overall prediction of in vivo OMD of the 122 calibration silages was slightly lower (R^2 for uncorrected = 0.68; R^2 for corrected = 0.67). Accordingly, the NB48 OMD uncorrected values were used in this work. However, it was felt that where the in situ method may potentially be applied (for example as an intermediate standard), the inclusion of an internal standard would be useful. Mehrez and Ørskov (1977) reported that the greatest source of variation for substrate disappearance from the bags was that between sheep followed by between days and the least between bags. In such cases, the inclusion of an internal standard with bags incubated in the rumen would be of value so that these variations can be corrected for.

The length of incubation period may influence in situ results. However, this depends on the material being incubated. For forages, the incubation period tends to be long so that complete digestion of the samples can be obtained. Van Keuren and Heinmann (1962) reported large variations between bags at short incubation times for various forages. These variations diminished as the incubation times increased up to 72 hours. Ørskov et al (1980) recommended an incubation time of 24-60 hours for medium quality forages and 48-72 hours for poor quality roughages. These times are required to reach the asymptote (potential degradation).

In forage evaluation, longer incubation times were found to be a better predictor of in vivo digestibility. At the start of this study, 72 dried silages were subjected to a polyester bag study using 6 and 48 hours incubation time (Kridis et al, 1987). A more accurate prediction of in vivo OMD was found when 48 hours incubation was used

compared to 6 hours. These findings are in agreement with those reported by Chenost et al (1970) when they found that in vivo OMD of various forages was better correlated with 48 hours than with 12 and 24 hours incubation time. The 48 hours incubation time has been subsequently used by Aerts et al (1977) and Cottyn et al (1986). Therefore, that an incubation time of 48 hours was used in this study.

The use of nylon bags to predict in vivo OMD of the 122 calibration silages gave an R^2 and RSD% of 0.68 and 3.6% respectively (Table 4.1 and Figure 4.5). Few reports have been cited about the use of this method in silage evaluation. Aerts et al (1977) quoted an R^2 and RSD% of 0.79 and 3.4% respectively for the prediction of in vivo OMD of 56 grass silages. A more comprehensive study reported by Cottyn et al (1986) quoted an R^2 and CV% of 0.85 and 3.2% respectively for the prediction of in vivo OMD of 100 grass silages. However, the bags in these reports are further incubated for 48 hours in acid pepsin after removal from the rumen which may explain their precision.

Of the traditional methods, the IVOMD method gave the best prediction of in vivo OMD (R^2 and RSD% of 0.74 and 3.2 respectively) [Table 4.1 and Figure 4.6]. This method has been used extensively in forage evaluation and has been cited to be the best predictor of in vivo digestibility over a variety of other techniques. O'Shea et al (1972) found that the IVOMD method gave the best prediction of in vivo OMD of 31 grass silages over various techniques (R^2 = 0.66). For 20 grass silages, Morrison (1973) concluded that IVOMD was a better predictor of in vivo OMD than the LIGA method (R^2 = 0.74 compared to R^2 = 0.71 respectively). More recently, Givens et al (1989a) compared the ability of 11 laboratory methods to predict in vivo OMD and concluded that the IVOMD method

gave the highest R^2 and lowest SEP% of 0.76 and 3.1 respectively for 117 grass silages.

In contrast with the chemical and enzymic methods reported previously, both the NB48 OMD and IVOMD methods can describe all populations by a single regression equation (Table 4.1, Figures 4.5 and 4.6 respectively). These findings are in agreement with those reported by Terry et al (1978). They reported that for 48 grasses and 25 legumes, one regression equation can be used for both grasses and legumes when the in vitro DMD method was used to predict in vivo DMD.

4.3.3 The Use of Bivariate Relationships to Predict in vivo OMD

The results shown in Table 4.4 suggested that the combination of predictors in bivariate relationships can improve the prediction of in vivo OMD over the univariate relationship shown in Table 4.1. This is so for all possible bivariate relationships with the exception of the bivariate relationships of MADF with IVOMD, PCOMD, NCOMD and LIGA. The significant improvements in prediction over the univariate relationships suggest that each predictor of the bivariate measures a different aspect of digestion. However, the inclusion of other predictors with MADF failed to improve the prediction with the exception of the NB48 OMD + MADF bivariate relationship. This suggests that the IVOMD, PCOMD, NCOMD and LIGA methods measure feed characteristics which include those measured by MADF. These findings are in agreement with those reported by Kridis et al (1987) and Givens et al (1989a) where they indicated significant improvements in the prediction of in vivo OMD of grass silages when the major predictors of digestibility were combined in a bivariate relationship.

Although these results may suggest significant improvements in the prediction of *in vivo* OMD, this improvement does not provide sufficient justification for the determination of two methods simultaneously. The cost involved is a major setback against such a proposition for routine advisory work.

4.3.4 A Physical Method (NIR)

The use of NIR 8-term equation gave an R^2 and SEC % of 0.85 and 2.5 respectively (Table 4.1 and Figure 4.8). Analysis of variance showed that at least six terms must be included in the calibration equation to avoid significant between-population differences in the regression equations (Table 4.5).

The selection of an 8-term calibration equation was based on the validation procedure reported by Westerhaus (1985c) and Murray (1986) [see 4.2.5]. The validation process is a true criteria of the performance of equations selected by stepwise multiple linear regression and found to be essential to prevent overfitting, ie: the inclusion of random noise. The SEP will decrease to a point and then increase when overfitting occurs (Table 4.7). This is in contrast with the SEC which decreases progressively with each added term (Table 4.5) [Westerhaus, 1985b]. Another useful validation statistic is the slope of regression between *in vivo* OMD measured and *in vivo* OMD predicted (Table 4.7) [Westerhaus, 1985b; Murray, 1986]. The slope should be as close to 1.0 as possible. A large deviation from 1.0 will imply that high and low values will be over or under-estimated (Westerhaus, 1985b). The SEP% and slope for selected 8-term calibration equations were 2.6% and 0.93 respectively (Table 4.7 and Figure 4.10).

The F-statistic is an additional important regression statistic produced by stepwise multiple linear regression

(see Table 2.7). It indicates how much the given term contributed to the equation. Small F-values signify little contribution to the equation, except to fit random errors. Westerhaus (1985c) suggests that no explanatory wavelength should have an F-statistic less than 10 in the calibration equation. The selected 8-term equation reported in this study meets this criteria (Table 4.8).

The use of several terms in the calibration equation to predict digestibility have been reported previously. Norris et al (1976) used a 9-term calibration equation to predict in vivo DMD of 76 various forages. They reported an R^2 and SEC% of 0.78 and 3.6% respectively. Abrams et al (1987) also used a 9-term equation to predict the IVOMD of 100 forages with SEP% and bias of 2.4% and 0.12 respectively. It can be argued that several terms may be required to predict complex parameters like digestibility (Murray et al, 1987). This stems from the fact that a complete digestion process occurring inside the animal may require more spectral information to unravel its complexity. Additionally, there is no danger of using several terms in the calibration equation as long as the validation procedure is conducted adequately. Therefore, our selected 8-term equation would be perfectly valid for this type of study.

The NIR 8-term calibration equation was used to predict another unknown silage population which has been evaluated in vivo at the Department of Agriculture for Northern Ireland. These silages gave a validation statistic of R^2, SEP %, bias and slope of 0.76, 3.6, 1.74 and 0.99 respectively (Figure 4.12). This validation exercise will be considered another rigorous test of the validity of the selected 8-term calibration equation in predicting another set of UK silages obtained from a different source.

Table 4.6 shows the centred wavelengths which explain the 122 in vivo silages. It is clear that the second derivative segments centred at 1162 and 2266 nm were always the primary explanatory wavelengths followed by 1230 nm (Figure 4.9). The choice of 1662" nm and 2266" nm are well established to correlate favourably to digestibility. Norris et al (1976) found 1666 nm was the first explanatory wavelength and 2266 nm was the third explanatory wavelength to be selected for the prediction of in vivo DMD of 76 various forages by NIR. Shenk et al (1979) found the 1641 nm region was important in the prediction of IVDMD. Murray et al (1987) found the 1662" nm was the primary explanatory wavelength and 2266" nm was the third explanatory wavelength to be selected for the prediction of in vivo OMD of 72 grass silages. In the same study, the selection of 1662" nm as the primary explanatory wavelength was supported by NIR calibration with nylon bag 48 hours digestibility.

Out of the 8 explanatory wavelengths found in our 8-term calibration equation, the 1662" and 2266" nm regions will explain 60% of the variance in in vivo OMD prediction. This suggests the importance of these two regions in the prediction of digestibility.

The 1662 nm wavelength, using a 2, 24, 4, 4 math treatment (see 2.2.9.1), will include the change in Log $\frac{1}{R}$ values from 1634 to 1688 nm. This segment was found to measure the aromatic CH at 1680 nm which belongs to Lignin (Murray et al, 1987). The 2266" nm region may be associated to methylene groups ($-CH_2-$) and to the methoxyl groups (CH_3-O-) which are frequently found as ether side groups of Lignin. These two regions were always selected as the primary and secondary wavelengths for the prediction of acetyl bromide Lignin by NIR. Therefore, it is likely that these two regions may arise directly from Lignin (Murray et al, 1987).

4.3.5 Calibration of NIR with IVOMD

NIR calibration with *in vivo* measurements to predict digestibility is the ultimate target so that this technology can potentially be applied for practical purposes. However, using animals to measure digestibility is expensive and requires special facilities, and therefore cannot be used on a large number of samples on places which do not possess feed evaluation units. For these reasons NIR calibration with *in vivo* measurements will not be possible for many evaluation programs.

In this study, the IVOMD method was found to be the best predictor of *in vivo* OMD when compared to other traditional methods. It gave the best calibration statistics (Table 4.1), described all populations by a single regression equation (Figure 4.6) and gave the highest R^2 and lowest SEP in a validation test (Table 4.7 and Figure 4.11). Therefore, it was felt that this technique may have a potential value as an intermediate standard to replace the expensive *in vivo* determinations.

The results shown in Table 4.8 and Figure 4.13 indicated the precision of NIR to predict IVOMD. The 5-term calibration equation gave an R^2 and SEC % of 0.83 and 2.4 respectively. This equation was selected on the basis of the validation procedure shown in Table 4.9, which gave an R^2, SEP%, bias and slope of 0.71, 2.8%, +0.74 and 0.89 respectively (Figure 4.14).

The use of the IVOMD method to calibrate NIR has been reported previously in the literature. Norris *et al* (1976) reported an R^2 and SEC% of 0.90 and 2.6% respectively to predict IVDMD of 76 forages using 9-term equation. Brown and Moore (1987) reported an R^2 and SEC% of 0.89 and 2.3% respectively to predict IVOMD of 83 forage samples using 6-term equation. All these workers indicated successful NIR calibration with IVOMD.

According to these results, the IVOMD method is probably a successful candidate for NIR calibration. This suggests that the predicted IVOMD values can be used either to calculate in vivo digestibility using appropriate regression equations or its absolute values can be used to rank forages in many plant breeding programs.

4.4 Comparison of Methods

For many years, the search for a simple, economical and accurate laboratory method to predict the nutritive value of grass silages has been pursued vigorously by advisory services in the UK. Not surprisingly, advisers recognised the difficulty of predicting the nutritive value of grass silages when compared to other classes of forages. Factors like the use of additives, wilting and more importantly, the extent of fermentation which takes place during ensilage, may lead to variation in the nutritive value of the resulting silage. In fact, this variation is likely to be greater when it comes to real farm practices. These factors, coupled with the pre-harvest conditions like species, geographical location and stage of maturity, makes silage evaluation increasingly more difficult.

Many attempts have been made to compare the various laboratory methods in order to determine the most reliable method(s) of predicting the nutritive value of silages. Notably, the comparisons of Aerts et al (1977), Barber et al (1984), Cottyn et al (1986) and Givens et al (1989a) were the most comprehensive. These comparisons were previously mentioned in Chapter 1, Section 2.

For the purpose of this comparison, the methods studied will be compared with regard to two aspects:

a) Prediction accuracy of in vivo OMD and
b) The differences in population regression equations as

assessed by analysis of variance.

a) Prediction Accuracy

The comparisons performed in this work indicated clearly the superiority of the rumen liquor methods (NB48 OMD, IVOMD) when compared to methods involving the use of enzymes (NCOMD, PCOMD) or chemical methods (MADF, LIGA) (Table 4.1). This prediction superiority may be related to the sensitivity of the rumen micro-organisms to the known and unknown factors which might affect digestibility. The other methods will not recognise such unknown factors, since they rely heavily on measuring the major parameters which are found to affect digestibility (ie: cellulose, hemicellulose and lignin) [Aerts et al, 1977; Cottyn et al, 1986; McQueen, 1986; Givens et al, 1989a].

The precision of the NB48 OMD method was found to be less than in the case of the IVOMD method (Table 4.1). The observation may be explained by the uncontrolled variation associated with the NB48 OMD method, particularly losses of fines from the bags (Lindberg and Knutson, 1981) [see Chapter 1, Section 2, in situ method]. Although the samples were sieved through a 45 µm sieve, it was not possible to extract all fines present in the samples. I believe that the NB48 OMD method may give a better prediction if fresh minced silages were used, however such material was not available for this study.

Compared to the IVOMD method, the NB48 OMD method profits from a lesser variation due to rumen liquor activity, since bags are incubated in the rumen itself. In addition, with the NB48 OMD

method the rumen liquor does not undergo any treatment which might affect the rumen micro-organisms activity.

While the NB48 OMD and IVOMD methods improved the prediction precision, these methods, however, were found to be difficult to standardise (Barnes, 1973), slow and expensive, their use demands free access to fistulated animals and the use of surgically prepared animals is strongly objected to by the animal welfare organisations. These limitations make these methods unfavourable for routine use.

These difficulties have led to a search for a method which could utilise the cellulase enzyme as a means of replacing the rumen liquor. The methods of PCOMD and NCOMD were such a development (Jones and Hayward, 1975; Dowman and Collins, 1982). These techniques were found to predict digestibility of silage with relative success, however they were not as precise as the rumen liquor methods (Givens et al, 1989a). This conclusion is in agreement with findings reported in this work (Table 4.1).

For all 122 silages, the PCOMD regression performed slightly better than the regression of NCOMD. In addition, the individual regressions for the three populations were exceptionally better for PCOMD than the NCOMD (Table 4.1). This suggests that PCOMD method would be adequate for predictive purposes for grasses grown in one geographical location. These findings are in agreement with those reported by McLeod and Minson (1982) for grasses and legumes and by Barber et al (1984) and Givens et al (1989a) for grass silages.

McLeod and Minson (1982) commented about the better accuracy of the pepsin-cellulase method when compared to the neutral detergent cellulase method. For the prediction of in vivo DMD of 50 grasses, the two methods gave an RSD% of 2.6 and 3.3 respectively. Those workers further investigated this discrepancy and concluded that the pretreatment with neutral detergent introduced more analytical error than the pretreatment with acid pepsin, which accounts for the lesser prediction precision for the neutral detergent cellulase method.

The cellulase enzymes method benefits from its speed, precision and convenience relative to the rumen liquor method. However, the activity of the enzyme between batches and from different suppliers requires constant monitoring so that the method's accuracy can be maintained (Clark and Beard, 1977).

Plant cell wall components are the major limiting factors which affect digestibility of plants and the methods of MADF and LIGA were used to extract these important fractions. These methods have been used extensively to predict digestibility of grass silages and other forages (Barber et al, 1984; Givens, 1986; Murray, 1986), and speed, reproducibility and cheapness were the most attractive features of these techniques. However, their prediction precision was found to be insufficient. In this work, MADF and LIGA were inferior in prediction precision when compared to other methods (Table 4.1). Although the LIGA method performed considerably better than the MADF method, for population 3 the prediction accuracy was rather poor. Possible explanation of this is the presence in many of population 3 of an unknown proportion of clover which suggests the inaccuracies

of various lignin techniques when used to predict the digestibility of a mixture of grasses and legumes (Minson, 1982).

Of the methods studied in this work, NIR was the most precise predictor of in vivo digestibility of grass silages (Table 4.1). The ability of this technique to unravel the compositional information which might relate to digestibility and then the use of this information in a multi-variate way, is primarily behind its successful prediction. However, this technique has to be carefully calibrated and the resultant equations must be adequately validated, otherwise meaningless results may occur. The speed, accuracy, precision and unit cost of analysis (see Chapter 7) make this technique attractive for routine use.

b) The Differences in Population Regression Equations as Assessed by Analysis of Variance

The methods of MADF, LIGA, NCOMD and PCOMD gave between-population differences in the regression equations obtained (Table 4.1 and 4.2). These differences cannot be explained by between-laboratory differences, since each method was determined in one laboratory.

For the MADF and PCOMD methods, each population regression line is significantly different from the other in intercepts and in intercepts and slopes respectively (Table 4.2, Figures 4.1 and 4.4 respectively), whereas for LIGA it was only the population 3 regression line intercept which was significantly different from either population 2 or 4 (Figure 4.2, Table 4.2). Possible explanations of these differences are the combination effects of geographical locations and the heterogeneity of the

Rowett Research Institute silages (population 3), since this population contained unknown proportions of legumes.

Aldrich and Dent (1967) have shown an indication of higher digestibility at northern latitudes when compared to southern latitudes in the UK in cocksfoot. Deinum et al (1968) also found that digestibility of perennial ryegrass was significantly influenced by both light intensity and environmental temperature. These findings suggest that the digestibility - laboratory method relationship may be affected by geographical site. These explanations have been reported by other workers (Osbourn, 1978; Barber et al, 1984; Givens et al, 1989b).

Various workers indicated the need for separate regression equations for grasses and legumes when digestibility was predicted by MADF (Clancy and Wilson, 1966), lignin (Minson, 1982) and pepsin-cellulase (Terry et al, 1978). All these workers showed that the prediction precision was improved when grass and legume regression equations were considered separately. These findings are in agreement with those reported in this work.

The differences in population regression lines for the NCOMD method were somewhat different from the other methods. Population 4 was only significantly different from population 2, but population 3 was not significantly different from either population 2 or 4 (Figure 4.3, Table 4.2). This suggests that the NCOMD was possibly not influenced by either geographical region or the diversity of the RRI silages, since population 3 was not significantly different from population 2, and yet they were from

different geographical regions. Givens et al (1989b) found a similar conclusion when they reported that the neutral detergent cellulase method was not influenced by county of harvest when used to predict the digestibility of grasses grown throughout England and Wales. However, Givens et al (1989b) showed that the pepsin-cellulase method was also not influenced by county of harvest, an observation which disagrees with the findings reported in our study.

In contrast with the methods mentioned previously, the NB48 OMD, IVOMD and NIR methods can describe the three populations of silages by a single regression line (Table 4.1). This implies that these methods were not affected by either the geographical region, or by the heterogeneity of the RRI silages and so the methods are robust enough to be descriptors of in vivo OMD of grass silages. This agrees well with the findings of Terry et al (1978) for samples of dried forages using the IVOMD method.

4.5 Conclusions

In vivo OMD of grass silages was not accurately predicted by the MADF, LIGA, NCOMD and PCOMD methods. All gave between-population differences in the regression equations obtained. This suggests that their use to evaluate silages routinely may be unjustified. The accuracy of the PCOMD method for individual regressions implies its potential usefulness for predictive purposes.

NB48 OMD, IVOMD and NIR predicted silage digestibility more accurately than any other methods tested in this work. Their application requires one single regression equation to describe all populations.

The NIR technique performs substantially better than any other method tested, provided that it is calibrated in the manner described in this work. The speed, precision, accuracy and unit cost of analysis (see Chapter 7) are the most attractive features of this technique. Its application for silage evaluation is therefore strongly recommended.

The results obtained in this work suggest the benefit of the IVOMD method as an intermediate standard either to replace the expensive in vivo determinations or as a calibration method for NIR.

