More Accurate Laboratory Tests for Assessing Silage Quality

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Executive Summary

Accurate estimates of silage quality are needed by dairy farmers to formulate balanced diets for their cows, and as a management tool to assess the success of their silage making operation in producing silage of acceptable quality. Within the TopFodder Silage program farmers are being encouraged to have their silage tested to clearly establish the linkage between management practices and silage quality.

Currently, feed testing laboratories are using generalised procedures and prediction equations for estimating the feeding value of all forages. It has been assumed that these procedures are also suitable for silages but there is clear evidence that this is not the case, and that ME and protein content of silages are systematically underestimated. Silage differs from other ruminant feeds because it is a fermented product, and contains volatile compounds that are lost during conventional oven drying. Also, the type of silage fermentation will influence silage quality, voluntary intake (and palatability), and the utilisation of the silage nitrogen by animals, so some measure of silage fermentation quality is needed. Hence there are special issues to be considered when analysing silage, and the aim of this project was to develop laboratory procedures that will improve the accuracy of feed tests used to assess silage quality.

The project followed two lines of research. Firstly, 30 diverse silages, varying widely in forage type and expected digestibility, were produced for feeding experiments. Digestibility was measured in both sheep and cattle at close to the maintenance level of feeding, and the accuracy of various laboratory methods for predicting *in vivo* digestibility was then assessed. The second line of research investigated the errors in DM (and subsequently digestibility and ME content) and protein determination resulting from the oven drying of silage samples prior to analysis. Correction equations were developed to predict the "true" DM and protein content.

Although there were some differences between cattle and sheep in the digestibility of individual silages, overall the differences were small, and there was a close relationship between the two. There was a tendency for the digestibility of low quality silages to be higher in sheep, but the difference was small, and sheep could be used to predict silage digestibility in cattle.

Significant underestimation of silage ME (up to 1.5 MJ/kg DM) occurred when account was not taken of the volatile DM lost on oven drying, and inappropriate equations were used to estimate ME from digestibility data. Of the laboratory methods evaluated for the prediction of the *in vivo* digestibility of silages, those based on various fibre fractions (NDF, ADF and lignin) lacked precision and appear to be of little value for silage analyses. The pepsin cellulase method, which is currently used by a number of feed testing laboratories and appears to offer good precision with hays, did not perform as well with silages and explained only 52% of the variation in OMD (combined cattle and sheep data). The relationship for sheep only was a little better, explaining 66% of the variation. The summer growing forage crops (forage sorghums and pennisetum) appeared to be a problem for the pepsin cellulase method. If these data were removed the percentage of variation in *in vivo* OMD accounted for increased to 69% (74% for sheep only).

The *in vitro* digestibility method (using rumen fluid) was the most accurate laboratory method for estimating *in vivo* digestibility, accounting for 77% of the variation (combined sheep and

cattle data for OMD). This result compares favourably with UK data based on a much larger set of grass (predominantly ryegrass) silages.

Using hay standards produced at DPI, Hamilton, in an earlier RIRDC project, we compared the relationships between *in vivo* and *in vitro* digestibility and between *in vivo* and pepsin cellulase digestibility for the hays and silages. The silage and hay regressions were different but we believe this was due to the low N content of some the hays depressing *in vivo* digestibility. If the low N hays were removed from the data set the hay and silage regressions were similar, indicating that a combined hay/silage regression could be used by feed testing laboratories, provided the *in vivo* standards contain or are fed with sufficient rumen degradable N.

The difference between true DM (determined by Karl Fischer titration) and oven DM content was greater for low DM silages. A good relationship was found between the two, and an equation has been developed for feed testing laboratories to predict true DM from oven DM. Similarly an equation has been developed to predict the true N content of silages from N analyses conducted on oven dried silage samples. Both equations will be adopted by AFIA and incorporated into their Laboratory Methods Manual.

The results of this project will, when adopted by feed testing laboratories, improve the accuracy of silage analyses for farmers. In the longer term the best strategy for improving the accuracy of feed tests is to develop direct NIRS calibrations for *in vivo* digestibility. However the number of silages available at present is insufficient, so further *in vivo* work is recommended. Also the development of a set of silage samples (say 150) of known predicted *in vivo* digestibility, using the *in vitro* digestibility method and the currently available standards, would provide an opportunity for an NIRS calibration for use by feed testing laboratories. Other recommendations raised in the report are:-

- Determine whether an amylase digestion step will improve the capacity of the pepsin cellulase method to predict *in vivo* digestibility
- Determine whether the inclusion of gross energy in multiple regressions with *in vitro* or pepsin cellulase digestibility will improve the prediction of *in vivo* silage digestibility
- Develop a laboratory based method to estimate DM losses during the ensiling process.

Acknowledgements

We thank DRDC, MLA, RIRDC and NSW Agriculture for supporting this project, and Pacific Seeds Pty Ltd (member of Advanta), Heritage Seeds Pty Ltd, Snowy River Seeds Co-operative Ltd, Pioneer Hi-Bred Australia Pty Ltd and Seedco Australia Co-operative Ltd for donating seed for some of the silage crops. A number of local farmers and Haydn Kingston assisted with the provision of silages samples for some of the laboratory studies. We are greatly indebted to John Piltz for assistance with all aspects of the project, David Vonarx and Jean Smith for assistance with animal house experiments, the Wagga Wagga Agricultural Institute's farm staff for assistance with silage making, and Bev Orchard for advice on statistical analyses. We also thank Don Law and Peter Flinn for advice on chemical analyses – Peter Flinn also provided the hay standards for the silage *vs* hay comparisons and assisted with pepsin-cellulase analyses.

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Background and Introduction

Farmers and their advisers need accurate estimates of silage quality to formulate balanced diets for cows, and as a management tool to assess the success of their silage making operation in producing silage of acceptable quality. Hence within the TopFodder Silage program we are encouraging farmers to have their silage tested. This will clearly establish the linkage between management practices and silage quality. Our market research (early 2002) has shown that currently only 14% of dairy farmers normally test their silage. This is a major limitation to the adoption of best-practice silage management on dairy farms.

In the "Successful Silage" manual we have allocated a chapter to the use of feed testing to assess silage quality. This chapter covers the subject in some detail, with recommendations for farmers to:-

- Routinely test their silage
- Check that the estimated metabolisable energy (ME) and crude protein (CP) content take account of the volatiles lost on oven drying (we are currently developing correction equations)
- Ask for additional tests to assess silage fermentation quality – silage pH and ammonia-N content

Currently, most Australian feed testing laboratories are not taking account of silages volatiles in their analyses and are not providing tests to assess silage fermentation quality. So the TopFodder Silage program will work with both farmers and laboratories to ensure that the number of silage samples submitted to the laboratories will increase, and that appropriate silage tests are adopted by the industry.

How is Silage Different?

Silage differs from other ruminant feeds because it is a fermented product. The type of silage fermentation will influence silage quality, voluntary intake (and palatability), and the utilisation of the silage nitrogen by animals. Consequently, the potential high level of animal production possible from a given silage with a high ME and high CP content may not be realised if there has been a poor fermentation. Therefore, the conventional quality measures (digestibility or ME, and CP) used for other ruminant feeds are not sufficient for silage samples. Some measure of ***fermentation quality*** is also needed.

An additional issue is the volatile nature of some of the silage fermentation products. These are lost on oven drying and this can effect the accuracy of silage ME and CP estimates.

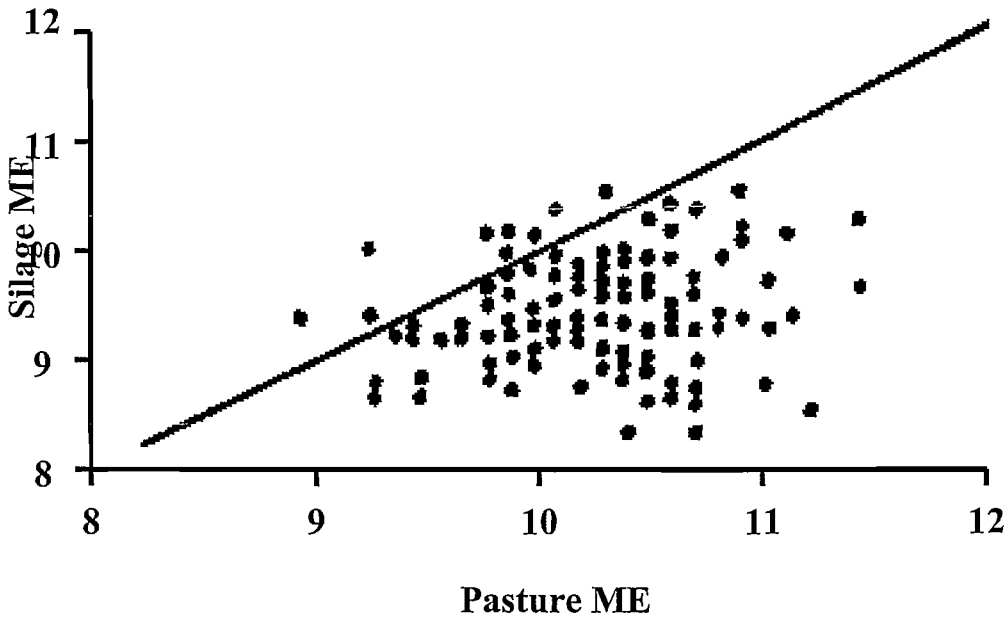
The Accuracy of Current Laboratory Methods