CHAPTER FIVE

THE EFFECT OF EXTERNAL FACTORS IN THE RELATIONSHIP BETWEEN *IN VIVO* OMD AND ITS PREDICTORS

5.1 Introduction

Plant growth and environment play a major role in determining the nutritive value of forages and it is these two factors which could alter the composition of the standing crop and hence the resulting silage. In the literature, it has been long recognised that environmental and other external factors could influence *in vivo* - laboratory method relationship. These factors include geographical location (Aldrich and Dent, 1967; Osbourn, 1978; Barber *et al*, 1984), stage of maturity (Deinum *et al*, 1968), year of harvest (Morgan and Stakelum, 1987; Givens *et al* 1989b) and age of ley (Givens *et al*, 1989b).

In this study, it was possible to accumulate some characteristics regarding the history of the silages studied. These characteristics will be used to assess the effect of environmental and other factors on *in vivo* OMD - laboratory method relationship. The latter aspect forms the theme of this chapter.

5.2 Methods

One hundred grass silages evaluated at ADAS Feed Evaluation Unit were categorised according to the known characteristics for these silages. These characteristics are listed in Table 5.1. The number of silages used in this study are shown in Appendix 18.

The silages were grouped according to their characteristics and the regression equations which resulted from regressing *in vivo* OMD on the laboratory method for each group were assessed by analysis of variance which was then used to compare the regression equations. This allowed the detection of significant differences in intercept and/or slope according to the statistical models described in 2.4.

Table 5.1

Characteristics of Silages Studied*

	Characteristics	Number of Silages
1	Year of Harvest	
	- 1978	10
	- 1980	19
	- 1982	17
	- 1983	12
	- 1985	15
	- 1986	13
2	Method of Ensiling	
	- Clamp	72
	- Big Bale	19
3	Cut Number	
	- First Cut	48
	- Second Cut	22
	- Third Cut	7
4	Wilting Time (Hours)	
	- ≤ 12	13
	- > 12-40	52
	- > 40	13
5	Age of Ley (Years)	
	- ≤ 1	17
	- > 1-5	32
	- > 5	17
	- Permanent Pasture	16
6	Additive Application	
	- None	38
	- Add-F	20
	- Sylade + F100	21
7	Fertilisation (kgN/ha)	
	- ≤ 100	28
	- > 100-200	49
	- > 200	9

* Not all silages have the known characteristics.

5.3 Results

The effects of different characteristics of silages on the in vivo OMD - laboratory methods relationships are summarised in Table 5.2.

For both the NCOMD and IVOMD methods, the between years regression equations were significantly ($P < 0.001$) different in intercepts. The NIR 8-term Log $\frac{1}{R}$ values were less affected by years, being significant at $P < 0.05$. However, the regression equations were significantly different in slopes. Other predictors were found to be unaffected by years.

For cut number, the regression equations based on the MADF and LIGA methods were significantly ($P < 0.01$ and $P < 0.001$ respectively) different in intercepts. Other predictors were unaffected by cut number.

For age of ley, only the IVOMD method gave regression equations which differed significantly ($P < 0.05$) in intercepts, whereas other methods were unaffected.

The method of ensiling, wilting time, additives application and nitrogen fertilisation gave no effect on the regression equations for all predictors.

Analysis of variance results are shown in Appendix 19.

5.4 Discussion

The regression analyses were rather complex and therefore may need a large number of samples for each characteristic to obtain a valid conclusion. This is mainly because the variable being predicted is fairly complex (ie: digestibility) and thus an appreciable amount of error would be attached to it. Therefore, the regression equations obtained may require a sizeable

Table 5.2

The Effect of Different Silage Characteristics on The Regression Equations Between in vivo OMD and the Laboratory Methods Studied in this Work

Characteristics	Statistical Models	Predictors[1] MADF	LIGA	NCOMD	PCOMD	IVOMD	NIR 8-term
1 Year of Harvest	More than one Line	NS	NS	***	NS	***	*
	Differences in Intercepts	-	-	***	-	***	NS
	Differences in Slopes	-	-	NS	-	NS	*
2 Method of Ensiling	More than one Line	NS	NS	NS	NS	NS	NS
	Differences in Intercepts	-	-	-	-	-	-
	Differences in Slopes	-	-	-	-	-	-
3 Cut Number	More than one Line	*	*	NS	NS	NS	NS
	Differences in Intercepts	**	***	-	-	-	-
	Differences in Slopes	NS	NS	-	-	-	-
4 Wilting Time	More than one Line	NS	NS	NS	NS	NS	NS
	Differences in Intercepts	-	-	-	-	-	-
	Differences in Slopes	-	-	-	-	-	-
5 Age of Ley	More than one Line	NS	NS	NS	NS	*	NS
	Differences in Intercepts	-	-	-	-	*	-
	Differences in Slopes	-	-	-	-	NS	-
6 Additives Application	More than one Line	NS	NS	NS	NS	NS	NS
	Differences in Intercepts	-	-	-	-	-	-
	Differences in Slopes	-	-	-	-	-	-
7 Fertilisation	More than one Line	NS	NS	NS	NS	NS	NS
	Differences in Intercepts	-	-	-	-	-	-
	Differences in Slopes	-	-	-	-	-	-

*** = Significant at P < 0.001
** = Significant at P < 0.01
* = Significant at P < 0.05
NS = Not Significant at P = 0.05
1 = NB48 OMD method not reported here because of missing data.

number of samples to control that error. However, in many situations this requirement may sometimes seem difficult to achieve and thus the researcher is obliged to accept the available information at his disposal and conclusions based on the analysis can be only provisional.

In this experiment therefore, we were not able to increase the number of samples for each silage characteristic so that our conclusions can be fundamentally sound. This may be attributed to the various groups of silages studied with each group having different characteristics (Table 5.1) and more importantly, the variable was an in vivo parameter which did not permit us to acquire more samples easily.

The results reported in this chapter indicated that the regression equations based on the IVOMD and NCOMD methods have been strongly affected by the year of harvest (significant at $P < 0.001$, Table 5.2). These findings are in agreement with those reported by Barber et al (1984), where they reported a parallel lines relationship when various laboratory methods including the in vitro and neutral detergent cellulase methods were used to predict in vivo DOMD of hays. For fresh grasses, Morgan and Stakelum (1987) also found significant intercept differences when the IVOMD method was used to predict in vivo OMD of 35 grasses. However in the same study, the NCOMD method was found to be unaffected by years. These workers showed better predictions when each year was considered alone for the IVOMD method.

The effect of years has also been reported by Givens et al (1989b). Those workers showed significant differences not only in intercepts but also in slopes of the regressions obtained for the IVDOMD method when used to predict in vivo OMD of various grasses. For the neutral detergent cellulase method, Givens et al (1989b) reported

significant differences only in intercepts, a finding which has been shown in this work (Table 5.2). However, for the regression equations based on the PCDOMD and MADF methods Givens et al (1989b) showed a significant difference in intercepts and slopes and intercepts respectively, an observation which disagrees with the findings reported in this study (Table 5.2). However, our results must be viewed from the fact that all of the analytical work was done in the same year in contrast to other studies so that a significant difference between years suggests a real difference in the in vivo OMD - laboratory method relationship rather than a variation between years in analytical techniques.

The years effect on the IVOMD method raises queries about the stability of this regression over varying years. This instability may also be applied to the NCOMD method. However, the fact that the PCOMD method, another enzyme method, was not affected by years did not validate this conclusion. The disagreement between the two enzymic methods may be related to the technical differences in the actual determinations for each method. McLeod and Minson (1982) indicated that the pretreatment of grass samples with neutral detergent introduced more analytical error than the pretreatment with acid pepsin. This error accounted for the lesser accuracy of predicting in vivo DMD of 50 grasses by the neutral detergent cellulase method when compared to the pepsin-cellulase method.

Slope differences were also noted when the NIR 8-term equation was used as a predictor. However, this method was noticed to be less affected by years when compared to the previous methods (significant at $P < 0.05$, Table 5.2).

The second notable factor which was found to affect in vivo OMD - laboratory method relationship is cut number

(Table 5.2). Significant differences in intercepts were found for the regression equations based on the MADF ($P < 0.01$) and LIGA ($P < 0.001$) methods. This is in agreement with work reported by Deinum et al (1968), where they found a strong influence of stage of maturity on regression equations based on ADF and Lignin for 12 grasses. This has been confirmed lately by Morgan and Stakelum (1987) when ADF was used to predict in vivo OMD of 35 grasses. The effect of cut number on the MADF and LIGA relationships is not surprising since we know that cell wall components increase as plant advances in maturity.

For age of ley, intercept differences were found when the IVOMD method was used as a predictor. However, this effect was slightly less when compared to the years effect (significant at $P < 0.05$). This is in agreement with a study reported by Givens et al (1989b). However, those workers found that the regression equations based on the MADF method were found to be significantly different in intercepts and slopes, findings which disagree with results reported in this work (Table 5.2). Givens et al (1989b) also reported that the PCDOMD and NCDOMD methods were not affected by age of ley. This is in agreement with results shown in this study.

For nitrogen fertilisation, all predictors studied in this work were unaffected by this parameter (Table 5.2). This is in close agreement with results reported by Deinum et al (1968) with regards to the ADF and Van Soest's Lignin methods.

The method of ensiling, wilting time and additive application were found not to affect the regression equations of any predictor reported in this work (Table 5.2). This may not be surprising since these characteristics were expected to effect fermentation quality

rather than digestibility.

5.5 Conclusions

The IVOMD and NCOMD methods were strongly affected by year of harvest which also showed a slight effect on NIR. Only the MADF and LIGA methods were affected by cut number. The IVOMD method was slightly affected by age of ley.

The method of ensiling, wilting time, additive application and nitrogen fertilisation were found not to affect the regression equations of any predictors studied in this work.

The fact that the number of silages for each characteristic was small and variable in number may warrant further investigation. A large population of silages with known characteristics is advisable so that the conclusions presented in this work may be tested more fully.

CHAPTER SIX

Prediction of *in vivo* DOMD and DE of Grass Silages

SECTION 1

Prediction of DOMD of Grass Silages

6.1.1 Introduction

The term DOMD (or 'D' value) is the most widely used digestibility measurement among farmers and advisory services in the UK (Barber et al, 1984). Those services have adopted the practice of predicting OMD, but to measure the ash content of silages and then calculate DOMD. The measurement of the ash content of silages was found to be useful so as to alert the farmer about the extent of soil contamination in his silage.

In Chapter 4, it has been reported that the NIR method was the most accurate predictor of in vivo OMD. In this section it is intended to explore further the possibility of using NIR to predict in vivo DOMD and examine the optional routes available to achieve this target.

DOMD can be predicted by using 3 optional routes which all involve the use of NIR:

Route 1

Direct prediction of DOMD by NIR, ie: NIR is calibrated directly against in vivo DOMD.

Route 2

Indirect approach which requires:

a) OMD prediction from NIR OMD calibration equation and

b) Ash prediction from NIR ash calibration equation.

From a) and b) DOMD can then be calculated as:

DOMD% = OMD NIR predicted * OM (100 - Ash% NIR predicted)

Route 3

As route 2 but ash is actually measured in muffle furnace.

Figure 6.1 summarises the 3 optional routes available for this study.

6.1.2 Methods

For each route, the NIR calibration equations were produced using the 122 calibration silages and then cross-validated using the 48 validation silages (see Table 2.9). The calibration and the validation statistics for the prediction of in vivo DOMD, in vivo OMD and ash are shown in Tables 6.1, 6.2 and 6.3 respectively. The NIR regression equations produced by stepwise multiple linear regression for the prediction of in vivo DOMD and ash are shown in Appendices 20 and 21 respectively. According to the validation statistics described in 4.2.4, the calibration equation based on 8, 8 and 3-term equations were the best to describe the prediction of in vivo DOMD, in vivo OMD and ash content of grass silages studied. These selected NIR calibration equations and its subsequent validation procedure formed the basis by which the predictive routes were compared.

6.1.3 Statistical Analysis

The actual and predicted DOMD values of the validation silages were used to assess the prediction performance of each route. For this purpose, 4 statistical terms were calculated:

1 **Bias** $= \dfrac{\Sigma\,(\text{Actual DOMD} - \text{Predicted DOMD})}{N}$

2 **SEP** uncorrected for bias $= \sqrt{\dfrac{\Sigma\,(\text{Actual DOMD} - \text{Predicted DOMD})^2}{N}}$

Figure 6.1

The Three Optional Routes Available to Predict DOMD

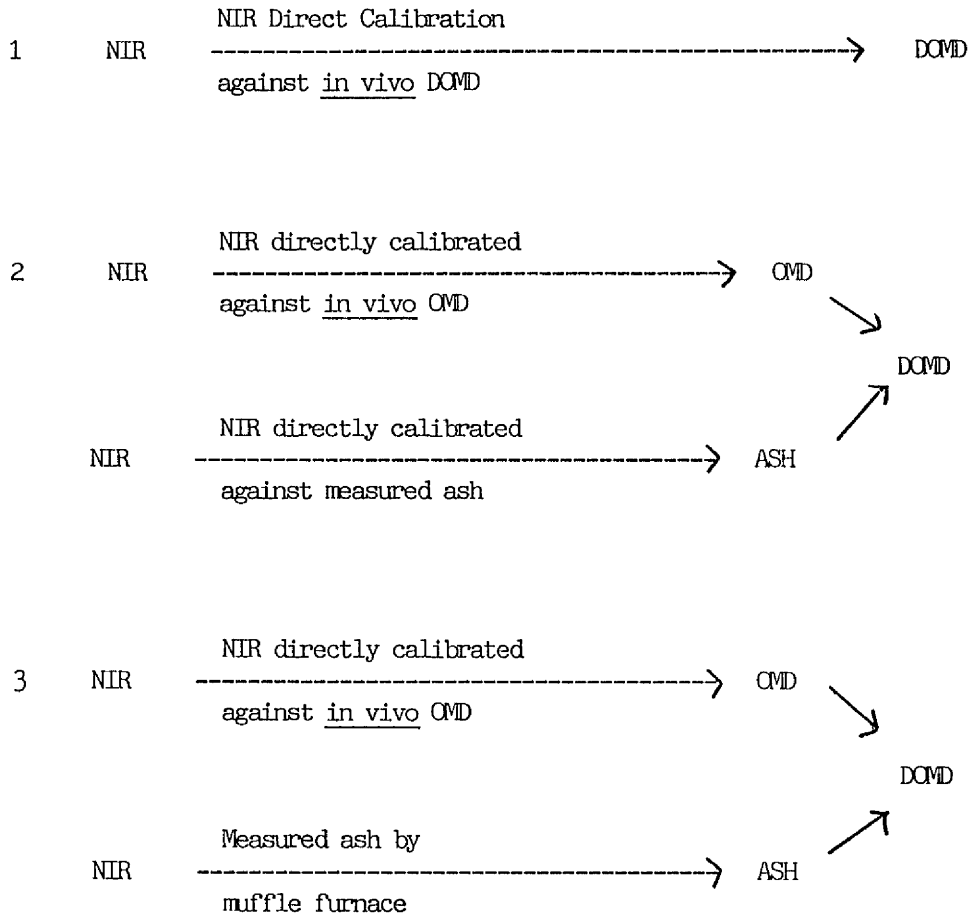


Table 6.1

Calibration and Validation Statistics for The Prediction of *in vivo* DOMD of Grass Silages

No of Terms	Calibration Statistics		Validation Statistics			
	R^{2a}	SEC%	R^{2a}	SEP%[b]	Bias[c]	Slope[d]
1	0.48	4.2	0.30	4.3	-0.81	0.84
2	0.60	3.7	0.51	3.8	-1.38	0.84
3	0.71	3.2	0.62	3.4	-1.24	0.81
4	0.75	3.0	0.64	3.6	-1.61	0.77
5	0.76	2.9	0.65	3.4	-1.11	0.76
6	0.78	2.7	0.56	3.6	-0.87	0.76
7	0.80	2.6	0.62	3.1	-0.21	0.95
8	0.83	2.4	0.64	2.9	-0.16	0.93
9	0.83	2.4	0.64	3.2	-1.13	0.94

a = R^2 adjusted for degrees of freedom.
b = SEP standard error of prediction not corrected for bias.
c = Mean bias between measured and predicted DOMD.
d = Slope of regression between measured and predicted DOMD.

Table 6.2

Calibration and Validation Statistics for The Prediction of *in vivo* OMD of Grass Silages

No of Terms	Calibration Statistics		Validation Statistics			
	R^{2a}	SEC%	R^{2a}	SEP%[b]	Bias[c]	Slope[d]
1	0.52	4.4	0.33	4.2	0.22	0.81
2	0.64	3.8	0.46	3.8	-0.05	0.79
3	0.71	3.4	0.52	3.5	-0.29	0.91
4	0.76	3.1	0.61	3.3	-0.81	0.86
5	0.79	2.9	0.68	3.1	-0.14	0.78
6	0.82	2.7	0.76	2.7	-1.04	0.89
7	0.83	2.6	0.71	3.0	-0.03	0.78
8	0.85	2.5	0.76	2.6	-0.79	0.93
9	0.85	2.4	0.73	2.8	-0.70	0.88

a = R^2 adjusted for degrees of freedom.
b = SEP standard error of prediction not corrected for bias.
c = Mean bias between measured and predicted OMD.
d = Slope of regression between measured and predicted OMD.

Table 6.3

Calibration and Validation Statistics for The Prediction of Ash Content of Grass Silages

No of Terms	Calibration Statistics		Validation Statistics			
	R^{2a}	SEC%	R^{2a}	SEP%[b]	Bias[c]	Slope[d]
1	0.30	1.3	0.08	2.1	1.08	0.96
2	0.40	1.2	0.33	1.7	0.65	1.40
3	0.46	1.1	0.50	1.5	0.54	1.60
4	0.50	1.1	0.43	1.6	0.59	1.30
5	0.60	1.0	0.27	1.8	0.66	0.69
6	0.63	0.95	0.34	1.8	0.57	0.62
7	0.65	0.93	0.39	1.7	0.68	0.67
8	0.69	0.88	0.35	1.9	1.10	0.97
9	0.72	0.83	0.22	2.2	1.40	0.66

a = R^2 adjusted for degrees of freedom.
b = SEP standard error of prediction not corrected for bias.
c = Mean bias between measured and predicted ash.
d = Slope of regression between measured and predicted ash.

3 **SEP** corrected for bias =

$$\sqrt{\frac{(\text{Actual DOMD} - \text{Predicted DOMD} - \text{Bias})^2}{N - 1}}$$

4 **SEP** corrected for intercept and slope =

$$\sqrt{\frac{(\text{Actual DOMD} - \text{intercept} - \text{slope} * \text{Predicted DOMD})^2}{N - 2}}$$

Here the intercept and slope were obtained from the regression equation of actual DOMD vs Predicted DOMD for each route.

The calculated SEPs obtained from steps 2, 3 and 4 were compared by analysis of variance to examine the significance of bias and/or intercept and slope correction.

6.1.4 Results

The results of the in vivo DOMD prediction using the 3 routes are summarised in Table 6.4.

The mean bias between actual and predicted in vivo DOMD were the lowest for route 1 (-0.16) followed by route 3 (-0.65) then route 2 (-1.019). In terms of prediction precision, the R^2 and SEP% not corrected for bias were the highest for route 3 (0.78, 2.43) followed by route 2 (0.71, 2.87) then route 1 (0.64, 2.97). Analysis of variance suggests that routes 1 and 3 produced non-significant bias correction, whereas in route 2 the bias correction was required (significant at $P < 0.05$). All routes gave a non-significant intercept and slope correction. Analysis of variance test is shown in Appendix 22.