(a) Digestibility and ME

Some concern has been expressed about the accuracy of current procedures used by feed testing laboratories to evaluate silages, and this was identified as a high priority area for research at DRDC's 1998 Silage Workshop. The frequency with which anomalous results occur is unknown, and it is possible that some low ME values are due to poor sampling procedures or to deterioration of the sample before it reaches the laboratory. However there is

evidence from a large field study by Jacobs (1997)[1] that raises doubts about the relationship between pasture ME and silage ME. In this study no relationship was found between the two (see Fig. 1). This result is surprising and contrary to other published literature that shows that silage ME increases with pasture/forage ME. Various interpretations can be placed on these results. Firstly significant losses during the ensiling process may have reduced silage ME and masked the relationship with parent forage ME. While it is feasible that losses occurred, at least some silages would have been unaffected, so some high ME pastures would have been expected to yield high ME silages. This is not evident in the figure. The second possible explanation is that the laboratory tests may have given inaccurate results for at least some of the silages. This possibility needs to be investigated.

Figure 1. Relationship between metabolisable energy content (ME, MJ/kg DM) of the initial pasture and the ME content of the resulting silage



Experience in the UK has shown that ME estimates for silages have varied with the laboratory method used to determine digestibility (Adamson and Givens 1989)[2], and with the prediction equation used to estimate ME from digestibility. It has generally been accepted that the ME content of high quality silages has been underestimated, although more recently this problem has been overcome by the correction for silage volatiles lost during oven drying, and the direct calibration of NIR for *in vivo* digestibility. However the UK work has focused on ryegrass silage. The forage base used on Australian dairy farms for silage production is

[1] Jacobs, J.L. (1997). In: Proceedings of the Target 10 "Keys to Successful Silage" Seminar, September 1997, pp. 21-35.
[2] Adamson, A.H. and Givens. D.I. (1989). In: "Silage for Milk Production", ed. C.S. Mayne, British Grassland Society Occasional Symposium No. 23, pp.20-30.

considerably more diverse, and a recent survey showed that forages from12 different categories are ensiled (Kaiser and Evans 1997)[1]. This diversity of silages represents a major challenge for Australian feed testing laboratories especially given the unavailability of *in vivo* standards for calibration purposes. The provision of standards would improve the accuracy of ME estimates from individual feed testing laboratories, reduce variation between laboratories and provide the opportunity for laboratories to directly calibrate their NIR for *in vivo* digestibility.

(b) Implications for Commercial Feed Testing

A number of other issues related to commercial testing of silage samples need to be addressed. At present most laboratory tests are conducted on oven dried silage samples. This results in an underestimation of true DM content, and because the volatiles lost during oven drying are completely digestible, there is also an underestimation of silage digestibility and ME content. As the volatile components of silage generally reduce with increasing silage DM content, the error (DM, digestibility, ME) associated with oven drying is smaller with drier silages. While there are methods for determining the true DM content of silages, these are too expensive for routine use in commercial feed testing laboratories. However there are correction equations that can be used to predict true DM from oven DM. Kaiser *et al.* (1995)[2] developed such an equation that accurately ($r^2 = 0.995$) predicted true DM, as measured by Karl Fischer titration. Although this relationship covered a range of species and silage DM contents, it was only based on 13 silages so it needs to be further developed to cover a much larger number of silages.