6.1.5 Discussion

The use of NIR to predict directly in vivo DOMD (route 1) gave an R^2 and SEP% in a validation test of 0.64 and 2.97

Table 6.4

Predictions of *in vivo* DOMD Using Various Routes by NIR

Routes	Bias[a]	R^{2}[b]	SEP not Corrected for Bias	SEP Corrected for Bias	SEP Corrected for Intercept and Slope	Significance of Bias Correction	Significance of Intercept and Slope Correction
Route 1	-0.16	0.64	2.97	3.00	3.00	NS	NS
Route 2	-1.019	0.71	2.87	2.71	2.73	*	NS
Route 3	-0.65	0.78	2.43	2.37	2.37	NS	NS

a = Mean Bias between actual and predicted *in vivo* DOMD

b = R^{2} adjusted for degrees of freedom.

* = Significant at $P < 0.05$.

NS = Not Significant at $P = 0.05$.

respectively. This is inferior to the NIR prediction of *in vivo* OMD (R^2 = 0.76 and SEP% = 2.6). However for DOMD, this is not surprising since NIR will attempt to predict both OMD and ash content of silages and hence more spectral information is required to predict both parameters. The precision of *in vivo* OMD prediction was better for route 2 (R^2 = 0.71 and SEP% = 2.87) and route 3 (R^2 = 0.78 and SEP% = 2.43) than for route 1 (R^2 = 0.64 and SEP% = 2.97).

The lesser prediction precision for route 2 compared to route 3 was entirely due to the rather weak prediction of ash content by NIR (Table 6.3). However, this inferior prediction of ash can be viewed from the fact that minerals do not have reflectance spectra and any correlation existing between NIR and minerals is related to the variation of these minerals in accordance with the variations of some organic constituents present in the samples (Shenk *et al*, 1979) The selected 3-term ash calibration equation gave an R^2 = 0.46 and SEC% = 1.1, and the validation test gave an R^2 = 0.50 and SEP% = 1.5 (Table 6.3). This inferior prediction of ash gave a significant ($P < 0.05$) bias correction for route 2 when compared to either routes 1 or 3 (Table 6.4). This type of observation makes route 2 unfavourable for the prediction of *in vivo* DOMD.

The choice of NIR DOMD prediction either directly (route 1) or indirectly (route 3) is a matter of debate. Route 1 will offer a simple and economical approach since no ash measurement is required. However, precision has to be sacrificed. On the other hand, it can be argued that route 3 is more desirable from an advisory point of view. This stems from the fact that this route is more precise than route 1 and, more importantly, the measurement of ash content of a farmer's silage in itself is an essential requirement for advisory services to give the farmer an

indication about the extent of soil contamination in his silage.

6.1.6 Conclusions

NIR can predict directly *in vivo* DOMD, however with lesser precision than predicting *in vivo* OMD. This approach is simpler and more economical since ash determination is not required. However, the calculation of *in vivo* DOMD by NIR prediction of *in vivo* OMD and then measuring ash content, will provide a more precise prediction and also the ash measurement in itself is a useful indicator of the extent of soil contamination in silage samples. For this set of silages, ash content was not predicted with sufficient precision by NIR.

SECTION 2

Prediction of the DE of Grass Silages

6.2.1 Introduction

The literature contains few reports about the prediction of the DE of grass silages from laboratory measurements. Those reports include that of Alderman et al (1971) who predicted the DE of 45 grass silages from various laboratory methods. In addition, O'Shea et al (1972) reported DE prediction of 31 grass silages from the IVOMD and other fibre methods. In a study by Morrison (1973), the use of IVOMD, MADF and LIGA methods to predict the DE of 20 grass silages was reported. More recently, NIR has also been used to predict the DE of 60 various forages including silages (Eckman et al, 1983).

While these reports attempted to predict DE with various degrees of accuracy, the number of silages used were limited and it may be that the herbages used to make the silages were grown in a confined area.

The objective of this section is to report the ability of the various laboratory methods studied in this work to predict in vivo DE using a large number of silages obtained from different sources around the UK.

6.2.2 Methods

One hundred and forty silages which have been evaluated in vivo for DE, were collected from different sources around the UK. Table 6.5 shows the sources of silage populations used for this study.

The individual values for in vivo DE, chemical and biological parameters used in this study are shown in Appendix 23.

Table 6.5

Sources and *in vivo* DE Distribution of Silages Used in This Study

Source	DE MJ kg^{-1} oven DM			Number
	Mean	Range	SD	
ADAS Feed Evaluation Unit - Pre-1980	14.6	10.8-17.4	1.67	27
ADAS Feed Evaluation Unit - Post-1980	15.0	9.5-20.6	2.03	71
Rowett Research Institute	15.4	13.1-17.5	1.02	42
TOTAL	15.0	9.5-20.6	1.73	140

6.2.3 Statistical Analysis

The significant differences between population regression lines were assessed by analysis of variance as described in 2.4.

6.2.4 Results

The results of using the laboratory methods to predict in vivo DE are shown in Table 6.6. Of the conventional methods, the best predictors of in vivo DE were PCOMD (R^2 = 0.32, RSD = 1.43) and IVOMD (R^2 = 0.31, RSD = 1.44) followed by LIGA (R^2 = 0.22, RSD = 1.53), NCOMD (R^2 = 0.16, RSD = 1.6) and MADF (R^2 = 0.09, RSD = 1.65).

For all these methods, the regression lines obtained for the three populations of silages differed significantly in intercepts, being significant at $P < 0.001$ for MADF, LIGA and NCOMD, significant at $P < 0.01$ for PCOMD and significant at $P < 0.05$ for IVOMD.

The best predictor of in vivo DE found in this study was the use of NIR (Table 6.7). The 9-term equation gave an R^2 of 0.72 and SEC of 0.91. In contrast to the conventional methods, one regression line can be used to describe all data. This is true for all NIR terms with the exception of term 1 which gave significant ($P < 0.001$) regression line differences in intercepts.

Analysis of variance of the regression equations for the 3 populations used in the calibration set for the prediction of in vivo DE by conventional predictors is shown in Appendix 24. Analysis of variance for the NIR regression equation is shown in Appendix 25. The NIR regression equations produced by stepwise multiple linear regression are shown in Appendix 26.

Table 6.6

Overall Regression Statistics and Significance of Between-Population Differences in Laboratories Methods Regression Equations[1] for the Prediction of *in vivo* DE (MJ kg^{-1} oven DM) (n = 140)

Predictors[1]	R^2	RSD	More than One Line	Differences in Intercepts	Differences in Slopes
MADF	0.09	1.65	**	***	NS
LIGA	0.22	1.53	***	***	NS
NCOMD	0.16	1.60	**	***	NS
PCOMD	0.32	1.43	**	**	NS
IVOMD	0.31	1.44	*	*	NS
NIR 8-Term	0.71	0.93	NS	-	-

R^2 adjusted for degrees of freedom

*** = Significant at P < 0.001

** = Significant at P < 0.01

* = Significant at P < 0.05

NS = Not Significant at P = 0.05

1 = NB method was not reported here because of missing data

Table 6.7

Overall Population Statistics and Significance of Between-Population Differences in NIR Regression Equations for the Prediction of Silage DE (MJ kg^{-1} oven DM) (n = 140).

No of NIR Terms	R^2	SEC	More than One Line	Differences in Intercepts	Differences in Slopes
1	0.38	1.36	**	***	NS
2	0.49	1.24	NS	-	-
3	0.54	1.17	NS	-	-
4	0.59	1.11	NS	-	-
5	0.63	1.05	NS	-	-
6	0.66	1.01	NS	-	-
7	0.70	0.95	NS	-	-
8	0.71	0.93	NS	-	-
9	0.72	0.91	NS	-	-

R^2 adjusted for degrees of freedom

*** = Significant at P < 0.001

** = Significant at P < 0.01

NS = Not Significant at P = 0.05

6.2.5 Discussion

The use of the MADF and LIGA methods to predict in vivo DE gave R^2 and RSD of 0.09, 1.65 and 0.22, 1.53 respectively (Table 6.6). These figures are in close agreement with those reported by Alderman et al (1971) and Morrison (1973). Alderman et al (1971) found an R^2 of 0.04 and 0.23 when in vivo DE of 45 grass silages was predicted by MADF and Deriaz Lignin respectively. Similarly, Morrison (1973) reported an R^2 of 0.03 and 0.19 for the prediction of in vivo DE of 20 grass silages by the MADF and LIGA methods respectively. For the NCOMD and PCOMD methods, a striking difference between the two methods in their predictive ability to in vivo DE was observed (R^2 of 0.16 for NCOMD to 0.32 for PCOMD).

The use of the IVOMD method to predict in vivo DE gave an R^2 and RSD or 0.31 and 1.44 respectively. While this prediction is relatively poor, the study conducted by O'Shea et al (1972) gave a better prediction (R^2 = 0.64) for 31 grass silages. However, this better prediction may be related to the limited number of silages used by O'Shea et al (1972).

Brown and Radcliffe (1971) commented about the poor predictability of silage DE from digestibility measurements and concluded that the energy values of the various organic compounds present in silages varied independently of digestibilities.

In this study, NIR proved to be the best predictor of in vivo DE. The 9-term equation gave an R^2 of 0.72 and RSD of 0.9, a precision which is substantially better than the conventional methods used in this work. Eckman et al (1983) obtained a similar result when they predicted the DE of 60 various forages with an R^2 of 0.67 using the 2-term NIR equation.

The precision of NIR to predict DE may be related to its ability to predict both silage digestibility and GE, even though the samples were dried. This suggests that the GE of grass silages is largely variable and could be accounted for when it is used with a digestibility measurement to predict DE (Givens et al, 1989a). To demonstrate this aspect, the GE of silages used in this study was combined with the IVOMD method in a bivariate relationship to predict DE. The prediction power was increased from an R^2 of 0.32 for IVOMD alone to 0.80 when IVOMD and GE were in combination, suggesting the importance of GE in energy evaluation of silages (see 1.1.3, GE section).

In addition to the low predictability of DE by the conventional methods, the regression lines obtained from the three populations of silages differed significantly in intercept (Table 6.6). Only NIR described all populations by a single regression line (Table 6.7).

The above results clearly highlight the difficulty of DE prediction for silages. Methods other than NIR are unsatisfactory predictors of DE. In fact, attempting to predict ME will be more difficult since the error in ME prediction is greater than DE prediction error. Large proportions of this error can be accounted for by the GE of silages, suggesting the importance of GE in the evaluation process.

While the use of NIR makes a substantial improvement in DE prediction (R^2 = 0.72), however this is inferior to the NIR prediction of in vivo OMD (R^2 = 0.85).

6.2.6 Conclusions

The DE of grass silages was poorly predicted by the laboratory methods studied in this work. This suggests that the DE prediction (and similarly ME) is more

complex and cannot be explained by a simple digestibility measurement. Only the multi-term NIR equation makes a significant improvement in DE prediction. The GE of silages plays an important role in energy evaluation of silages.

CHAPTER SEVEN

GENERAL DISCUSSION

ME and digestibility are the most useful indicators of the nutritive value of silages and the former is the basis for allocating energy allowances for ruminants in the UK. Ideally, the use of animals to measure these parameters is the ultimate measurement however, this approach is labour intensive, expensive and requires special facilities and consequently, it is not likely to be a practical proposition. Therefore, a rapid, simple, accurate and comparatively inexpensive laboratory method(s) to predict these parameters is an absolute requirement.

ADAS Feed Evaluation Unit proposed several regression equations to predict ME from simple laboratory methods (Barber et al, 1984; Givens, 1986). However, advisers have long recognised that those equations were unreliable and gave an ME value which resulted in an over-prediction of animal performance (Barber et al, 1983). Four plausible reasons were postulated to explain this over-prediction, namely:

a) The GE of silage is widely variable;

b) The fate of the energy dense silage volatiles is unknown;

c) The accuracy of predicting methane energy by Blaxter and Clapperton's (1965) equation is questionable; and

d) The unreliability of the current efficiency values of ME utilisation for silages. (see 1.1.4)

This situation created some disagreement among advisers about which direction the prediction of the nutritive value of grass silages should proceed. The routes illustrated in Figure 1.4 indicated the two paths that may be used by the advisory organisations in the UK in calculating ME.

The choice of any path shown in Figure 1.4, is a matter of debate. However, the uncertainties attached to the direct

prediction of ME, which have been stated previously, may prevent the immediate adoption of route II. Until these uncertainties are fully understood and then removed, the adoption of route I will provide an alternative simple approach which has been found to be more understandable and can deliver sound practical advice (Barber et al, 1983). It is important therefore, for the advisory services to find a reliable predictive method which can predict in vivo digestibility of grass silages accurately and inexpensively.

The search for such a laboratory method(s) has been a major concern of the different advisory organisations in the UK. Distinguished attempts to develop prediction equations for OMD of grass silages were reported by Barber et al (1984) and Givens et al (1989a).

Until recently, the different advisory laboratories in the UK have used different laboratory methods to predict digestibility of grass silages with various degrees of accuracy. Table 7.1 illustrates the methods used by those laboratories in the UK.

Commercial NIR instruments became an accepted methodology in forage evaluation and recently ADAS, SAC and DANI have adopted this technique in their silage evaluation programs. However, instead of a direct calibration of NIR on in vivo digestibility, those laboratories used NIR to predict fibre parameters (MADF, LIGNIN and MADF respectively). Digestibility and ME were then calculated using the appropriate regression equation. The overall error of silage evaluation is increased by this indirect approach because the error of the NIR prediction must be added to the error of the prediction equation relating fibre content to digestibility.

The work described in this thesis was intended to examine this problem by testing the ability of various laboratory methods, including those used routinely by advisory services in the UK, to predict in vivo OMD of a large population of silages gathered from

Table 7.1

Methods Used by Advisory Laboratories in the UK

Laboratory	Methods Used
ADAS	MADF NIR (MADF)
SAC	LIGA NIR (LIGA)
DANI	MADF NIR (MADF)

different sources around the UK (Table 2.1). A longer term aim of this work was to explore the possibility of establishing an improved predictive technique which could be used by all advisory laboratories in the UK.

For all 122 calibration silages studied in this work (Chapter 4), the rumen liquor methods (NB48 OMD, IVOMD) gave more precise predictions than either the enzymic methods (NCOMD, PCOMD) or the chemical methods (MADF, LIGA). This better prediction may be related to the sensitivity of the rumen micro-organisms to the known and unknown factors which might affect digestibility. Other predictors will not be expected to recognise these unknown factors since they rely heavily on measuring the major components which are found to affect digestibility (ie: cellulose, hemicellulose and Lignin).

For individual population regressions, the PCOMD method was found to give superior prediction of in vivo OMD (Table 4.1). This suggests that this technique might be adequate for predictive purposes for grasses grown in one geographical region.

The LIGA method gave a rather poor prediction of in vivo OMD of silages obtained from the Rowett Research Institute (R^2 = 0.20). This inferior prediction may be explained by the presence in many of these silages of unknown proportions of clover. This suggests the imprecision of the LIGA method for the prediction of the digestibility of a mixture of grasses and legumes (Minson, 1982).

The NB48 OMD method gave less precise predictions when compared to the IVOMD method (Table 4.1). This inferior prediction of the NB48 OMD method may be related to the inherent uncontrolled variations associated with this technique. Two important sources of variation may be important to improve the predictive power of this technique. Firstly, the washing procedure after bags are removed from the rumen. This aspect has been investigated in detail in Chapter 3 where polyester bags were subjected to a range of different washing reagents. The results indicated that

the combination of machine washing with non-biological washing powder after bags were removed from the rumen reduced considerably the between-bag variability without altering the form of the degradation curve. This washing procedure was found to be both cheap and convenient.

The second source of variation is the differential losses of fines from bags during rumen incubation. It was felt that the use of freshly minced silage may improve the prediction. However, such material was not available for this study.

Of the methods tested in this study, the NIR method gave the most precise prediction of in vivo OMD of grass silages (Table 4.1). The ability of this technique to unravel compositional information contained in the light reflected off samples and then analyse this information by multiple regression analysis is primarily behind its accurate prediction.

Analysis of variance of the regression equations obtained for the populations of silages used in the calibration set indicated that significant differences in intercepts were found for the regression equations based on the MADF, LIGA and NCOMD methods, and in both intercepts and slopes for the PCOMD method (Table 4.1). One regression line can be used to describe all data when the NB48 OMD, IVOMD and NIR techniques were used as predictors. This implies the robustness of these methods as predictors of digestibility. The fact that the MADF, LIGA, NCOMD and PCOMD methods gave between-population differences in the regressions obtained suggests the inadequacy of these techniques as predictors of digestibility, and their use to evaluate silages routinely may not be justified.

On the basis of this investigation, only the NIR method can provide a reliable alternative to the conventional methods studied in this work. Provided that this technique is calibrated and validated in the manner described in this work, the speed, precision and cost effectiveness are the most attractive features of

this method. These qualities may give strong justification for NIR to be applied on a national basis.

With increasing economic pressures on advisory laboratories, the selected method needs to provide an economic and fast service for farmers. Tables 7.2 and 7.3 illustrate the unit cost of analysis and laboratory turn-around time at its best for some of the methods tested in present work. These tables show the relatively low unit cost of analysis for NIR when compared to other methods. Even though the MADF method has been found to be cheap and fast to operate, the NIR method can provide a competitive price, and greater speed and accuracy than the MADF method. In terms of labour input, only NIR requires the minimal labour when compared to other methods (Dixon, Personal Communication). To scan a silage sample, which takes less than a minute, simple drying and grinding are the only required procedures to report ME and other determinations.

Practical Evaluation of MADF and NIR Regression Equations

The error involved in using the MADF and NIR 8-term regression equations for the prediction of *in vivo* OMD of the 122 calibration silages was assessed to determine the effect of such error on two aspects:

1 The Effect on ME Calculation and
2 The Effect on Ration Formulation.

1 **The Effect on ME Calculation**

The use of MADF and NIR 8-term regression equations to predict *in vivo* OMD gave an RSD% and SEC% of 5.1 and 2.5 respectively (Table 4.1).

From the normal distribution curve (Figure 7.1), the percentage of the actual values which will be more or less than a given range from a predicted ME value of 10.4

Table 7.2

Unit Cost of Analysis and Laboratory Turn-Around Time for Some of the Methods Tested in This Work (Dixon, Personal Communication)

Method	Unit Cost of Laboratory Analysis (£)*	Turn-Around Time (Working Days)
MADF	2.80	2-3
NCD	7.40	3-4
IVOMD	3.90**	12-15
NIR	See Below	2

* Excluding cost of sample preparation and laboratory overheads.
** Excludes the cost of maintaining surgically modified animals.

TABLE 7.3

Unit Cost of NIR Analyis*

	Annual Costs	£/Annum
1	Capital Cost of Equipment £87,500 depreciated over 5 years	17,500
2	Interest on Capital at 20% APR	17,500
3	Labour Requirement (1 full-time technician)	10,000
4	Maintenance & Insurance	5,000
	TOTAL AMOUNT OF EXPENDITURE	50,000

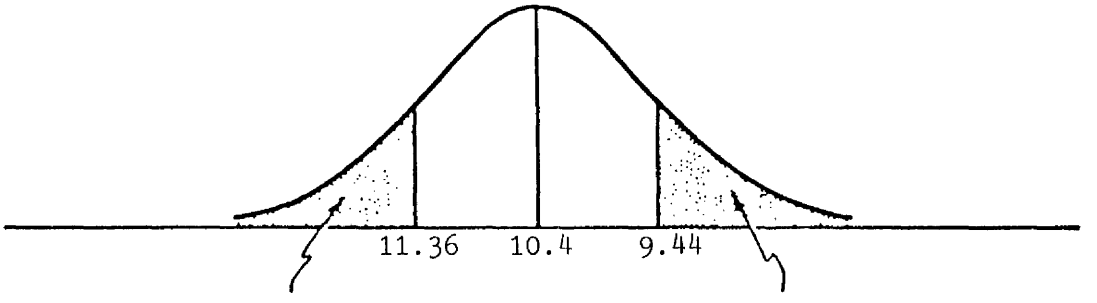
200 Samples can be scanned/day for 250 days
= 50,000 samples/annum.

Unit Cost at Full Capacity £1/sample
at Half Capacity £2/sample

* It is assumed that silage analysis is only a small proportion of the total use of the instrument.

FIGURE 7.1

The Normal Distribution Curve for the Prediction of Silage ME (MJ kg^{-1} DM).



Shaded areas are percentages of actual values which will be out by $\pm$ 0.96 of ME from the value predicted.

MJ kg^{-1} DM were calculated. For ME prediction, Figure 7.2 shows the percentage of the actual values which will be greater than the predicted ME value by the stated number of ME units. For example, whereas 12% of the actual values will be more than 0.96 MJ of ME from the predicted value when MADF is used as a predictor, the corresponding figure for NIR is only 1%.

These calculations assume a conversion factor of 0.16 to calculate ME from DOMD%. Therefore, the true error of ME prediction will be more than that since these calculations assume that urine and methane losses can be predicted without error.

2 **The Effect on Ration Formulation**

The effect of prediction error on ration formulation (and so on the advice which could be given to farmers) has been shown using the SAC dairy cow rationing software.

The amount of concentrate required for a 100 cow dairy herd during a 182 day winter feeding was calculated for a silage (ME = 10.4 MJ Kg^{-1} DM) and concentrate (ME = 13 MJ kg^{-1} DM).

As with ME, similar calculations can be performed here. Figure 7.3 shows the percentage of cases in which over-purchase of concentrate for winter feeding will result. For example, the advice given using the MADF regression equation will result in over-purchase of concentrate of 29.5 tonnes in 12% of cases, whereas for NIR it is only 1% of cases which will result in over-purchase.

The above comparisons clearly highlights the magnitude of the prediction error when translated to real farm practice. The use of either the MADF or NIR methods as a predictor of the energy value of grass silages can have serious financial consequences to the farmer.

FIGURE 7.2

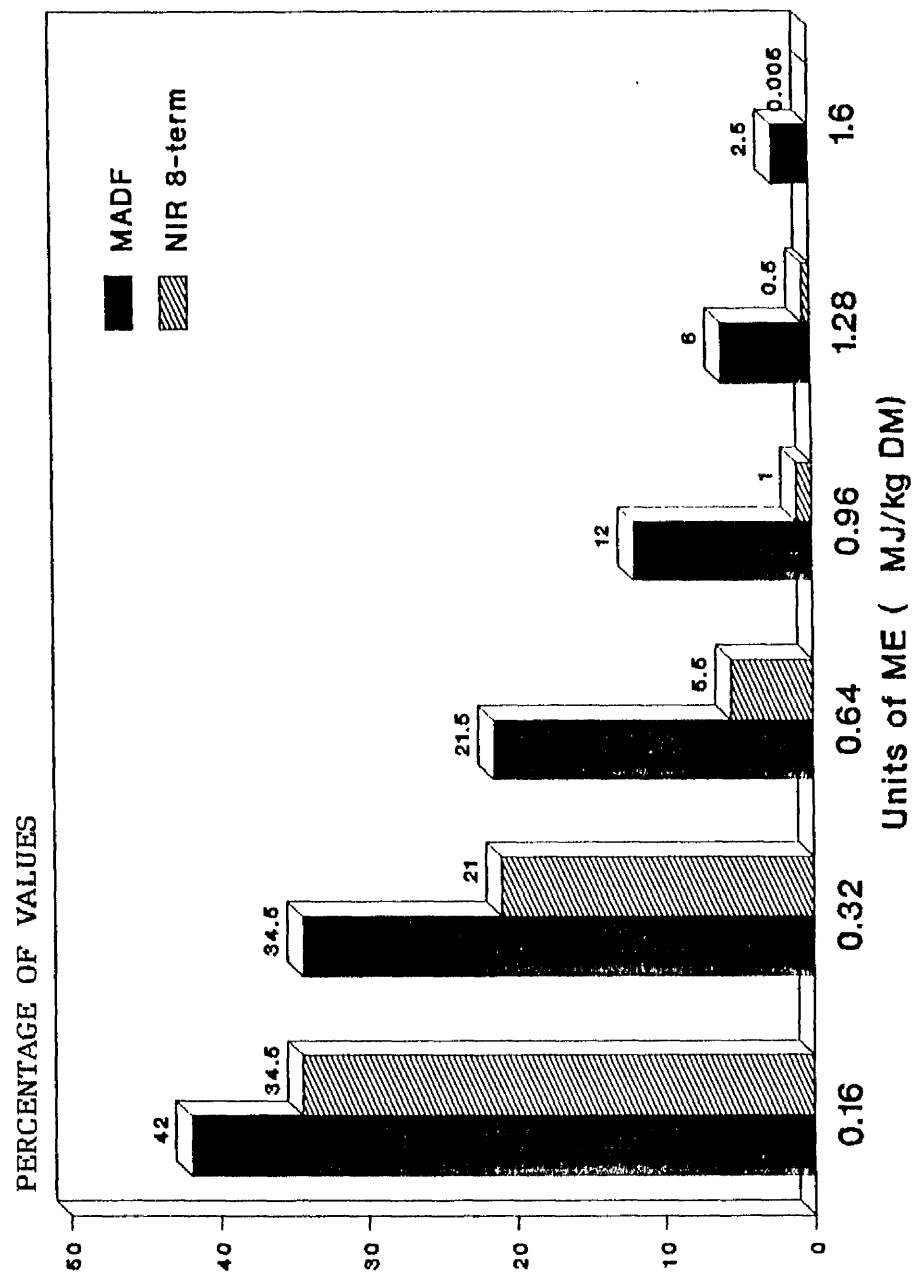
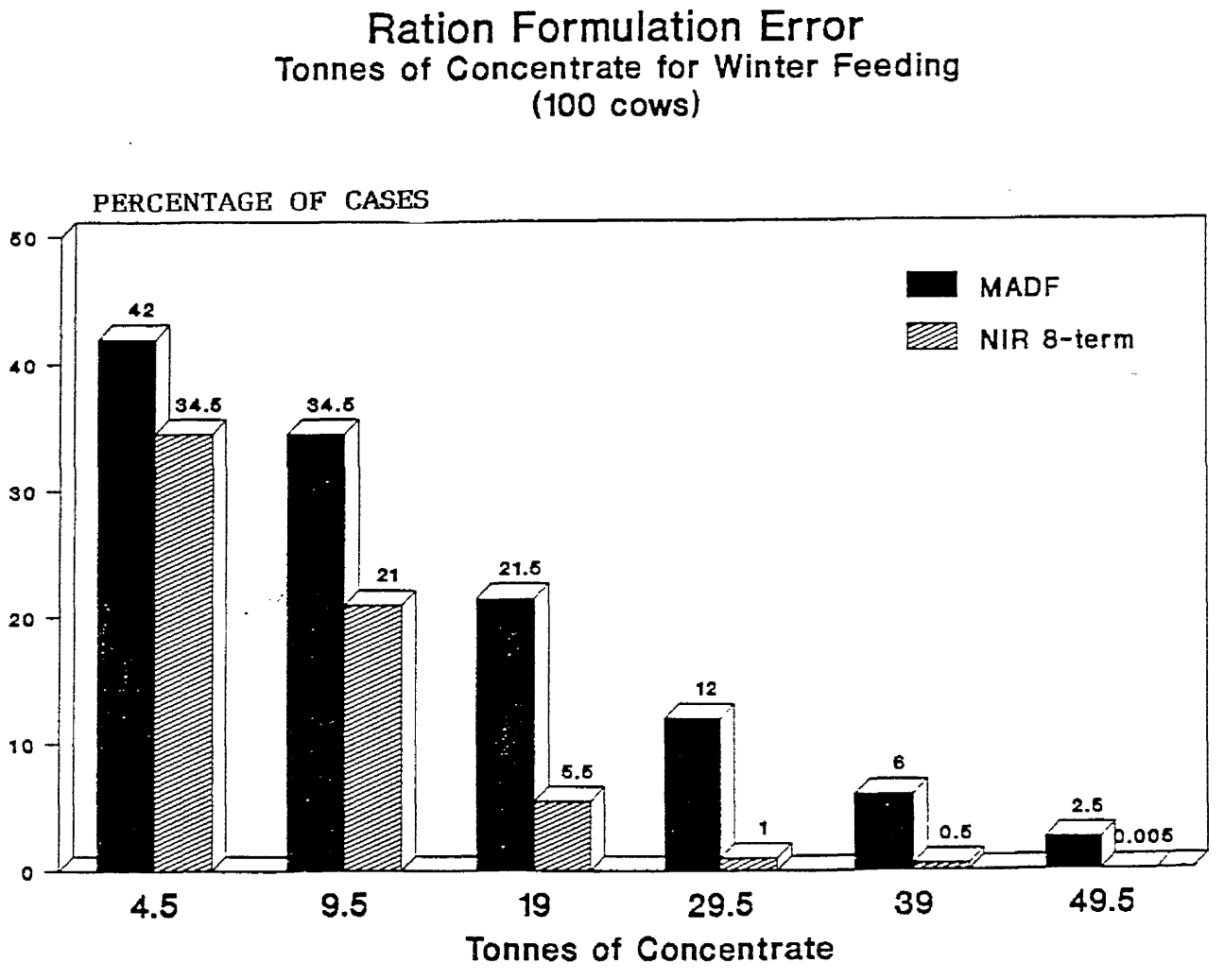

ME Prediction Error
PERCENTAGE OF VALUES
MADF
NIR 8-term
50
40
30
20
10
0
42
34.5
34.5
21
21.5
5.5
12
1
6
0.5
2.5
0.005
0.16
0.32
0.64
0.96
1.28
1.6
Units of ME (MJ/kg DM)

FIGURE 7.3


Ration Formulation Error
Tonnes of Concentrate for Winter Feeding
(100 cows)
PERCENTAGE OF CASES
50
40
30
20
10
0
MADF
NIR 8-term
42
34.5
34.5
21
21.5
5.5
12
1
6
0.5
2.5
0.005
4.5
9.5
19
29.5
39
49.5
Tonnes of Concentrate

Assuming that the current price of one tonne of concentrate is equal to £170, this means that 12% of the farmers will needlessly pay an extra £5,015 for buying extra concentrate for winter feeding for a herd of 100 cows if the MADF method was used as a predictor of ME, whereas with NIR it is only 1% of the farmers who will pay that amount.

The work described in Chapter 5 suggested that the in vivo OMD - laboratory method relationship can be influenced by environmental and other external factors. A factor like year of harvest was found to affect the relationships based on the IVOMD and NCOMD methods. Another noticeable factor was cut number which was found to affect the relationships based on the MADF and LIGA methods. Factors like the method of ensiling, wilting time, additive application and nitrogen fertilisation were not found to affect the relationship between in vivo OMD and its predictors.

In Chapter 4, it was reported that the NIR method was the most precise predictor of in vivo OMD. In Chapter 6, Section 1, it was demonstrated that NIR can be further used to predict in vivo DOMD. However, the in vivo DOMD prediction was found to be inferior to in vivo OMD prediction. The direct prediction of in vivo DOMD using NIR was found to be simpler and more economical since ash determination is not required. However, the NIR prediction of in vivo OMD and then measuring the ash content, was found to give more precise prediction of in vivo DOMD. Additionally, the measurement of the ash content in itself is an important indicator of the extent of soil contamination in silage samples, and a useful guide to the quality of silage making techniques.

The comparison procedure performed between in vivo OMD predictors (Chapter 4) was repeated in Chapter 6, Section

2 in an attempt to demonstrate the ability of the laboratory methods studied in this work to predict in vivo DE of grass silages by using a large number of silages. The results indicated that the DE of grass silages was poorly predicted by the conventional methods. This poor prediction highlighted the difficulty of the DE prediction (and similarly ME) using a simple digestibility measurement. It has been found that the GE of silages plays an important role in energy evaluation of silages and can account for much of the variation in silage DE. However, this aspect warrants further investigation. Of the methods tested in this Chapter, the NIR method proved to be the best predictor of in vivo DE.

POSSIBILITIES FOR FUTURE RESEARCH

In view of the results obtained from this thesis, the following section describes the avenues of research which are potentially important and may provide valuable and interesting results. These avenues include:

i) The in situ method is recognised as being of potential value in forage evaluation. This method has been shown in this work to be as precise as the IVOMD method. However, it is felt that this method may surpass the precision of the IVOMD method if fresh minced forage was used, provided that it is used in the manner described in Chapter 3.

ii) A method for accurate and precise in vivo ME prediction for routine silage evaluation is urgently required. Digestibility predictors were found to give poor predictions of in vivo DE (Chapter 6, Section 2) and similarly, in vivo ME (Givens et al, 1989a) of grass silages. Only NIR was found to give a significant improvement in the DE prediction. It is therefore possible that, with the help of NIR, the ME prediction can be improved by:

a) Direct Calibration of NIR with large in vivo ME populations in the manner described in Chapter 4;

b) Where a) is not possible, the NIR method can be of value to predict the GE of silages since the measurement of this parameter is rather difficult for grass silages. If this prediction is found to be accurate, this will then allow the establishment of a quick and cheap method for the determination of the GE of silages. A further aim of this requirement is to study the exact role of GE in the energy evaluation of silages. This stems from the fact that

GE was found to play an important role in ME prediction and can account for almost 50% of the variability in ME (Givens and Brunnen, 1987).

However, the prediction of 'correct' ME values for silage must be combined with 'correct' values for efficiency of utilisation of ME at the rationing stage and further information on this aspect is also needed.

iii) Between-laboratory differences are an important aspect of the choice of any predictive method(s). It is therefore important to investigate this problem before any predictive method(s) can be applied between laboratories. This aspect is particularly important if the NIR method is to be used on a national basis. The matching of NIR instruments and then the need for continuous monitoring of their performance is required. NIR instrument matching has already been made by other workers (Shenk et al, 1985).

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APPENDICES

APPENDIX 1

Program Used to Calculate the Derivatised $\text{Log } \frac{1}{R}$ Segments (W values) for NIR Regression Equations

```
f$=" ####.###";f1$="###"
def fna(x)=sgn(x)*int(abs(x)*10000+.5)/10000
dim r(701),a1%(10,10),b1%(10,10),a2%(10,10),b2%(10,10),c2%(10,10),wls%(701)
dim an1%(10,10),bn1%(10,10),an2%(10,10),bn2%(10,10),cn2%(10,10),w(10),dr%(10)


print:print
input "enter WAVELENGTH DATA source file name";file2$
print:print
input "enter RAW DATA source file name";file$
print:print
input "enter OUTPUT file name";file1$
create file1$ as 2
open file$ as 1
OPEN FILE2$ AS 3
READ #3;TERM%

w1%=1100
for t%=1 to 700
wls%(t%)=w1%
w1%=w1%+2
next t%

for b%=1 to TERM%
READ #3;DR%(b%)
IF DR%(b%)<>1 THEN 1.1
FOR T%=1 TO 4:READ #3;A1%(b%,T%):NEXT T%
FOR T%=1 TO 4:READ #3;B1%(b%,T%):NEXT T%
goto 1.2

1.1 REM b%>1
FOR T%=1 TO 4:READ #3;A2%(b%,T%):NEXT T%
FOR T%=1 TO 4:READ #3;B2%(b%,T%):NEXT T%
FOR T%=1 TO 4:READ #3;c2%(b%,T%):NEXT T%

1.2 rem skip

for t%=1 to 4
for tt%=1 to 700

if dr%(b%)<>1 then 1.3
rem first
if a1%(b%,t%)=wls%(tt%) then an1%(b%,t%)=tt%
if b1%(b%,t%)=wls%(tt%) then bn1%(b%,t%)=tt%
goto 1.4

rem second
1.3 if a2%(b%,t%)=wls%(tt%) then an2%(b%,t%)=tt%
if b2%(b%,t%)=wls%(tt%) then bn2%(b%,t%)=tt%
if c2%(b%,t%)=wls%(tt%) then cn2%(b%,t%)=tt%

1.4 next tt%
next t%
next b%
```

```
if end #1 then 2
for ttt%=1 to 200

read #1;n%
print "sample ";n%
for tt%=1 to 700
read #1;r(tt%)
next tt%

FOR b%=1 TO TERM%

sega1=0:sega2=0:segb1=0:segb2=0:segc2=0

for tt%=1 to 4
IF DR%(b%)<>1 THEN 1.6
sega1=sega1+(r(an1%(b%,tt%)))
segb1=segb1+(r(bn1%(b%,tt%)))
goto 1.7

1.6 sega2=sega2+(r(an2%(b%,tt%)))
segb2=segb2+(r(bn2%(b%,tt%)))
segc2=segc2+(r(cn2%(b%,tt%)))
1.7 next tt%

IF DR%(b%)=1 then w(b%)=(10000#(sega1-segb1))/4
IF DR%(b%)=2 then w(b%)=(10000#(sega2-2#segb2+segc2))/4

next b%
on term% goto 11,12,13,14,15,16,17,18,19

11 print using f1$+f$;#2;n%,w(1):goto 20
12 print using f1$+f$+f$;#2;n%,w(1),w(2):goto 20
13 print using f1$+F$+f$+f$;#2;n%,w(1),w(2),w(3):goto 20
14 print using f1$+f$+F$+f$+f$;#2;n%,w(1),w(2),w(3),w(4):goto 20
15 print using f1$+f$+f$+F$+f$+f$;#2;n%,w(1),w(2),w(3),w(4),w(5):goto 20
16 print using f1$+f$+f$+f$+F$+f$+f$;#2;n%,w(1),w(2),w(3),w(4),w(5),w(6):goto 20
17 print using f1$+f$+f$+f$+f$+F$+f$+f$;#2;n%,w(1),w(2),w(3),w(4),w(5),w(6),w(7):goto 20
18 print using f1$+f$+f$+f$+f$+f$+F$+f$+f$;#2;n%,w(1),w(2),w(3),w(4),w(5),w(6),w(7),w(8):goto 20
19 print using f1$+f$+f$+f$+f$+f$+f$+F$+f$+f$;#2;n%,w(1),w(2),w(3),w(4),w(5),w(6),w(7),w(8),w(9):goto 20
20 next ttt%
2 close 1
close 2
close 3
stop
```

This program was written by N W Offer at the Department of Nutrition and Microbiology.

APPENDIX 2

Detailed Washing Procedures for the Polyester Bags Experiment

Treat Code	Description
A	After Incubation the bags were washed with cold water in a washing machine which consume approximately 107.5 l of water and run for 1½ hours.
NDI	Before incubation, the bags were treated with neutral detergent solution as follows: 8 bags were put in previously boiled 2.5 l neutral detergent solution (Van Soest and Wine, 1967), kept at 95°C and stirred continuously for 2 hours. Each gram of sample in the bag received 56.8 ml detergent. Then the bags were washed in the washing machine, as in treatment A, to remove excess detergent (we found it necessary to do this step before the bags went into the sheep, because it was difficult to remove excess detergent with hand washing only). The bags were then incubated in the rumen and after incubation they were washed in the washing machine as in A.
NDIND	Before incubation the bags were treated with neutral detergent only, as in NDI, then washed in the washing machine, as in A, then incubated in the rumen, then treated again with neutral detergent, as in NDI, then washed in the washing

machine, as in A.

IND — Bags were incubated, then treated with neutral detergent, as in NDI, then washed in the washing machine, as in A.