No such corrections are available to convert CP determined on an oven-dried sample to a true CP value (as determined on a fresh silage sample). Unfortunately most, if not all, feed testing laboratories conduct analyses on dried samples even though it is known that this results in an underestimation of CP content. The extent of volatile N losses during oven drying will vary with the DM content of the silage and the volatility of the N fraction. Having an accurate estimate of silage CP content is important when assessing the CP status (RDP and UDP) of the diet, and the need for supplementary protein.

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[1] Kaiser, A.G. and Evans, M.J. (1997). "Forage Conservation on Australian Dairy Farms". Animal Industries Report No. 3 (NSW Agriculture, Orange), 18 pp.

[2] Kaiser, A.G., Mailer, R.J. and Vonarx, M.M. (1995). *Journal of the Science of Food and Agriculture* **69**: 51-59.

Objectives and Their Achievement

The broad objective of the project was to improve the accuracy of feed tests used to assess silage quality. Specific objectives were as follows:-

1. Produce silage standards of known *in vivo* digestibility and use these to develop more accurate laboratory tests. Make these standards available to feed testing laboratories for calibration purposes.

Achievement:
The digestibility of 30 silages was determined in sheep and cattle. A range of laboratory measures were evaluated in terms of their precision for predicting *in vivo* digestibility. The various fibre analyses did not provide an accurate indication of digestibility. The pepsin cellulase method, while suitable for hays, lacked sufficient precision for silages. The most accurate method for silages was the *in vitro* digestibility method based on rumen fluid and pepsin. The standards of known *in vivo* digestibility can be made available to feed testing laboratories once the stakeholders have confirmed that the proposed policy can be implemented.

2. Develop methods for accurately estimating the true DM content of silage, and the true N content of silage from an N analysis conducted on an oven-dried silage sample.

Achievement:
The true DM content of 60 silages was determined by Karl Fischer titration and related to the DM content determined by oven drying. The greatest difference between true and oven DM was with lower DM silages. An equation was derived for predicting true DM from oven DM and this accounted for 99% of the variation. A second prediction equation was derived to predict true N content, determined on a fresh silage sample, from an N analysis conducted on an oven dried sample. This relationship accounted for 98% of the variation, and showed that the underestimation of the true N content of a silage, due to conducting a N analysis on an oven dried silage sample, increased with silage N content. Both prediction equations are to be adopted by AFIA and will be incorporated in their Laboratory Methods Manual.

Methodology

1. Silage Digestibility Studies with Cattle and Sheep

1.1 Silages

Silages were produced at the Wagga Wagga Agricultural Institute over the period 1998-2001 from both dry-land and irrigated crops and pastures. Some crops and pastures were specifically grown for the project while others were harvested opportunistically. The primary aim was to cover a diverse range of species and forage qualities, and in some cases harvest was deliberately delayed to produce silages of low digestibility. A description of the silages and their botanical composition at harvest are provided in Tables 1 and 2 respectively

1.2 Harvesting

All forages were harvested using precision chop forage harvesters – a Claas® 'Jaguar 62' for silages 1 and 2 and a Kverneland Taarup® TA622 for the remainder. The wilted pasture and forage crop silages were harvested using a windrow pick-up front while the maize and sorghum silages were direct harvested using a 2-row row-crop front on the forages harvesters.

Chop lengths for most particles in the majority of silages ranged from about 1-4cm. However some silages, particularly the weather damaged oats, pasture silages containing a significant proportion of *Vulpia*, and some winter cereal silages contained a significant proportion of particles 10 cm or greater in length.

The silages (approximately 5-12 t DM), after rolling to achieve adequate compaction, were stored in small above-ground buns sealed with 160 µm laminated black/white silage plastic sheeting. Tyres (touching) were placed on top of the plastic sheet to maintain good contact between the sheeting and silage. The edges of the silages buns were sealed by running the plastic sheeting into a shallow trench and burying it with soil.

1.3 Crop and forage sampling

Parent crops and pastures were sampled at mowing and again at chopping. Three to four bulk samples (based on sub samples taken from across the paddock) were collected at mowing. Botanical composition was determined on a portion of each sample (see Table 2), and the remainder was dried at 80°C for 24 hours in a forced draught oven to determine DM content.