API — Before incubation the bags were treated with acid pepsin solution as follows: 8 bags were put in 2.5 l of acid pepsin solution (Tilley and Terry, 1963) in a water bath (40°C) for 96 hours with occasional stirring. Each gram of sample in the bags received 56.8 ml acid pepsin. Then the bags were washed in the washing machine, as in A, then incubated in the rumen. After incubation they were washed in the washing machine, as in A.

APIAP — Before incubation the bags were treated with acid pepsin, exactly as in API, then washed in the washing machine, as in A, then incubated in the rumen, then treated again with acid pepsin, as in API, then washed in the washing machine, as in A.

IAP — Bags were incubated in the rumen, then treated with acid pepsin, as in API, then washed in the washing machine, as in A.

WPI — Before incubation the bags were treated with 60g of commercial washing powder (non-biological Persil brand) in the hot cycle (95°C, 3 hour run) of the washing machine. The bags were then incubated, and after incubation they were washed in the washing machine, as in A.

WPIWP — Before incubation the bags were treated with washing powder, as in WPI, then incubated in the rumen, then treated again with washing powder, as in WPI, then washed in the washing machine, as in A.

IWP — Bags were incubated in the rumen, then treated with washing powder, as in WPI, then washed in the washing machine, as in A.

APPENDIX 3

Analysis of Variance of % Organic Matter Disappearance of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Different Washing Techniques

Source of Variation	D.F.	Variance Ratio (F)			
		3 hrs	8 hrs	24 hrs	72 hrs
Sheep	3	2.685	0.104	7.422	10.260
Period	2	35.278	1.843	2.923	54.800
Sheep & Period	6	2.725	1.223	0.101	7.325
Treatments in Period 1	3	65.725	16.123	3.469	24.013
Treatments in Period 2	3	135.352	34.478	9.624	27.241
Treatments in Period 3	3	213.144	36.027	12.809	25.158
Residual MS	27	1.403	5.746	7.469	1.520

APPENDIX 4

Analysis of Variance of Effective Degradability (%) of Hay Organic Matter Disappearance from Polyester Bags Incubated in the Rumen of Sheep and Treated with Different Washing Techniques at Selected Outflow Rate

Source of Variation	Variance Ratio . (F)			
	D.F.	ED 2%	ED 4%	ED 8%
Sheep	3	9.139	3.641	2.416
Period	2	24.203	8.352	10.761
Sheep & Period	6	1.255	0.556	0.409
Treatments in Period 1	3	27.893	23.276	26.807
Treatments in Period 2	3	60.040	50.314	61.300
Treatments in Period 3	3	61.884	62.895	85.697
Residual MS	27	1.400	2.187	2.377

APPENDIX 5

Organic Matter Disappearances (%) and Effective Degradabilities (ED) (%) for All Washing Treatments.

Periods[a]	Sheep No	Treatments Code[b]	0 hrs	3 hrs	8 hrs	24 hrs	72 hrs	ED at 2%	ED at 4%	ED at 8%
1	1	01	11.68	18.78	30.97	59.27	67.96	56	47	36
1	2	01	11.68	17.72	29.61	55.80	71.53	56	45	34
1	3	01	11.68	18.76	30.03	55.66	72.14	56	46	35
1	4	01	11.68	19.37	37.46	61.33	72.39	59	50	39
1	1	02	21.97	29.80	39.96	63.83	71.79	61	53	44
1	2	02	21.97	30.31	40.12	65.97	79.46	64	55	44
1	3	02	21.97	28.13	41.84	59.90	73.59	60	52	43
1	4	02	21.97	25.16	40.08	61.31	77.56	62	52	42
1	1	03	23.69	27.88	41.99	62.09	75.69	62	54	44
1	2	03	23.69	29.78	42.19	64.78	81.08	66	56	46
1	3	03	23.69	29.11	44.95	63.39	76.19	64	56	46
1	4	03	23.69	29.01	43.62	64.13	80.11	65	56	45
1	1	04	21.97	26.66	38.68	59.64	69.63	58	50	41
1	2	04	21.97	28.49	41.09	64.08	76.15	62	54	45
1	3	04	21.97	26.06	39.07	53.86	74.20	59	50	40
1	4	04	21.97	27.26	41.09	62.92	76.99	63	54	43
2	1	01	11.68	16.88	33.93	61.17	74.85	59	49	38
2	2	01	11.68	15.73	27.74	63.92	73.53	58	48	36
2	3	01	11.68	18.79	32.02	49.58	73.34	55	44	33
2	4	01	11.68	16.26	30.49	61.16	73.99	59	48	36
2	1	05	25.01	29.18	43.73	67.00	81.64	67	58	47
2	2	05	25.01	30.62	46.15	64.20	79.87	66	57	48
2	3	05	25.01	34.91	42.42	66.22	77.26	65	57	48
2	4	05	25.01	27.75	44.09	67.58	80.01	66	57	47
2	1	06	27.67	31.95	47.90	67.17	82.05	68	59	50
2	2	06	27.67	30.61	47.65	68.65	79.02	67	59	49
2	3	06	27.67	33.37	46.91	65.73	82.15	68	58	48
2	4	06	27.67	33.16	46.58	68.58	81.54	68	59	50
2	1	07	25.01	26.21	41.27	58.58	77.21	59	51	43
2	2	07	25.01	27.21	40.60	64.98	77.37	64	55	45
2	3	07	25.01	28.96	41.50	57.98	75.43	60	51	42
2	4	07	25.01	26.51	35.66	60.04	77.16	61	51	41
3	1	01	11.68	16.43	28.18	58.99	73.58	57	46	34
3	2	01	11.68	17.25	28.48	53.90	74.70	57	45	34
3	3	01	11.68	17.41	30.44	49.77	74.67	55	44	33
3	4	01	11.68	16.66	31.86	56.89	78.48	59	47	35
3	1	08	28.21	32.32	41.65	62.31	81.52	65	55	45
3	2	08	28.21	33.23	42.32	62.72	82.11	66	55	46
3	3	08	28.21	32.34	39.00	57.16	79.29	63	52	43
3	4	08	28.21	33.41	42.34	62.13	80.72	66	56	47
3	1	09	35.42	36.31	48.30	64.92	82.80	68	60	51
3	2	09	35.42	36.14	46.97	68.65	82.11	69	60	52
3	3	09	35.42	37.84	48.81	66.52	80.35	67	60	52
3	4	09	35.42	37.50	42.85	65.74	84.24	68	58	49
3	1	10	28.21	29.10	39.36	62.85	77.62	64	54	44
3	2	10	28.21	29.53	48.04	64.54	78.83	65	57	48
3	3	10	28.21	30.70	40.76	59.97	77.08	63	54	44
3	4	10	28.21	30.79	38.72	65.90	79.45	64	55	46

[a] Period 1 = Neutral Detergent Washing
Period 2 = Acid Pepsin Washing
Period 3 = Washing Powder

[b] Treatments Code = 01 Control; 02 NDI; 03 NDIND; 04 IND: 05 API; 06 APIAP; 07 IAP; 08 WPI; 09 WPIWP; 10 IWP.

APPENDIX 6

Analysis of Variance of % Nitrogen Disappearance of Hay from Polyester Bags Incubated in the Rumen of Sheep and Treated with Different Washing Techniques

Source of Variation	D.F.	Variance Ratio (F)			
		3 hrs	8 hrs	24 hrs	72 hrs
Sheep	3	0.782	1.294	3.180	7.386
Period	2	243.144	44.869	33.531	155.584
Sheep & Period	6	0.883	0.640	2.538	10.694
Treatments in Period 1	3	84.337	53.788	47.544	161.775
Treatments in Period 2	3	417.427	143.276	96.701	119.890
Treatments in Period 3	3	214.922	105.829	113.594	138.332
Residual MS	27	7.343	13.110	5.306	0.773

APPENDIX 7

Analysis of Variance of % Nitrogen Concentration ($g\ Kg^{-1}$ DM) in Bag Residues

Source of Variation	D.F.	Variance Ratio (F)			
		3 hrs	8 hrs	24 hrs	72 hrs
Sheep	3	1.083	1.257	0.925	2.772
Period	2	249.364	48.554	45.731	141.768
Sheep & Period	6	0.813	0.666	2.756	6.161
Treatments in Period 1	3	30.352	29.496	42.485	94.736
Treatments in Period 2	3	243.153	96.616	97.889	79.960
Treatments in Period 3	3	86.998	53.034	73.076	129.083
Residual MS	27	0.170	0.3826	0.3331	0.1266

APPENDIX 8

Nitrogen Disappearances (%) and Nitrogen Concentration ($g\ kg^{-1}$ DM) in Bag Residues (NR) for All Washing Treatments.

Periods[a]	Sheep No	Treatments Code[b]	0 hrs	3 hrs	8 hrs	24 hrs	72 hrs	NR 0 hrs	NR 3 hrs	NR 8 hrs	NR 24 hrs	NR 72 hrs
1	1	01	16.35	32.47	45.26	63.43	78.08	9.8	8.7	8.3	9.2	7.0
1	2	01	16.35	24.19	40.43	66.41	78.83	9.8	9.6	8.8	7.8	7.6
1	3	01	16.35	28.99	41.52	66.40	79.91	9.8	9.1	8.7	7.8	7.4
1	4	01	16.35	24.22	40.25	62.92	80.34	9.8	9.8	9.9	9.8	7.3
1	1	02	37.12	46.24	58.83	70.19	81.46	8.4	8.2	7.3	8.7	6.9
1	2	02	37.12	48.67	55.64	65.57	86.20	8.4	7.8	7.9	10.6	7.1
1	3	02	37.12	49.61	58.28	71.07	82.66	8.4	7.5	7.6	7.6	7.0
1	4	02	37.12	42.08	60.25	67.74	86.66	8.4	8.2	7.0	8.9	6.3
1	1	03	44.89	58.51	79.41	81.77	89.79	7.6	6.1	3.8	5.1	4.4
1	2	03	44.89	57.39	65.05	78.74	93.34	7.6	6.4	6.4	6.4	3.7
1	3	03	44.89	57.38	73.59	82.44	91.24	7.6	6.4	5.1	5.1	3.9
1	4	03	44.89	54.56	73.79	80.64	93.27	7.6	7.4	4.9	5.8	3.6
1	1	04	37.12	50.51	64.24	78.31	85.32	8.4	7.1	6.2	5.7	5.1
1	2	04	37.12	51.38	70.55	79.64	91.17	8.4	7.2	5.3	6.0	3.9
1	3	04	37.12	42.69	63.15	78.27	89.48	8.4	8.2	6.4	5.0	4.3
1	4	04	37.12	48.93	63.83	81.46	91.23	8.4	7.4	6.5	5.3	4.0
2	1	01	16.35	20.32	39.89	68.79	79.45	9.8	10.0	9.5	8.3	8.3
2	2	01	16.35	24.62	38.54	69.13	80.01	9.8	9.3	8.9	8.7	7.7
2	3	01	16.35	26.29	39.42	56.09	78.39	9.8	9.4	9.3	9.0	8.3
2	4	01	16.35	24.93	41.89	63.96	78.27	9.8	9.4	8.7	9.5	8.5
2	1	05	77.86	75.53	76.38	74.23	83.54	3.1	3.6	4.4	8.0	9.0
2	2	05	77.86	78.15	74.71	73.96	85.72	3.1	3.3	4.9	7.5	7.2
2	3	05	77.86	74.36	72.54	72.04	82.22	3.1	4.1	5.0	8.5	8.0
2	4	05	77.86	77.57	76.18	70.72	82.01	3.1	3.2	4.5	9.3	9.0
2	1	06	83.89	81.39	90.57	90.10	90.08	2.3	2.9	1.9	3.1	5.8
2	2	06	83.89	84.36	87.95	89.38	89.94	2.3	2.4	2.4	3.5	4.9
2	3	06	83.89	81.76	86.08	83.40	89.44	2.3	2.9	2.7	5.1	6.1
2	4	06	83.89	80.51	89.78	89.26	89.20	2.3	3.1	2.0	3.6	6.1
2	1	07	77.86	80.55	84.85	86.92	88.91	3.1	2.8	2.7	3.3	5.0
2	2	07	77.86	79.65	82.98	87.51	88.65	3.1	2.9	3.0	3.7	5.2
2	3	07	77.86	79.02	80.73	85.02	87.41	3.1	3.1	3.5	3.7	5.3
2	4	07	77.86	77.74	79.13	86.96	87.33	3.1	3.2	3.4	3.4	5.7
3	1	01	16.35	21.08	41.80	68.93	85.52	9.8	9.9	8.5	7.9	5.6
3	2	01	16.35	23.30	33.03	63.02	82.73	9.8	9.7	9.8	8.3	7.1
3	3	01	16.35	21.07	40.26	57.30	81.94	9.8	10.0	8.9	8.8	7.2
3	4	01	16.35	26.52	27.58	63.63	84.65	9.8	9.2	11.1	8.7	7.2
3	1	08	46.15	56.98	67.75	77.92	90.49	7.9	6.7	5.9	6.2	5.5
3	2	08	46.15	53.35	68.88	78.56	89.15	7.9	7.4	5.7	6.1	6.5
3	3	08	46.15	56.47	61.20	76.95	87.20	7.9	6.9	6.7	5.7	6.5
3	4	08	46.15	53.12	70.14	80.44	87.96	7.9	7.5	5.5	5.5	6.5
3	1	09	63.09	67.60	77.77	89.79	96.48	6.0	5.4	4.6	3.1	2.2
3	2	09	63.09	70.50	76.44	90.97	95.25	6.0	4.9	4.7	3.1	2.8
3	3	09	63.09	73.80	81.01	90.36	94.22	6.0	4.5	4.0	3.1	3.1
3	4	09	63.09	68.12	75.78	91.19	95.43	6.0	5.4	4.5	2.7	3.1
3	1	10	46.15	55.02	69.47	88.13	93.41	7.9	6.7	5.4	3.4	3.1
3	2	10	46.15	58.96	75.41	87.62	94.12	7.9	6.2	5.0	3.7	3.0
3	3	10	46.15	52.88	72.02	85.91	92.08	7.9	7.3	5.0	3.7	3.7
3	4	10	46.15	56.01	66.18	88.35	93.43	7.9	6.7	5.9	3.6	3.4

[a] Period 1 = Neutral Detergent Washing
Period 2 = Acid Pepsin Washing
Period 3 = Washing Powder

[b] Treatments Code = 01 Control; 02 NDI; 03 NDIND; 04 IND: 05 API; 06 APIAP; 07 IAP; 08 WPI; 09 WPIWP; 10 IWP.

APPENDIX 9

Analysis of Variance of the Regression Equations for the 3 Populations Used in the Calibration Set for the Conventional Predictors of *in vivo* OMD.

Models		MADF	LIGA	NCOMD	PCOMD	NB48 OMD	IVOMD
Model 1	DF	120	120	117	120	120	120
	MS	26.19	19.07	18.39	17.82	12.73	10.44
Model 2	DF	118	118	115	118	118	118
	MS	20.27	10.91	17.12	10.21	12.93	10.57
Model 3	DF	116	116	113	116	116	116
	MS	19.81	10.63	16.95	8.911	12.27	9.942

APPENDIX 10

Analysis of Variance of the Regression Equations for the 3 Populations Used in the Calibration Set for the NIR Regression Equations

Models		1-term	2-term	3-term	4-term	5-term	6-term	7-term	8-term	9-term
Model 1	DF	120	119	118	117	116	115	114	113	112
	MS	18.97	14.13	11.38	9.496	8.387	7.303	6.769	6.13	5.88
Model 2	DF	118	117	116	115	114	113	112	111	110
	MS	16.39	9.097	9.051	7.563	7.293	6.982	6.331	5.833	5.632
Model 3	DF	116	113	110	107	104	101	98	95	92
	MS	16.42	8.903	8.529	7.434	7.011	6.747	6.44	5.574	5.489

APPENDIX 11

Analysis of Variance of the Individual Regression Equations for Each Population Used in the Calibration Set

Models		MADF			LIGA			NCOMD			PCOMD		
		Pop 2 & 4	Pop 2 & 3	Pop 4 & 3	Pop 2 & 4	Pop 2 & 3	Pop 4 & 3	Pop 2 & 4	Pop 2 & 3	Pop 4 & 3	Pop 2 & 4	Pop 2 & 3	Pop 4 & 3
Model 1	DF	97	93	48	97	93	48	94	92	46	97	93	48
	MS	23.78	25.21	23.49	10.32	21.59	19.87	20.02	15.59	18.96	18.91	12.92	13.10
Model 2	DF	96	92	47	96	92	47	93	91	45	96	92	47
	MS	21.54	18.58	21.11	10.16	12.27	9.793	18.21	15.46	18.33	10.01	9.343	11.91
Model 3	DF	95	91	46	95	91	46	92	90	44	95	91	46
	MS	21.42	18.28	19.53	10.09	12.08	8.878	18.11	15.43	17.64	9.193	8.838	8.476

APPENDIX 12

The Individual Values for in vivo OMD, in vivo DOMD, Chemical and Biological Parameters for the 122 Calibration Silages Used in this Study