Each truckload of forage was sampled on delivery to the storage site. A portion of the sample was dried (as above) to determine DM content, and the remainder was frozen for later analysis. For the direct cut crops (maize and sorghum) random whole plant samples (approximately 36) were collected on the day before harvest. Fresh whole plant weights were recorded, and cobs were then removed from the maize plants and seed heads from the grain and sweet sorghums. All plant components were dried and weighed, and the grain was hand stripped from the cobs and seed heads and weighed, and crop grain content calculated (see Table 3).

All dried forage samples were ground through a 1 mm screen prior to analysis.

Table 1. Description of silages produced for the animal feeding experiments.

Silage No.	Forage species	Mown	Wilting period (hrs)*	DM content at ensiling (g/kg)
1	Subclover (Goulburn and Junee)	13/10/98	48	435.0
2	Oat (Cooba) weather damaged)	21/9/99	192	368.5
3	Maize (early cut Pioneer 3335)	27/3/01	Direct chop	317.7
4	Maize (late cut Pioneer 3335)	11/4/01	Direct chop	399.4
5	Annual ryegrass (ARG)/ oat (Cooba), early cut	5/10/00	48	375.8
6	Annual ryegrass (ARG)/ oat (Cooba), late cut	31/10/00	24	283.0
7	Subclover/ annual ryegrass/ lucerne, early cut	6/10/00	48	448.4
8	Annual ryegrass/ subclover, late cut	31/10/00	24	300.3
9	Sweet sorghum (Sugargraze)	10/4/01	Direct chop	260.7
10	Sweet sorghum (Sugargraze), delayed sealing	10/4/01	Direct chop	271.5
11	Forage sorghum × sorghum (Chopper)	10/4/01	Direct chop	344.8
12	Grain sorghum (Buster)	11/4/01	Direct chop	381.5
13	Wheat (Petrel)/ annual ryegrass	14/11/01	Direct chop	529.6
14	Barley (Dictator)/ annual ryegrass	29/10/01	Direct chop	383.1
15	Oat (Cooba)/ pea (Morgan)	29/10/01	24	520.7
16	Wheat (Petrel) / vetch (Popany)/ annual ryegrass	14/11/01	Direct chop	574.3
17	Subclover/ annual ryegrass/ lucerne	9/10/00	48	368.5
18	Italian ryegrass (Marbella), early cut	30/10/01	24	324.1
19	Italian ryegrass (Marbella), late cut	14/11/01	24	410.1
20	White clover (Nusiral)/ annual ryegrass	14/11/01	24	262.4
21	Maize (Snowy River SR.85)	27/3/01	Direct chop	380.5
22	Subclover / Silver grass/ lucerne	9/10/00	48	361.0
23	Annual ryegrass	29/10/01	24	459.9
24	Mixed annual grasses and cocksfoot	25/10/01	Direct chop	349.6
25	Wheat with annual grass weeds, late cut	7/11/00	Direct chop	382.7
26	Sorghum × Sudan grass (Brown midrib)	5/3/02	Direct chop	204.5
27	Sudan grass (Superdan)	6/3/02	Direct chop	227.0
28	Sorghum × Sudan grass (Sweet Jumbo)	5/3/02	Direct chop	195.5
29	Pennisetum millet (Nutrifeed)	6/3/02	Direct chop	176.6
30	Maize (Pioneer 3153)	2/5/02	Direct chop	358.0
31†	Lucerne, irrigated 2nd cut	11/3/02	48	530.0

* Interval from mowing to harvest

† *In vivo* data from this silage not used in the statistical analyses.

Table 2. Botanical composition of the crops and pastures harvested for silage.

Silage No.	Species 1	Species 2	Species 3	Other species
1	Subclover, 80%	Silver grass, 19%	Capeweed, 1%	
2	Oats, 100%			
3	Maize, 100%			
4	Maize, 100%			
5	Annual ryegrass, 72%	Oats, 26%		Great brome, 2%
6	Annual ryegrass, 66%	Oats, 28%	Great brome, 6%	Barley grass, 1%
7	Annual ryegrass, 42%	Subclover, 25%	Lucerne, 19%	Capeweed, 7% Dead material, 5% Barley grass, 3%
8	Annual ryegrass, 52%	Subclover, 28%	Silver grass, 13%	Capeweed, 6% Dead material, 1%
9	Sweet sorghum, 100%			
10	Sweet sorghum, 100%			
11	Forage sorghum, 100%			
12	Grain sorghum, 100%			
13	Wheat, 49%	Annual ryegrass, 47%	Radish, 3%	Wild oats, 1%
14	Barley, 79%	Annual ryegrass, 20%	Radish, 1%	Dead material, 1%
15	Oats, 49%	Pea, 45%	Annual ryegrass, 5%	Great brome, 1% Barley grass, 1%
16	Wheat, 57%	Annual ryegrass, 22%	Purple vetch, 21%	
17	Subclover, 38%	Annual ryegrass, 29%	Lucerne, 17%	Wild oats, 10% Dead material, 6%
18	Italian ryegrass, 91%	Amsinckia, 9%		
19	Italian ryegrass, 93%	Amsinckia, 7%		
20	White clover, 63%	Annual ryegrass, 25%	Amsinckia, 13%	
21	Maize, 100%			
22	Subclover, 48%	Silver grass, 23%	Lucerne, 18%	Barley grass, 7% Annual ryegrass, 3%
23	Annual ryegrass, 94%	Wild radish, 4%	Great brome, 2%	Persian clover, 1%
24	Great brome, 39%	Annual ryegrass, 32%	Cocksfoot, 7%	Silver grass, 7% Barley grass, 4% Soft brome, 3% Other spp., 8%
25	Wheat, 46%	Wild oats, 30%	Annual ryegrass, 20%	Great brome, 4%
26	Sorghum × Sudan grass, 100%			
27	Sudan grass, 100%			
28	Sorghum × Sudan grass, 100%			
29	Pennisetum millet, 81%	Barnyard grass, 19%		
30	Maize, 100%			
31	Lucerne, cut late summer, estimated close to 100%			

Table 3. Grain content of the maize and sorghum crops harvested for silage.

Silage No.	Crop description	Grain content (% DM)
3	Maize (Pioneer 3335) cut early, MLS = 0.4*	37
4	Maize (Pioneer 3335) cut late, MLS = 2.5	59
11	Forage sorghum (Chopper, tall grain type)	29
12	Grain sorghum (Buster)	41
21	Maize (SR 85), MLS = 4.2	52
30	Maize (Pioneer 3153), MLS = 2.2	36

* MLS = milk line score

1.4 Animal House Experiments

The experimental program comprised:-

- Experiment 1. 12 silages (1 to 12) fed to both cattle and sheep
- Experiment 2. 12 silages (13 to 24) fed to both cattle and sheep
- Experiment 3. 6 silages fed to cattle

All animal experiments were approved by NSW Agriculture's Animal Care and Ethics Committee, Orange, NSW.

1.4.1 Animals and management

Steers approximately 9-11 months of age were sourced from producers in southern NSW. The first experiment used 24 Angus and Murray Grey steers with a mean initial live weight of 277 kg. The second experiment used 24 Angus steers with a mean initial live weight of 278 kg, and the third experiment used 12 Murray Grey steers with a mean initial live weight of 304 kg.

The Merino wethers were sourced from the Institute's flock. The 32 wethers used in the first experiment had a mean initial live weight of 50.5 kg and an estimated age of 3 to 4 years. The wethers used in the second experiment had a mean initial live weight of 45 kg and an estimated age of 1½ to 2½ years.

At the commencement of the experiments both cattle and sheep were ear-tagged and drenched. Cattle were vaccinated twice with Coopers® 7 in 1 vaccine and treated with Eprinex® backline drench and Fasinex® flukicide, while wethers in both experiments were drenched with Ivomec®, a general-purpose white drench.

1.4.2 Experimental design

Experiments 1 and 2: Sheep and cattle remained in the experiments for a total of 86 days. In order to match numbers of animals with the available digestibility pens/crates, they were divided into 2 equal groups, each consisting of 12 cattle and 16 sheep. Group 1 animals entered the experiment 14 days ahead of Group 2, with each group spending a total of 72 days in the animal house. Animals were allocated to groups by stratified randomisation based on initial live weight.

Each experiment was divided into three periods, each of 24 days. Hence during the course of an experiment, each animal was fed three of the twelve experimental silages. The first 10 days

of each period comprised an adaptation to the new silage at the *ad lib* level of feeding. Intake was then reduced to 16.5g DM/ kg live weight (near maintenance), with digestibility being measured during the last 9 days.