Pop No*	Silage No	In vivo OMD	In vivo DOMD	MADF	LIGA	NCOMD	PCOMD	NB48 OMD	IVOMD	Ash %
2	79047	68.50	60.3485	330	2.745	71.7174	64.68	88.27	66.31	11.90
2	80022	71.93	65.4563	362	2.790	68.7019	63.89	81.40	70.80	9.00
2	80031	65.54	59.1826	415	2.803	54.8235	51.38	82.57	62.78	9.70
2	80170	68.58	61.9277	330	3.034	67.2223	57.72	82.10	64.45	9.70
2	80179	68.69	62.1645	282	2.195	76.0360	70.88	93.03	69.68	9.50
2	81003	52.80	47.4672	424	3.829	43.9377	36.83	61.47	47.53	10.10
2	81005	72.00	63.8640	326	2.693	72.3843	69.10	91.00	70.01	11.30
2	81006	75.93	70.6149	283	2.511	77.7419	67.71	87.83	69.27	7.00
2	81007	58.56	51.7085	353	3.105	61.1657	58.31	85.03	63.45	11.70
2	81012	82.79	73.6831	261	1.817	84.7389	81.23	96.83	75.06	11.00
2	81014	62.70	58.2483	385	3.081	56.3630	55.34	80.30	60.61	7.10
2	81020	72.31	66.3806	318	2.761	63.0074	62.01	87.30	64.77	8.20
2	82004	63.89	57.1177	399	2.977	55.8652	54.38	76.77	60.72	10.60
2	82005	65.73	59.9458	385	3.176	*	49.53	71.53	56.71	8.80
2	82008	64.74	60.1435	348	2.717	59.6340	53.28	78.90	60.95	7.10
2	82013	65.92	60.4486	368	2.913	65.1036	56.25	81.50	61.68	8.30
2	82014	66.57	61.1778	392	3.013	62.8944	54.66	72.17	60.44	8.10
2	82015	72.85	66.6578	353	2.647	75.1913	66.47	88.07	68.83	8.50
2	82016	74.40	68.3736	342	2.544	68.5704	67.73	90.43	69.21	8.10
2	82022	66.58	60.2549	351	2.644	60.1360	61.57	81.50	66.21	9.50
2	82023	67.41	61.7476	373	2.658	64.8920	57.88	79.50	69.27	8.40
2	82025	72.35	65.7662	335	2.493	72.2772	67.23	88.27	68.39	9.10
2	82027	69.01	61.6259	334	2.429	66.7996	63.42	85.20	67.51	10.70
2	82031	71.86	66.4705	338	2.534	70.4865	65.57	87.37	68.48	7.50
2	82033	67.10	61.7991	371	2.825	62.1064	57.33	82.57	63.09	7.90
2	82057	61.00	57.7060	409	3.212	52.7029	50.53	73.80	56.04	5.40
2	82065	67.46	62.7378	408	2.612	44.7819	58.35	76.43	63.52	7.00
2	82074	66.87	60.2499	393	2.830	62.5567	59.95	83.70	65.91	9.90
2	82079	77.97	70.5810	257	2.151	77.1125	76.18	93.47	72.97	9.50
2	83007	70.18	63.7234	303	2.448	67.6808	67.36	83.80	69.38	9.20
2	83008	61.85	55.6650	366	2.954	52.6040	53.78	70.67	59.39	10.00
2	83013	79.59	72.1881	279	2.053	73.9203	77.82	93.87	74.40	9.30
2	83014	78.04	71.9529	304	1.981	68.0589	74.16	91.30	73.42	7.80
2	83021	72.63	65.1491	334	2.363	65.7921	67.68	90.80	69.81	10.30
2	83026	79.51	74.1033	301	2.229	68.2661	68.94	88.47	70.64	6.80
2	83027	69.53	62.9246	407	2.385	53.5362	61.20	85.73	60.08	9.50
2	83031	52.91	48.4127	397	3.430	50.6896	44.33	67.23	50.70	8.50
2	83036	73.51	67.2617	313	2.312	71.0478	71.28	90.90	71.58	8.50
2	83049	77.75	71.6855	322	2.724	70.3801	67.43	87.43	65.49	7.80
2	83065	67.62	60.3170	343	2.835	63.0456	61.20	80.10	63.38	10.80
2	83110	78.49	70.2485	298	2.222	75.8631	75.04	92.80	71.83	10.50
2	84018	66.30	61.9242	377	3.045	54.3314	56.51	77.00	62.55	6.60
2	84074	68.79	61.3607	333	2.654	69.0694	65.48	82.30	66.10	10.80
2	84087	61.61	57.0509	377	2.959	57.3031	56.96	76.70	62.02	7.40
2	85016	66.81	60.8639	306	2.418	68.5526	67.02	81.20	66.76	8.90
2	85017	82.09	74.7019	230	1.774	80.5738	80.79	95.97	77.87	9.00
2	85018	61.38	55.9172	322	2.538	55.6531	57.81	79.73	55.62	8.90
2	85022	64.72	57.9244	348	3.021	57.7654	51.17	75.37	57.02	10.50
2	85024	79.72	73.8207	312	2.364	69.7624	64.64	82.67	65.84	7.40
2	85026	80.92	74.5273	285	1.897	74.5797	76.66	94.27	75.38	7.90
2	85030	66.51	59.9255	367	2.862	58.8235	55.41	80.63	63.82	9.90
2	85033	61.17	55.6035	375	3.148	57.5446	55.83	76.67	60.43	9.10
2	85036	71.15	65.3157	381	2.551	52.5599	61.44	83.03	63.05	8.20
2	85037	65.01	60.9794	359	2.582	52.0548	56.40	77.27	57.34	6.20
2	85038	78.95	71.1340	321	2.171	66.2528	69.86	91.10	71.74	9.90
2	85041	78.56	72.1181	287	2.261	74.3790	74.53	92.83	72.75	8.20
2	85043	67.77	60.8575	317	2.283	65.7016	63.54	85.63	57.30	10.20
2	85048	71.90	63.9191	349	2.485	67.6041	65.24	81.00	65.12	11.10
2	85057	80.08	73.0330	291	1.991	75.8744	73.33	87.70	75.60	8.80
2	86005	73.16	66.5024	332	2.435	73.6673	66.94	86.03	69.92	9.10

APPENDIX 12 (cont)

Pop No	Silage No	In vivo OMD	In vivo DOMD	MADF	LIGA	NCOMD	PCOMD	NB48 OMD	IVOMD	Ash %
2	86015	70.35	62.8929	360	2.710	73.4141	55.40	81.07	60.24	10.60
2	86016	77.10	70.3923	335	1.833	79.6474	73.94	93.73	70.92	8.70
2	86018	74.99	69.2908	374	2.458	71.1850	64.29	84.50	67.26	7.60
2	86020	75.92	69.7705	319	2.330	73.9977	69.19	88.70	72.24	8.10
2	86021	75.84	70.2278	314	2.472	73.9618	67.40	87.63	69.13	7.40
2	86026	76.12	70.4110	338	2.125	76.1845	71.88	92.20	73.75	7.50
2	86029	70.92	63.1897	333	2.379	64.1975	60.86	85.77	62.67	10.90
2	86033	68.14	63.5065	383	2.640	64.0412	59.75	82.07	63.56	6.80
2	86036	75.61	66.9149	332	2.065	78.6869	71.66	89.50	71.02	11.50
2	86037	73.98	65.9902	354	2.435	68.7220	65.18	82.77	67.06	10.80
2	86042	78.42	69.4801	298	1.935	83.5214	73.19	92.10	72.56	11.40
2	86067	69.20	60.2040	328	2.165	79.0805	71.40	88.77	69.81	13.00
3	2	68.70	60.6552	423	3.140	66.8303	54.70	85.50	65.10	11.71
3	4	75.10	68.8442	373	2.850	66.7322	58.83	84.00	64.10	8.33
3	5	74.90	66.9232	353	2.740	75.2949	64.77	90.80	72.30	10.65
3	7	78.70	73.0729	366	2.580	75.6981	68.75	90.90	71.20	7.15
3	8	74.50	65.0758	338	2.570	77.4925	66.82	88.60	70.50	12.65
3	9	68.10	62.8767	424	3.420	53.9727	45.56	76.10	55.00	7.67
3	10	68.70	61.0056	364	3.010	69.7237	60.02	83.60	67.30	11.20
3	11	72.60	64.8318	406	3.020	65.1504	53.53	83.30	64.90	10.70
3	12	70.10	64.5972	386	2.850	66.4041	57.70	87.40	69.00	7.85
3	13	63.00	56.3787	410	3.160	62.2761	50.56	81.30	60.50	10.51
3	15	67.90	61.4902	428	3.170	62.3755	48.12	81.00	59.00	9.44
3	16	72.90	64.4363	400	3.150	70.0059	57.30	89.30	67.50	11.61
3	18	70.60	66.2228	390	3.100	67.6350	60.02	87.50	68.30	6.20
3	28	74.20	67.4478	309	2.530	79.7580	71.12	95.10	77.40	9.10
3	37	76.70	69.4902	382	2.860	69.4088	65.78	92.70	72.60	9.40
3	38	77.80	70.4090	358	3.185	71.9930	67.96	92.40	74.50	9.50
3	40	76.50	69.8445	355	3.000	68.6999	67.14	90.70	73.60	8.70
3	41	76.10	67.9573	414	3.160	64.1020	60.25	86.00	67.90	10.70
3	42	72.20	65.4854	344	2.875	71.9492	67.20	85.10	69.30	9.30
3	44	74.50	66.5285	351	2.765	68.5165	64.17	91.30	68.40	10.70
3	45	68.60	62.5632	382	3.185	62.8661	52.30	88.30	67.70	8.80
3	46	76.90	71.5939	368	2.915	67.6315	63.48	90.30	70.70	6.90
3	47	77.40	68.6538	370	3.060	68.4013	58.57	91.30	71.70	11.30
4	33	78.90	71.0889	331	2.160	69.5894	62.68	93.23	76.20	9.90
4	34	74.10	66.4677	380	2.300	67.3356	63.41	90.53	72.90	10.30
4	35	76.80	71.3472	351	2.370	68.7836	61.35	89.47	73.90	7.10
4	36	55.20	50.7840	418	3.380	49.6739	44.61	77.93	56.40	8.00
4	37	62.80	58.8436	396	2.840	52.7215	45.54	73.77	57.50	6.30
4	38	62.00	56.7920	384	3.250	53.2751	46.79	76.23	57.50	8.40
4	39	63.50	57.9120	372	3.140	52.5219	47.07	79.80	58.60	8.80
4	40	63.10	58.3675	374	3.240	53.9459	46.74	80.10	58.20	7.50
4	41	66.90	61.6818	441	2.890	48.4816	44.61	78.40	59.00	7.80
4	42	72.70	67.2475	357	2.630	56.8649	54.56	85.53	68.30	7.50
4	43	77.60	72.1680	319	2.350	69.4624	59.57	83.57	70.90	7.00
4	44	77.20	71.4100	325	2.310	69.2973	55.15	90.37	68.00	7.50
4	45	75.00	67.2750	365	2.410	53.7347	58.77	87.77	71.90	10.30
4	46	70.50	63.4500	344	2.470	54.0000	56.57	87.43	67.70	10.00
4	47	86.70	81.1512	357	1.800	69.6581	64.32	95.37	74.40	6.40
4	48	73.20	67.6368	366	2.610	61.3636	52.53	84.63	67.60	7.60
4	49	74.80	68.9656	345	2.390	66.2670	58.56	90.93	72.60	7.80
4	50	72.20	65.1966	334	2.310	65.3378	59.32	86.97	70.80	9.70
4	51	75.30	69.4266	317	2.370	63.8659	57.09	85.97	70.50	7.80
4	52	74.30	68.9504	338	2.610	69.7198	56.02	89.53	69.10	7.20
4	53	72.90	66.7764	362	2.270	71.8341	55.42	90.87	69.80	8.40
4	54	62.40	57.2208	399	3.000	58.6696	44.65	79.27	63.90	8.30
4	55	62.60	56.7156	426	3.300	58.1678	45.61	79.80	60.60	9.40
4	56	64.20	59.1924	380	2.940	61.6052	47.50	80.67	60.40	7.80
4	57	74.00	69.4120	342	2.330	*	57.39	89.70	70.10	6.20
4	58	72.50	66.7000	373	2.490	*	55.83	83.80	68.20	8.00
4	59	78.00	71.5260	353	2.090	73.1734	57.97	90.67	71.20	8.30

* Population Numbers = See Table 2.1

APPENDIX 13

Analysis of Variance to Test Whether the Bivariate Relationship has a Significant Improvement over the Univariate Relationship for <u>in vivo</u> Predictors Studied in this Work.

	IVOMD & NB48 OMD	IVOMD & PCOMD	IVOMD & NCOMD	IVOMD & MADF	IVOMD & LIGA	NB48 OMD & PCOMD	NB48 OMD & NCOMD	NB48 OMD & MADF	NB48 OMD & LIGA	PCOMD & NCOMD	PCOMD & MADF	PCOMD & LIGA	NCOMD & MADF	NCOMD & LIGA	LIGA & MADF
Res. DF for Univariate	120	120	117	120	120	120	117	120	120	117	120	120	117	117	120
Res. DF for Bivariate	119	119	116	119	119	119	116	119	119	116	119	119	116	116	119
Res. SS for Univariate	1252.4	1252.4	1252.4	1252.4	1252.4	1527	1527	1527	1527	2138.4	2138.4	2138.4	2151.7	2151.7	2288.9
Res. SS for Bivariate	1141.4	1191.8	1180.3	1223.4	1105.4	1370.4	1376.4	1471.4	1344.4	1841.4	2137.8	1857.9	2092.1	1630.9	2267.6
DF change from Univariate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SS change from Univariate	111	60.6	72.1	29	147	156.6	150.6	55.3	182.6	297	0.6	280.5	59.6	520.8	21.3
$MS = \frac{\text{SS change}}{\text{DF change}}$	111	60.6	72.1	29	147	156.6	150.6	55.3	182.6	297	0.6	280.5	59.6	520.8	21.3
Res. MS for Bivariate	9.592	10.01	10.17	10.28	9.29	11.52	11.87	12.37	11.30	15.87	17.96	15.61	18.04	14.06	19.06
$F = \frac{MS}{\text{Res. MS for Bivariate}}$	11.57	6.05	7.09	2.82	15.82	13.59	12.69	4.47	16.16	18.71	0.03	17.97	3.30	37.04	1.12

APPENDIX 14

700 Log $\frac{1}{R}$ Values (Data Points) taken at 2 nm Intervals Between 1100 and 2500 nm for One Silage Sample Only.

0.1664419025,0.1661406755,0.1659163088,0.1656093001,0.1653319299,0.1650668830
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0.5776500702,0.5785942078,0.5792701244,0.5796839595,0.5799936056,0.5798938274
0.5795146227,0.5791345835,0.5784724951,0.5776566867

APPENDIX 15

NIR Equations Produced by Stepwise Multiple Linear Regression

Coefficient		F	Wavelength	Math Treatment
Term 1				
B(0)	= 68.039			
B(1)	= -995.086	133.12	2290.	2 24. 4. 4.
Term 2				
B(0)	= 57.140			
B(1)	= -9740.772	77.64	1262.	1 16. 4. 4.
B(2)	= 2453.427	198.04	1662.	2 24. 4. 4.
Term 3				
B(0)	= 62.306			
B(1)	= 210.092	29.74	1910.	2 24. 4. 4.
B(2)	= -7679.309	52.32	1262.	1 16. 4. 4.
B(3)	= 2824.252	274.05	1662.	2 24. 4. 4.
Term 4				
B(0)	= 63.108			
B(1)	= -4580.140	37.46	1230.	2 24. 4. 4.
B(2)	= -6863.108	41.68	1274.	1 16. 4. 4.
B(3)	= 3571.792	202.59	1662.	2 24. 4. 4.
B(4)	= 799.446	54.38	2266.	2 24. 4. 4.
Term 5				
B(0)	= 67.510			
B(1)	= -5938.944	37.76	1262.	1 16. 4. 4.
B(2)	= 4410.001	183.22	1662.	2 24. 4. 4.
B(3)	= 917.587	68.18	2266.	2 24. 4. 4.
B(4)	= -1108.577	16.44	1646.	2 24. 4. 4.
B(5)	= -4583.659	40.06	1230.	2 24. 4. 4.
Term 6				
B(0)	= 74.250			
B(1)	= 3785.838	19.28	1150.	2 24. 4. 4.
B(2)	= -7890.381	59.17	1274.	1 16. 4. 4.
B(3)	= 3653.640	106.30	1662.	2 24. 4. 4.
B(4)	= 1008.835	96.94	2266.	2 24. 4. 4.
B(5)	= -1327.126	25.67	1646.	2 24. 4. 4.
B(6)	= -4478.543	43.00	1230.	2 24. 4. 4.

Coefficient		F	Wavelength	Math Treatment
Term 7				
B(0)	= 62.140			
B(1)	= 1113.195	13.01	2354.	2 24. 4. 4.
B(2)	= -5481.905	35.40	1274.	1 16. 4. 4.
B(3)	= 4281.137	187.92	1662.	2 24. 4. 4.
B(4)	= 1340.853	117.65	2266.	2 24. 4. 4.
B(5)	= -1415.554	30.48	1646.	2 24. 4. 4.
B(6)	= -4118.615	38.07	1230.	2 24. 4. 4.
B(7)	= 1696.556	30.45	2426.	2 24. 4. 4.
Term 8				
B(0)	= 63.973			
B(1)	= -4311.132	15.47	1842.	1 16. 4. 4.
B(2)	= -6062.110	35.83	1274.	1 16. 4. 4.
B(3)	= 3244.727	93.18	1662.	2 24. 4. 4.
B(4)	= 1027.880	104.43	2266.	2 24. 4. 4.
B(5)	= -1716.427	43.90	1646.	2 24. 4. 4.
B(6)	= -4355.047	46.93	1230.	2 24. 4. 4.
B(7)	= 1921.474	23.83	2426.	2 24. 4. 4.
B(8)	= 3245.068	14.57	1150.	2 24. 4. 4.
Term 9				
B(0)	= 63.560			
B(1)	= 882.543	5.81	1738.	2 24. 4. 4.
B(2)	= -4228.508	15.50	1842.	1 16. 4. 4.
B(3)	= -6258.256	39.55	1274.	1 16. 4. 4.
B(4)	= 3648.929	97.52	1662.	2 24. 4. 4.
B(5)	= 988.268	97.91	2266.	2 24. 4. 4.
B(6)	= -2244.549	44.82	1646.	2 24. 4. 4.
B(7)	= -4400.404	49.90	1230.	2 24. 4. 4.
B(8)	= 1888.862	23.98	2426.	2 24. 4. 4.
B(9)	= 3843.929	19.57	1150.	2 24. 4. 4.

APPENDIX 16

The Individual Values for in vivo OMD, in vivo DOMD, Chemical and Biological Parameters for the 48 Validation Silages Used in this Study.

Pop No*	Silage No	In vivo OMD	In vivo DOMD	MADF	LIGA	NCOMD	PCOMD	IVOMD	Ash %
1	77007	72.6278	63.8398	315.309	2.350	81.6637	62.04	70.1	12.10
1	78001	65.7616	58.6593	325.714	2.750	71.0762	54.18	63.8	10.80
1	78002	73.6872	65.1395	307.339	2.330	84.1629	60.19	72.2	11.60
1	78003	74.3995	66.2156	291.431	2.230	80.8989	66.36	73.7	11.00
1	78004	78.1094	68.9706	273.472	1.880	80.7475	70.78	73.2	11.70
1	78005	75.0695	67.5836	302.103	2.310	83.6848	62.21	72.7	9.90
1	78022	70.8724	63.1473	348.947	2.450	80.6958	60.28	73.2	10.90
1	78023	72.9512	66.6945	298.523	2.580	80.2048	59.38	71.8	8.70
1	78024	59.2022	49.2562	436.700	2.900	72.7164	55.37	57.6	16.80
1	78025	73.3333	66.0000	320.808	2.480	77.5556	63.23	71.8	10.00
1	78047	73.3293	64.0898	299.062	2.390	77.2311	62.85	70.2	12.60
1	79035	71.6568	61.4099	258.665	2.156	77.7130	63.12	68.1	14.30
1	79036	76.6455	66.9882	253.969	1.930	84.8970	68.98	71.8	12.60
1	79053	76.2389	68.4625	299.512	2.530	78.3984	63.61	70.1	10.20
1	B0021	73.5417	65.6727	352.083	2.590	70.3247	62.92	72.0	10.70
1	B0156	66.2402	58.0927	376.775	2.970	*	51.40	66.0	12.30
1	B0032	63.8563	56.7044	360.656	2.720	71.6216	54.12	63.2	11.20
1	B0049	75.9737	67.3887	285.657	2.370	77.4521	64.25	71.7	11.30
1	B0055	73.4447	66.3940	303.582	2.570	75.6637	59.17	69.7	9.60
1	B0056	68.7237	61.7139	382.840	2.950	*	55.15	69.9	10.20
1	B0099	63.1933	57.0003	354.444	2.890	68.5144	54.62	64.4	9.80
1	B0169	74.1511	66.0686	312.658	2.230	80.6958	67.56	69.8	10.90
1	B0178	62.1939	54.6062	367.015	3.120	66.0592	52.13	64.2	12.20
1	B0168	65.4938	59.0099	344.457	2.880	73.0300	53.67	66.2	9.90
1	B0171	71.5300	64.0193	359.934	2.900	77.0950	51.95	66.7	10.50
1	B0172	68.9405	60.7366	392.109	2.850	71.1691	55.38	68.9	11.90
1	B0175	61.3912	54.2698	391.490	3.170	59.8416	51.93	63.3	11.60
1	B0180	65.2946	59.1569	375.070	2.790	59.0508	55.41	65.5	9.40
3	1	64.3000	59.8826	453.000	3.520	53.7707	45.49	56.6	6.87
3	3	65.0000	59.2735	434.000	3.360	55.8668	45.45	60.0	8.81
3	6	60.9000	53.0195	352.000	2.390	68.7844	58.96	58.3	12.94
3	14	73.8000	67.9551	424.000	3.100	63.8727	53.85	62.8	7.92
3	19	66.3000	59.0070	385.000	3.450	63.6916	46.63	64.7	11.00
3	20	70.6000	64.5990	345.000	3.010	74.7372	65.85	73.7	8.50
3	21	70.8000	64.1448	349.000	2.950	71.4358	66.06	73.1	9.40
3	22	78.6000	71.1330	337.000	2.960	79.4038	73.20	78.2	9.50
3	23	75.5000	67.2705	351.000	2.860	75.7071	69.98	76.3	10.90
3	25	72.8000	67.1944	379.000	2.695	69.3263	63.16	71.0	7.70
3	26	68.3000	62.3579	414.000	3.260	65.6650	54.82	67.1	8.70
3	27	75.7000	68.9627	381.000	3.050	69.5411	59.93	71.6	8.90
3	29	71.6000	64.5116	422.000	3.195	62.2760	53.11	61.4	9.90
3	30	76.3000	69.5856	314.000	2.595	75.7597	72.31	75.2	8.80
3	32	81.5000	75.7950	336.000	2.875	76.6559	73.39	75.8	7.00
3	33	75.1000	68.7165	327.000	3.225	73.2692	69.13	72.2	8.50
3	39	74.0000	66.0820	367.000	2.960	64.1123	60.53	65.5	10.70
3	48	73.2000	68.2956	410.000	3.060	60.4990	57.13	67.2	6.70
3	50	71.4000	65.1882	381.000	3.165	61.9768	57.56	68.4	8.70
3	51	69.5000	63.6620	389.000	2.965	62.4572	52.24	62.9	8.40

*Population Numbers = See Table 2.1

APPENDIX 17

NIR Regression Equations Produced by Stepwise Multiple Linear Regression for the Prediction of IVOMD of the 122 Calibration Silages.

Coefficient		F	Wavelength	Math Treatment
Term 1				
B(0)	= 54.196			
B(1)	= -3455.186	123.82	2106.	1 16. 4. 4.
Term 2				
B(0)	= 53.523			
B(1)	= -4777.497	132.07	1454.	1 16. 4. 4.
B(2)	= -4947.764	77.70	1626.	2 24. 4. 4.
Term 3				
B(0)	= 56.183			
B(1)	= -6458.558	58.40	1286.	1 16. 4. 4.
B(2)	= -5378.479	235.62	1454.	1 16. 4. 4.
B(3)	= -3489.314	63.54	1626.	2 24. 4. 4.
Term 4				
B(0)	= 49.890			
B(1)	= -2144.556	27.80	1602.	1 16. 4. 4.
B(2)	= 1719.540	57.34	2378.	2 24. 4. 4.
B(3)	= -7156.548	71.54	1286.	1 16. 4. 4.
B(4)	= -3717.746	87.27	1454.	1 16. 4. 4.
Term 5				
B(0)	= 60.305			
B(1)	= 3854.424	123.14	2386.	2 24. 4. 4.
B(2)	= -4859.981	35.38	1286.	1 16. 4. 4.
B(3)	= -4617.785	164.55	1454.	1 16. 4. 4.
B(4)	= -4420.929	32.15	1258.	2 24. 4. 4.
B(5)	= -3262.613	115.83	2398.	2 24. 4. 4.
Term 6				
B(0)	= 53.539			
B(1)	= 2725.027	91.02	2382.	2 24. 4. 4.
B(2)	= -6197.911	70.36	1286.	1 16. 4. 4.
B(3)	= -5340.161	187.13	1454.	1 16. 4. 4.
B(4)	= 1637.996	38.88	1190.	2 24. 4. 4.
B(5)	= -1766.767	62.50	2398.	2 24. 4. 4.
B(6)	= -1945.402	14.36	1750.	2 24. 4. 4.

APPENDIX 17 (cont)

Coefficient		F	Wavelength	Math Treatment
Term 7				
B(0)	= 60.389			
B(1)	= -2220.655	26.56	1550.	1 16. 4. 4.
B(2)	= 3502.146	86.64	2378.	2 24. 4. 4.
B(3)	= -5929.965	66.52	1286.	1 16. 4. 4.
B(4)	= -3497.159	55.98	1454.	1 16. 4. 4.
B(5)	= -3682.150	24.92	1782.	1 16. 4. 4.
B(6)	= -1823.521	47.87	2390.	2 24. 4. 4.
B(7)	= -2907.229	34.26	1750.	2 24. 4. 4.
Term 8				
B(0)	= 59.150			
B(1)	= -3504.207	32.55	1778.	1 16. 4. 4.
B(2)	= -1950.031	50.85	2390.	2 24. 4. 4.
B(3)	= -3008.902	38.36	1750.	2 24. 4. 4.
B(4)	= -1709.148	11.07	1290.	2 24. 4. 4.
B(5)	= -2033.611	17.08	1554.	1 16. 4. 4.
B(6)	= 3809.010	100.52	2378.	2 24. 4. 4.
B(7)	= -5480.321	58.69	1286.	1 16. 4. 4.
B(8)	= -3981.551	67.52	1454.	1 16. 4. 4.
Term 9				
B(0)	= 63.340			
B(1)	= 1954.072	5.57	1294.	2 24. 4. 4.
B(2)	= -3599.017	35.57	1778.	1 16. 4. 4.
B(3)	= -2042.255	56.82	2390.	2 24. 4. 4.
B(4)	= -3136.075	42.81	1750.	2 24. 4. 4.
B(5)	= -3976.556	13.44	1290.	2 24. 4. 4.
B(6)	= -2146.583	19.61	1554.	1 16. 4. 4.
B(7)	= 3846.112	106.44	2378.	2 24. 4. 4.
B(8)	= -6218.856	65.57	1286.	1 16. 4. 4.
B(9)	= -3685.737	56.28	1454.	1 16. 4. 4.

APPENDIX 18

Characteristics of Silages Studied
With Their Corresponding Numbers

Silage No	Y	MEn	CN	WT	AL	NF	Add.
77087	3	1	1	2	1	0	3
78001	4	1	2	2	1	3	1
78002	4	1	1	1	1	3	1
78003	4	1	1	3	2	2	2
78004	4	1	2	2	2	2	2
78005	4	1	1	0	2	0	2
78022	4	0	0	0	0	0	0
78023	4	1	1	1	2	3	1
78024	4	1	0	2	4	2	1
78025	4	1	1	2	1	2	1
78047	4	1	2	2	2	3	1
79035	5	1	3	2	2	2	1
79036	5	1	1	3	2	2	1
79053	5	1	1	1	0	2	2
80021	6	1	1	2	2	1	1
80156	6	1	1	2	1	2	3
80032	6	1	2	2	1	1	3
80049	6	1	1	2	3	1	2
80055	6	1	2	2	2	2	0
80056	6	1	1	0	2	2	0
80099	6	1	2	2	1	1	3
80169	6	1	3	2	2	1	1
80178	6	1	2	0	2	2	0
80168	6	1	2	2	2	1	1
80171	6	1	0	2	2	3	2
80172	6	1	1	2	2	2	1
80175	6	1	1	3	1	2	0
80180	6	0	0	0	0	0	0
79047	5	1	1	3	3	1	1
80022	6	1	1	2	2	0	0
80031	6	1	1	2	1	2	3
80170	6	1	2	2	3	2	0
80179	6	1	3	2	3	1	1
81003	6	2	1	3	3	0	1
81005	7	1	2	2	2	1	1
81006	7	1	1	2	2	2	1
81007	7	1	1	2	2	2	3
81012	7	1	1	2	1	1	2
81014	7	1	1	2	1	1	2
81020	7	1	0	2	0	1	1
82004	8	1	2	2	2	2	3
82005	8	2	0	2	4	1	1
82008	8	2	1	3	3	1	1
82013	8	2	1	3	4	2	1
82014	8	2	1	3	4	2	1
82015	8	1	2	2	3	2	2
82016	8	1	2	2	3	2	2
82022	8	1	0	0	0	2	2
82023	8	0	1	0	3	2	2
82025	8	2	1	1	4	3	1
82027	8	1	0	2	1	2	2
82031	8	2	1	3	4	2	1
82033	8	2	1	3	3	2	1
82057	8	1	0	1	2	2	2
82065	8	1	0	1	2	2	2
82074	8	0	0	0	0	0	0
82079	8	1	1	0	0	3	3
83007	9	1	1	2	2	2	3
83008	9	1	2	2	2	1	3
83013	9	1	1	2	2	1	0
83014	9	1	1	0	2	1	0
83021	9	1	1	1	3	2	0
83026	9	1	1	0	4	0	2

APPENDIX 18 (cont)

Silage No	Y	MEn	CN	WT	AL	NF	Add.
83027	9	1	0	2	3	2	3
83031	9	1	1	2	4	1	1
83036	9	0	0	0	0	0	0
83049	9	1	0	0	0	2	2
83065	9	1	0	0	2	2	3
83110	9	1	0	2	0	3	3
84018	10	1	1	2	3	1	3
84074	10	2	1	3	1	2	1
84087	10	2	3	3	1	2	1
85016	11	1	0	2	4	2	2
85017	11	1	2	2	3	1	3
85018	11	1	3	2	4	0	3
85022	11	2	2	2	2	3	1
85024	11	1	2	2	1	2	2
85026	11	1	1	2	3	1	1
85030	11	2	1	2	4	0	1
85033	11	2	1	2	1	2	1
85036	11	1	1	0	0	0	0
85037	11	1	2	0	0	0	0
85038	11	0	0	0	0	0	0
85041	11	2	1	2	1	1	1
85043	11	2	2	3	0	1	1
85048	11	2	2	2	2	1	1
85057	11	0	0	0	0	0	0
86005	12	0	0	0	0	0	0
86015	12	1	2	1	4	1	0
86016	12	1	1	1	4	2	3
86018	12	1	1	1	4	2	3
86020	12	1	1	2	3	2	3
86021	12	1	1	1	4	2	3
86026	12	1	1	2	2	2	3
86029	12	2	3	1	4	1	1
86033	12	1	3	2	2	2	0
86036	12	1	0	1	2	1	2
86037	12	2	0	0	0	0	1
86042	12	2	2	0	3	1	1
86067	12	0	0	0	0	0	0

Y = Year of harvest - 3 = 1977; 4 = 1978; 5 = 1979; 6 = 1980;
7 = 1981; 8 = 1982; 9 = 1983; 10 = 1984; 11 = 1985; 12 = 1986.

MEn = Method of Ensiling - 0 = No data; 1 = clamp; 2 = big bale.

CN = Cut number - 0 = No data; 1 = first cut; 2 = second cut;
3 = third cut.

WT = wilting time - 0 = No data; 1 $\leq$ 12 hrs; 2 = > 12-40 hrs;
3 = > 40 hrs.

AL = Age of Ley (Years) - 0 = No data; 1 = $\leq$ 1; 2 = > 1-5; 3 = > 5;
4 = Permanent Pasture.

NF = Nitrogen Fertilisation (KgN/ha) - 0 = No data; 1 = $\leq$ 100;
2 = > 100-200; 3 = >200.

Add = Additive Use - 0 = No data; 1 = None; 2 = Add-F;
3 = Sylade + F100.

APPENDIX 19

Analysis of Variance of the Effect of Different Characteristics of Silages on the *in vivo* OMD : Laboratory Methods Relationships.

Characteristics	Prediction Models		MADF	LIGA	NCOMD	PCOMD	IVOMD	NIR 8-TERM
Years	Model 1	DF	84	84	81	84	84	77
		MS	17.47	10.95	20.59	9.508	10.74	5.297
	Model 2	DF	79	79	76	79	79	72
		MS	16.39	11.39	14.33	9.261	7.752	5.255
	Model 3	DF	74	74	71	74	74	32
		MS	16.46	11.12	13.18	8.754	7.743	3.319
Method of Ensiling	Model 1	DF	89	89	86	89	89	82
		MS	18.24	11.16	19.24	10.28	10.62	5.194
	Model 2	DF	88	88	85	88	88	81
		MS	18.28	11.26	19.43	10.31	10.46	5.229
	Model 3	DF	87	87	84	87	87	73
		MS	17.89	11.38	18.84	10.41	10.54	5.151
Cut Number	Model 1	DF	75	75	73	75	75	68
		MS	18.31	9.453	16.81	9.315	10.41	5.117
	Model 2	DF	73	73	71	73	73	66
		MS	16.91	7.771	16.62	8.883	10.43	5.100
	Model 3	DF	71	71	69	71	71	52
		MS	16.48	7.918	16.95	9.127	10.37	4.996
Wilting Time	Model 1	DF	76	76	74	76	76	69
		MS	18.59	10.89	17.36	10.77	9.596	5.350
	Model 2	DF	74	74	72	74	74	67
		MS	16.99	10.92	16.91	10.41	9.372	5.271
	Model 3	DF	72	72	70	72	72	51
		MS	17.33	10.95	16.36	10.62	9.257	5.262

APPENDIX 19 (cont)

Characteristics	Prediction Models		MADF	LIGA	NCOMD	PCOMD	IVOMD	NIR 8-TERM
Age of Ley	Model 1	DF	80	80	77	80	80	73
		MS	18.43	9.537	18.78	10.48	9.720	4.998
	Model 2	DF	77	77	74	77	77	70
		MS	18.99	9.736	18.69	10.[illegible]	8.936	4.815
	Model 3	DF	74	74	71	74	74	46
		MS	19.03	9.624	18.74	10.99	8.760	4.592
Additive Application	Model 1	DF	77	77	75	77	77	70
		MS	19.12	11.62	18.29	10.95	10.49	5.381
	Model 2	DF	75	75	73	75	75	68
		MS	18.33	11.37	17.13	10.65	10.57	5.237
	Model 3	DF	73	73	71	73	73	52
		MS	18.62	11.55	17.30	10.93	10.71	5.078
N Fertilisation	Model 1	DF	80	80	77	80	80	73
		MS	16.60	10.42	18.29	10.25	11.23	5.335
	Model 2	DF	78	78	75	78	78	71
		MS	16.79	10.41	18.61	9.915	11.24	5.475
	Model 3	DF	76	76	73	76	76	55
		MS	15.82	10.59	18.66	9.623	11.46	6.286

APPENDIX 20

NIR Regression Equations Produced by Stepwise Multiple Linear Regression for the Prediction of *in vivo* DOMD of the 122 Calibration Silages.

Coefficient		F	Wavelength	Math Treatment
Term 1				
B(0)	= 62.116			
B(1)	= -894.834	113.49	2290.	2 24. 4. 4.
Term 2				
B(0)	= 61.536			
B(1)	= -3985.890	37.72	1198.	1 16. 4. 4.
B(2)	= -1191.807	183.50	2290.	2 24. 4. 4.
Term 3				
B(0)	= 52.448			
B(1)	= 1802.993	51.35	1666.	2 24. 4. 4.
B(2)	= -6231.985	90.77	1206.	1 16. 4. 4.
B(3)	= -1039.753	165.87	2290.	2 24. 4. 4.
Term 4				
B(0)	= 70.706			
B(1)	= -5956.012	86.37	1210.	1 16. 4. 4.
B(2)	= -880.455	116.60	2290.	2 24. 4. 4.
B(3)	= 1404.923	22.16	2258.	1 16. 4. 4.
B(4)	= 3953.349	79.66	1666.	2 24. 4. 4.
Term 5				
B(0)	= 69.150			
B(1)	= 2299.533	6.18	1254.	2 24. 4. 4.
B(2)	= -6583.203	94.85	1210.	1 16. 4. 4.
B(3)	= -846.068	109.16	2290.	2 24. 4. 4.
B(4)	= 1386.306	22.52	2258.	1 16. 4. 4.
B(5)	= 3718.450	70.26	1666.	2 24. 4. 4.
Term 6				
B(0)	= 56.523			
B(1)	= 3717.525	143.87	1666.	2 24. 4. 4.
B(2)	= -4786.052	169.17	1550.	1 16. 4. 4.
B(3)	= 2203.421	20.57	1194.	2 24. 4. 4.
B(4)	= 6430.177	29.84	1254.	2 24. 4. 4.
B(5)	= -5966.962	87.29	1210.	1 16. 4. 4.
B(6)	= -5192.953	64.04	1842.	1 16. 4. 4.

Coefficient		F	Wavelength	Math Treatment
Term 7				
B(0)	= 40.814			
B(1)	= -1895.510	23.20	1638.	2 24. 4. 4.
B(2)	= 914.450	100.11	2270.	2 24. 4. 4.
B(3)	= 6074.395	27.61	1254.	2 24. 4. 4.
B(4)	= -4981.553	54.88	1214.	1 16. 4. 4.
B(5)	= -4516.571	19.17	1842.	1 16. 4. 4.
B(6)	= 3192.590	80.70	2422.	2 24. 4. 4.
B(7)	= 1848.087	27.88	1666.	2 24. 4. 4.
Term 8				
B(0)	= 42.377			
B(1)	= 609.605	20.88	1402.	2 24. 4. 4.
B(2)	= -2038.191	31.27	1638.	2 24. 4. 4.
B(3)	= 1000.800	134.09	2270.	2 24. 4. 4.
B(4)	= 5515.801	26.39	1254.	2 24. 4. 4.
B(5)	= -4694.963	56.67	1214.	1 16. 4. 4.
B(6)	= -5064.583	27.86	1842.	1 16. 4. 4.
B(7)	= 2551.743	51.18	2422.	2 24. 4. 4.
B(8)	= 2136.619	42.15	1666.	2 24. 4. 4.
Term 9				
B(0)	= 47.371			
B(1)	= -2402.929	14.36	1226.	2 24. 4. 4.
B(2)	= -5361.297	28.83	1842.	1 16. 4. 4.
B(3)	= 2580.007	49.89	2422.	2 24. 4. 4.
B(4)	= 1472.308	21.84	1666.	2 24. 4. 4.
B(5)	= -5306.342	44.82	1246.	1 16. 4. 4.
B(6)	= 648.392	21.84	1402.	2 24. 4. 4.
B(7)	= -2582.279	52.21	1638.	2 24. 4. 4.
B(8)	= 857.777	96.35	2270.	2 24. 4. 4.
B(9)	= 5986.215	23.95	1254.	2 24. 4. 4.

APPENDIX 21

NIR Regression Equations Produced by Stepwise Multiple Linear Regression for the Prediction of Ash Content of the 122 Calibration Silages

Coefficient		F	Wavelength	Math Treatment
Term 1				
B(0)	= 9.814			
B(1)	= 1126.983	53.20	1354.	2 24. 4. 4.
Term 2				
B(0)	= 11.677			
B(1)	= -839.474	21.53	1746.	2 24. 4. 4.
B(2)	= 977.457	44.59	1354.	2 24. 4. 4.
Term 3				
B(0)	= 11.416			
B(1)	= 1163.756	33.19	1350.	2 24. 4. 4.
B(2)	= -463.117	30.41	1970.	2 24. 4. 4.
B(3)	= -1052.198	38.20	1730.	1 16. 4. 4.
Term 4				
B(0)	= 10.978			
B(1)	= -501.344	10.23	1654.	1 16. 4. 4.
B(2)	= 1247.942	40.41	1350.	2 24. 4. 4.
B(3)	= -428.700	27.60	1970.	2 24. 4. 4.
B(4)	= -1594.957	45.69	1730.	1 16. 4. 4.
Term 5				
B(0)	= 9.924			
B(1)	= -1690.048	40.29	1650.	1 16. 4. 4.
B(2)	= 1408.094	60.94	1350.	2 24. 4. 4.
B(3)	= -511.948	47.90	1970.	2 24. 4. 4.
B(4)	= -2638.294	82.70	1730.	1 16. 4. 4.
B(5)	= -570.620	29.93	1594.	2 24. 4. 4.
Term 6				
B(0)	= 9.779			
B(1)	= -2045.767	58.82	1650.	1 16. 4. 4.
B(2)	= 858.485	13.45	1350.	2 24. 4. 4.
B(3)	= -720.777	61.39	1966.	2 24. 4. 4.
B(4)	= -2784.137	99.19	1730.	1 16. 4. 4.
B(5)	= -722.736	44.62	1594.	2 24. 4. 4.
B(6)	= 1011.146	14.75	1310.	1 16. 4. 4.