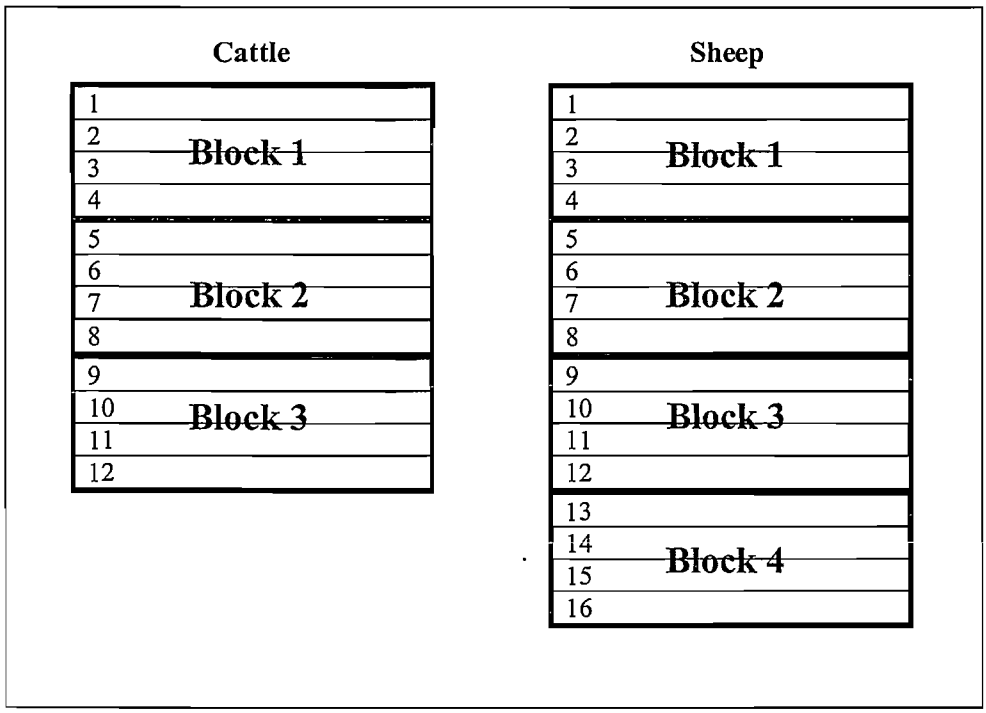
It was not possible to feed each of the 12 silages for the duration of the experiment because the slow rate of feeding would have inevitably resulted in deterioration of the silages (due to aerobic spoilage), compromising the results. To overcome this problem four silages were fed per period, restricting the total feeding duration for each silage to 38 days (covering Groups 1 and 2). The allocation of silages to be fed in each period was as follows:-

	Experiment 1	Experiment 2
Period 1	1	13
	2	14
	3	15
	4	16
Period 2	5	17
	6	18
	7	19
	8	20
Period 3	9	21
	10	22
	11	23
	12	24

Animals were housed in individual pens. The 12 metabolism pens for cattle were divided into 3 blocks of 4 pens, and the 16 metabolism cages/pens for sheep into 4 blocks of 4 pens (see Fig. 2). Silages were then randomly allocated to animals ensuring that each of the 4 silages for that period appeared in every block, and that between groups, silages did not appear in the same pen twice. Each group of animals was re-randomised at the commencement of a new period, ensuring that each animal received 3 different silages and appeared in each of the 3 blocks during an experiment.

Experiment 3: This experiment used one group of 12 steers and 3 periods, each 24 days. In each period the first 10 days allowed for an adaptation to the new silage at the *ad lib* level of feeding. Intake was then reduced to 16.5g DM/ kg live weight (near maintenance), and digestibility was measured during the final 9 days of the period. Two animals in each period were randomly allocated one of the 6 silages in each block. Silages and animals were randomised over blocks (of 6 pens) for each period to ensure that no animal received the same silage or appeared in the same block (or the same pen) twice.

Figure 2. Pen layout and allocation to blocks in Experiments 1 and 2.