APPENDIX 21 (cont)

Coefficient			F	Wavelength	Math Treatment
Term 7					
B(0)	=	10.279			
B(1)	=	-65.058	5.97	1898.	2 24. 4. 4.
B(2)	=	-1865.691	47.27	1650.	1 16. 4. 4.
B(3)	=	795.848	11.91	1350.	2 24. 4. 4.
B(4)	=	-729.128	65.44	1966.	2 24. 4. 4.
B(5)	=	-2615.032	85.80	1730.	1 16. 4. 4.
B(6)	=	-688.541	41.52	1594.	2 24. 4. 4.
B(7)	=	1429.274	21.34	1310.	1 16. 4. 4.
Term 8					
B(0)	=	11.342			
B(1)	=	-854.734	50.21	1890.	1 16. 4. 4.
B(2)	=	-809.134	51.14	1966.	2 24. 4. 4.
B(3)	=	-893.614	41.18	1730.	1 16. 4. 4.
B(4)	=	1041.033	67.04	1426.	2 24. 4. 4.
B(5)	=	-646.456	24.17	1398.	1 16. 4. 4.
B(6)	=	1563.721	22.13	1298.	1 16. 4. 4.
B(7)	=	1284.778	26.06	1866.	1 16. 4. 4.
B(8)	=	565.039	48.43	1910.	1 16. 4. 4.
Term 9					
B(0)	=	8.290			
B(1)	=	546.444	50.05	1910.	1 16. 4. 4.
B(2)	=	-590.868	13.43	1650.	1 16. 4. 4.
B(3)	=	-980.446	67.29	1890.	1 16. 4. 4.
B(4)	=	-796.440	54.94	1966.	2 24. 4. 4.
B(5)	=	-1250.521	58.01	1730.	1 16. 4. 4.
B(6)	=	1136.707	84.76	1426.	2 24. 4. 4.
B(7)	=	-754.803	34.63	1398.	1 16. 4. 4.
B(8)	=	1331.748	17.13	1298.	1 16. 4. 4.
B(9)	=	1737.466	41.74	1866.	1 16. 4. 4.

APPENDIX 22

Analysis of Variance of the Prediction of *in vivo* DOMD Using Various Routes by NIR

1 MS uncorrected for bias =

$$\frac{\Sigma(\text{Actual DOMD} - \text{Predicted DOMD})^2}{N} = \frac{A}{N}$$

2 MS corrected for bias =

$$\frac{\Sigma(\text{Actual DOMD} - \text{Predicted DOMD} - \text{Bias})^2}{N - 1} = \frac{B}{N - 1}$$

3 MS corrected for intercept and slope =

$$\frac{\Sigma(\text{Actual DOMD} - \text{Intercept} - \text{Slope x Predicted DOMD})^2}{N - 2} = \frac{C}{N - 2}$$

$$\text{Error MS to do F Tests} = \frac{C}{(N - 2)} = D$$

F Tests

$$\text{To Compare 1 with 2} = \frac{(A - B)}{D} \text{ at DF of } 1,46$$

$$\text{To Compare 1 with 3} = \frac{(A - C)}{2 * D} \text{ at DF of } 2,46$$

$$\text{To Compare 2 with 3} = \frac{(B - C)}{D} \text{ at DF of } 1,46$$

Analysis of Variance Table

Routes	A	B	C	D	Calculated F		
					1 & 2	1 & 3	2 & 3
1	424.60	423.35	419.2	9.11	0.137	0.296	0.456
2	395.15	345.35	343.16	7.46	6.680	3.480	0.293
3	284.45	264.23	257.40	5.59	3.620	2.420	1.220

APPENDIX 23

The Individual Values for in vivo DE, Chemical and Biological Parameters Used for the Prediction of in vivo DE.

Pop No*	Silage No	in vivo DE	MADF	LIGA	NCOMD	PCOMD	IVOMD
1	77087	14.7108	315.309	2.350	81.6837	62.04	70.10
1	78001	13.5107	325.714	2.750	71.0762	54.18	63.80
1	78002	15.7524	307.339	2.330	84.1629	60.19	72.20
1	78003	15.3027	291.431	2.230	80.8589	66.36	73.70
1	78004	17.2415	273.472	1.880	80.7475	70.78	73.20
1	78005	16.4555	302.103	2.310	83.6848	62.21	72.70
1	78022	13.7956	348.947	2.450	80.6958	60.28	73.20
1	78023	16.2152	298.523	2.580	80.2848	59.38	71.80
1	78024	10.7836	436.700	2.900	72.7164	55.37	57.60
1	78025	16.7368	320.808	2.480	77.5556	63.23	71.80
1	78047	14.0869	299.062	2.390	77.2311	62.85	70.20
1	79035	14.3929	258.665	2.150	77.7130	63.12	68.10
1	79036	16.3296	253.969	1.930	84.8970	68.98	71.80
1	79053	14.0878	299.512	2.550	78.3564	63.61	70.10
1	80021	14.2350	352.083	2.590	70.3247	62.92	72.00
1	80156	14.5001	366.775	2.970	*	51.40	66.00
1	80032	15.1272	360.656	2.720	71.6216	54.12	63.20
1	80049	14.3853	285.657	2.370	77.4521	64.25	71.70
1	80055	16.0359	303.582	2.570	75.6637	59.17	69.70
1	80056	12.7871	382.840	2.950	*	55.15	69.90
1	80099	14.7667	354.444	2.890	69.5144	54.62	64.40
1	80169	17.3772	312.658	2.230	80.6956	67.56	69.80
1	80178	11.1271	367.015	3.120	66.0592	52.13	64.20
1	80168	13.2132	344.457	2.880	73.0300	53.67	66.20
1	80171	15.3009	359.934	2.900	77.0950	51.95	66.70
1	80172	14.1183	392.109	2.850	71.1691	55.38	68.90
1	80175	12.3255	391.490	3.170	59.8416	51.93	63.30
2	79047	14.2556	330.000	2.745	71.7174	64.68	66.31
2	80022	15.0782	362.000	2.790	68.7019	63.89	70.80
2	80031	14.0526	415.000	2.803	54.8235	51.38	62.78
2	80170	14.8684	330.000	3.034	67.2223	57.72	64.45
2	80179	14.0184	282.000	2.195	76.0360	70.88	69.68
2	81003	9.5316	424.000	3.829	43.9377	36.83	47.53
2	81005	15.1828	326.000	2.603	72.3843	69.10	70.01
2	81006	15.0258	283.000	2.511	77.7419	67.71	69.27
2	81007	13.0605	353.000	3.105	61.1657	58.31	63.45
2	81012	15.7175	261.000	1.817	84.7389	81.23	75.06
2	81014	12.0392	385.000	3.081	56.3630	55.34	60.61
2	81020	13.7589	318.000	2.761	63.0974	62.01	64.77
2	82004	15.6271	399.000	2.977	55.8652	54.38	60.72
2	82005	14.0136	385.000	3.176	*	49.53	58.71
2	82008	11.8367	348.000	2.717	59.6340	53.28	60.95
2	82013	13.8817	368.000	2.913	65.1036	56.25	61.68
2	82014	12.2187	392.000	3.013	62.8944	54.66	60.44
2	82015	13.1133	353.000	2.647	75.1913	66.47	68.83
2	82016	13.5995	342.000	2.544	68.5704	67.73	69.21
2	82022	13.7073	351.000	2.644	60.1360	61.57	66.21
2	82023	14.2425	373.000	2.658	64.8520	57.88	63.27
2	82025	14.6005	335.000	2.493	72.2772	67.23	68.39
2	82027	15.0936	334.000	2.429	66.7996	63.42	67.51
2	82031	14.1419	338.000	2.534	70.4865	65.57	68.48
2	82033	14.0947	371.000	2.825	62.1064	57.33	63.09
2	82057	13.9065	409.000	3.212	52.7029	50.53	56.04
2	82065	15.2040	408.000	2.612	44.7819	58.35	63.52
2	82074	16.7394	393.000	2.830	62.5567	59.95	65.91
2	82079	15.9974	257.000	2.151	77.1225	76.18	72.97
2	83007	15.0119	303.000	2.448	67.6808	67.36	69.38
2	83008	14.9631	366.000	2.754	52.6940	53.78	59.39
2	83013	17.7681	279.000	2.053	73.9203	77.82	74.40
2	83014	18.0979	304.000	1.781	68.0589	74.16	73.42

APPENDIX 23 (cont)

Pop No	Silage No	In vivo DE	MADF	LIGA	NCOMD	PCOMD	IVOMD
2	B3021	17.3774	334.000	2.363	65.7921	67.68	69.81
2	B3026	17.0312	301.000	2.229	68.2861	68.94	70.64
2	B3027	18.9344	407.000	2.385	53.5362	61.20	60.08
2	B3031	10.6224	397.000	3.430	50.6876	44.33	50.70
2	B3034	16.8437	313.000	2.312	71.0478	71.28	71.58
2	B3049	16.8739	322.000	2.724	70.3801	67.43	65.49
2	B3065	12.8325	343.000	2.835	63.0456	61.20	63.38
2	B3110	15.9381	298.000	2.222	75.8631	75.04	71.83
2	B4018	14.1509	377.000	3.045	54.3314	56.51	62.55
2	B4074	11.6966	333.000	2.654	69.0694	65.48	66.10
2	B5016	13.6989	306.000	2.418	68.5526	67.02	66.76
2	B5017	17.8308	230.000	1.774	80.5738	80.79	77.87
2	B5018	13.3615	322.000	2.538	55.6531	57.81	55.62
2	B5022	12.5564	348.000	3.021	57.7654	51.17	57.02
2	B5024	13.5575	312.000	2.364	69.7624	64.64	65.94
2	B5026	16.5319	285.000	1.897	74.5797	76.66	75.38
2	B5030	11.8578	367.000	2.862	58.8235	55.41	63.82
2	B5033	12.9363	375.000	3.148	57.5446	55.83	60.43
2	B5036	16.7603	381.000	2.551	52.5599	61.44	63.05
2	B5037	15.9694	359.000	2.582	52.0548	56.40	57.34
2	B5038	16.6381	321.000	2.171	66.2528	69.86	71.74
2	B5041	16.6503	287.000	2.261	74.3790	74.53	72.75
2	B5043	13.2948	317.000	2.283	65.7016	63.54	57.30
2	B5048	14.0000	349.000	2.485	67.6041	65.24	65.12
2	B5057	16.4209	291.000	1.991	75.8744	73.33	75.60
2	B6005	14.7491	332.000	2.435	73.8873	66.94	69.92
2	B6015	17.6207	360.000	2.710	73.4141	55.40	60.24
2	B6016	19.2503	335.000	1.833	79.6474	73.94	70.92
2	B6018	15.9797	374.000	2.458	71.1850	64.29	67.26
2	B6020	17.5638	319.000	2.330	73.9977	69.19	72.24
2	B6021	15.8518	314.000	2.472	73.9618	67.40	69.13
2	B6026	16.2298	338.000	2.125	76.1845	71.88	73.75
2	B6029	16.3439	333.000	2.379	64.1975	60.86	62.67
2	B6033	15.0564	383.000	2.640	64.0412	59.75	63.56
2	B6036	20.6300	332.000	2.965	78.6869	71.66	71.02
2	B6037	15.6059	354.000	2.435	68.7220	65.18	67.06
2	B6042	14.3817	298.000	1.935	83.5214	73.19	72.58
2	B6067	14.1505	328.000	2.165	79.0805	71.40	69.81
3	1	13.6948	453.000	3.520	53.7707	45.49	56.60
3	2	14.5237	423.000	3.140	66.8303	54.70	65.10
3	3	13.1103	434.000	3.360	55.8668	45.45	60.00
3	4	15.2058	373.000	2.850	66.7322	58.83	64.10
3	5	15.6981	353.000	2.740	75.2949	64.77	72.30
3	7	16.5442	366.000	2.580	75.6981	68.75	71.20
3	8	15.9908	338.000	2.570	77.4925	66.82	70.50
3	9	13.8951	424.000	3.420	53.9727	45.56	55.90
3	10	14.1690	364.000	3.010	69.7237	60.02	67.30
3	11	15.9789	406.000	3.020	65.1504	53.53	64.90
3	12	15.0308	386.000	2.850	66.4041	57.70	69.00
3	13	15.1756	410.000	3.160	62.2961	50.56	60.50
3	14	14.9597	424.000	3.100	63.8727	53.85	62.80
3	15	15.4536	428.000	3.190	62.3755	48.12	59.00
3	16	15.5412	400.000	3.150	70.0059	57.30	67.50
3	18	15.3636	390.000	3.100	67.6350	60.02	68.50
3	19	13.6010	385.000	3.450	63.6916	46.63	64.70
3	20	15.3276	345.000	3.010	74.7372	65.85	73.70
3	21	15.2295	349.000	2.950	71.4358	66.96	73.10
3	22	16.5714	337.000	2.960	79.4038	73.20	78.20
3	23	16.0908	351.000	2.860	75.7071	69.98	76.30
3	25	16.4475	379.000	2.895	69.3263	63.16	71.00
3	26	14.1493	414.000	3.260	65.6650	54.82	67.10
3	27	16.8300	381.000	3.050	69.5411	59.93	71.60
3	28	15.9624	309.000	2.530	79.7580	71.12	77.40
3	29	16.0950	422.000	3.195	62.2760	53.11	61.40

Pop No	Silage No	In vivo DE	MADF	LIGA	NCOMD	PCOMD	IVOMD
3	30	15.9848	314.000	2.595	75.7597	72.31	75.20
3	32	16.5393	336.000	2.875	76.6559	73.39	75.80
3	33	15.9537	327.000	3.225	73.2692	69.13	72.20
3	37	16.5137	382.000	2.860	69.4088	65.78	72.60
3	38	15.7178	358.000	3.185	71.9930	67.96	74.50
3	39	15.1317	367.000	2.960	64.1123	60.53	65.50
3	40	16.6439	355.000	3.000	68.6999	67.14	73.60
3	41	16.5462	414.000	3.160	64.1020	60.25	67.90
3	42	14.2392	344.000	2.875	71.9492	67.20	69.30
3	44	15.2672	351.000	2.765	68.5165	64.17	68.40
3	45	14.2956	382.000	3.185	62.8661	52.30	67.70
3	46	17.4698	368.000	2.915	67.6315	63.48	70.70
3	47	16.3028	370.000	3.060	68.4013	58.57	71.70
3	48	13.6416	410.000	3.060	60.4990	57.13	67.20
3	50	15.4287	381.000	3.165	61.9968	57.56	68.40
3	51	15.4762	389.000	2.965	62.4572	52.24	62.90

For population numbers see Table 2.1

APPENDIX 24

Analysis of Variance of the Regression Equations for the 3 Populations Used in the Calibration Set for the Conventional Predictors of *in vivo* DE

Models		MADF	LIGA	NCOMD	PCOMD	IVOMD
Model 1	DF	138	138	135	138	138
	MS	2.729	2.327	2.545	2.051	2.078
Model 2	DF	136	136	133	136	136
	MS	2.438	1.686	2.308	1.919	1.996
Model 3	DF	134	134	131	134	134
	MS	2.453	1.696	2.294	1.873	1.949

APPENDIX 25

Analysis of Variance of Regression Equations for the 3 Populations Used in the Prediction of *in vivo* DE by NIR

Models		1-term	2-term	3-term	4-term	5-term	6-term	7-term	8-term	9-term
Model 1	DF	138	137	136	135	134	133	132	131	130
	MS	1.852	1.539	1.379	1.240	1.104	1.026	0.8445	0.8608	0.8328
Model 2	DF	136	135	134	133	132	131	130	129	128
	MS	1.606	1.440	1.358	1.241	1.114	1.013	0.9013	0.8661	0.8407
Model 3	DF	134	131	128	125	122	119	116	113	110
	MS	1.616	1.469	1.397	1.214	1.169	1.049	0.9243	0.8738	0.8783

APPENDIX 26

NIR Regression Equations Produced by Stepwise Multiple Linear Regression for the Prediction of in vivo DE of 140 Silages.

Coefficient		F	Wavelength	Math Treatment
Term 1				
B(0)	= 11.219			
B(1)	= -487.390	87.00	2278.	1 16. 4. 4.
Term 2				
B(0)	= 10.179			
B(1)	= -1017.376	29.06	1198.	1 16. 4. 4.
B(2)	= -630.472	133.69	2278.	1 16. 4. 4.
Term 3				
B(0)	= 9.724			
B(1)	= -534.728	16.94	2154.	2 24. 4. 4.
B(2)	= -1129.987	39.11	1198.	1 16. 4. 4.
B(3)	= -444.373	41.95	2278.	1 16. 4. 4.
Term 4				
B(0)	= 6.524			
B(1)	= -415.127	33.54	2278.	1 16. 4. 4.
B(2)	= 611.623	35.39	2434.	2 24. 4. 4.
B(3)	= -1196.941	39.77	1246.	1 16. 4. 4.
B(4)	= -506.960	14.24	2154.	2 24. 4. 4.
Term 5				
B(0)	= 7.388			
B(1)	= -166.540	30.78	2290.	2 24. 4. 4.
B(2)	= 559.237	27.17	2434.	2 24. 4. 4.
B(3)	= -1477.377	55.23	1246.	1 16. 4. 4.
B(4)	= -700.845	38.07	2154.	2 24. 4. 4.
B(5)	= -700.273	23.83	1218.	2 24. 4. 4.
Term 6				
B(0)	= 8.237			
B(1)	= -751.372	40.69	1486.	2 24. 4. 4.
B(2)	= -623.249	40.50	2410.	2 24. 4. 4.
B(3)	= -229.447	57.71	2290.	2 24. 4. 4.
B(4)	= 979.402	61.67	2434.	2 24. 4. 4.
B(5)	= -1242.039	41.52	1246.	1 16. 4. 4.
B(6)	= -681.980	27.37	2138.	2 24. 4. 4.

APPENDIX 26 (cont)

Coefficient		F	Wavelength	Math Treatment
Term 7				
B(0)	= 8.654			
B(1)	= -568.823	33.09	2198.	2 24. 4. 4.
B(2)	= -1433.322	226.52	2138.	2 24. 4. 4.
B(3)	= -1404.757	79.92	1210.	1 16. 4. 4.
B(4)	= -1064.857	54.17	1710.	1 16. 4. 4.
B(5)	= -596.431	46.41	2406.	2 24. 4. 4.
B(6)	= -725.474	15.58	1754.	2 24. 4. 4.
B(7)	= 763.223	49.39	2434.	2 24. 4. 4.
Term 8				
B(0)	= 8.357			
B(1)	= -549.836	8.06	1754.	2 24. 4. 4.
B(2)	= 733.533	46.82	2434.	2 24. 4. 4.
B(3)	= -820.179	6.17	1246.	1 16. 4. 4.
B(4)	= -560.672	33.37	2198.	2 24. 4. 4.
B(5)	= -1298.805	144.70	2138.	2 24. 4. 4.
B(6)	= -938.330	14.92	1210.	1 16. 4. 4.
B(7)	= -1226.279	61.72	1710.	1 16. 4. 4.
B(8)	= -505.501	29.32	2406.	2 24. 4. 4.
Term 9				
B(0)	= 9.883			
B(1)	= -899.840	7.53	1250.	1 16. 4. 4.
B(2)	= -690.570	38.33	2198.	2 24. 4. 4.
B(3)	= -1401.194	151.99	2138.	2 24. 4. 4.
B(4)	= -1079.015	24.02	1210.	1 16. 4. 4.
B(5)	= -1678.402	47.10	1710.	1 16. 4. 4.
B(6)	= -623.062	37.02	2402.	2 24. 4. 4.
B(7)	= 104.815	7.05	2306.	2 24. 4. 4.
B(8)	= -836.475	18.26	1754.	2 24. 4. 4.
B(9)	= 692.997	49.44	2434.	2 24. 4. 4.