1.4.3 Feeding

The silages were fed once daily in the morning. Care was taken to discard any aerobically spoiled silage when silages were being removed from the buns. As indicated above the silages were fed *ad lib* for the first 10 days. On day 11 animals were weighed, and individual animal intakes were then restricted to16.5g DM/kg live weight (true DM basis) based on this weight. Animals were then re-weighed 2 days before a metabolism run and any necessary adjustments to restricted intake made. Animals were weighed again at the conclusion of the digestibility run which also coincided with the end of the period. The level of intake restriction of 16.5g/kg was expected to be close to maintenance for lower digestibility silages and a little above maintenance for higher digestibility silages. In the interests of using uniform procedures to measure *in vivo* digestibility it was considered desirable to maintain all animals on the same DM intake relative to live weight.

In order to ensure that digestibility was not compromised by an inadequate level of rumen degradable N in the diet, all silages were analysed for total N content prior to commencement of the experiments, and the requirement for supplementary urea calculated. The quantity of urea required to maintain adequate RDP intake was based on AFRC (1993)[1], and took into account silage N content and silage DM intake. The mean quantities of urea fed per head per day on the various silages are presented in Table 4. Animals were also fed a commercial mineral supplement (Nutrimin S1®) at 90g/day for cattle and 16g/day for sheep to ensure dietary requirements were met (ARC, 1980)[2]. Its composition is presented in Table 5.

[1] AFRC (1993). *Energy and Protein Requirements of Ruminants.* An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients, (CAB International: Wallingford, UK).
[2] ARC (1980). *The Nutrient Requirements of Ruminant Livestock,* (CAB: Slough, UK).

Table 4. Mean quantity of supplementary urea required by cattle and sheep on the various silage diets.

Silage No.	Urea required (g/day)*		Silage No.	Urea required (g/day)†		Silage No.	Urea required (g/day)§
	Sheep	Cattle		Cattle	Sheep		Cattle
1	Nil	Nil	13	37.9	5.9	25	60.3
2	17.9	71.4	14	42.2	7.3	26	59.0
3	8.4	55.1	15	34.5	5.4	27	72.5
4	17.2	86.2	16	34.9	5.4	28	55.8
5	Nil	Nil	17	Nil	Nil	29	32.8
6	Nil	Nil	18	12.6	1.8	30	94.9
7	Nil	Nil	19	41	6.4		
8	Nil	Nil	20	Nil	Nil		
9	15.4	77.4	21	66.4	10.4		
10	14.1	70.7	22	Nil	Nil		
11	11.6	58.4	23	12.2	1.7		
12	13.2	66.3	24	4.3	0.5		

*Based on 280kg steer and 56kg wether, intake 16.5g/kg live weight.
†Based on 280kg steer and 45kg wether, intake 16.5g/kg live weight.
§ Based on 304kg steer, intake 16.5g/kg live weight

Table 5. Composition of mineral supplement

Ingredient	Content (g/kg DM)
Calcium (Ca)	140
Magnesium (Mg)	70
Phosphorus (P)	40
Sulphur (S)	50
Salt (NaCl)	100
Molasses	20
Fluorine (F)	6
	(mg/kg DM)
Manganese (Mn)	2500
Zinc (Zn)	2500
Cobalt (Co)	10
Iodine (I)	50
Iron (Fe++)	1000
Copper (Cu)	100

1.4.4 Digestibility runs

The digestibility pens (cattle) and crates (sheep) used in the experiments were specially designed to enable total faeces collection over the 7-day collection period. Each days faeces collection was weighed and sub-sampled (10% of total), and the sub-samples were frozen.

The daily faeces sub-samples for an individual animal were bulked. Faeces collection was one day in arrears of intake measurement.

Daily samples of the silages offered were collected during the digestibility runs. A 6.5 kg sample of fresh silage was taken while feeds were being weighed out, and 6.0 kg was dried (80°C for 24 hours) and ground to form part of the laboratory standard for that silage, while the remaining 500 g was bulked over the period of the digestibility run and frozen.

Feed residues were collected each morning prior to feeding if required. During the digestibility runs when animals were on restricted intake, the majority of silage diets had very few refusals, with the exception of the weather damaged oats (Silage 2) when fed to the sheep and some of sorghum silages (particularly both sweet sorghums) fed to cattle and sheep. In the case of the oats this was possibly due to the longer chop length, while in the sorghum silages heating (aerobic spoilage) was noted on several occasions affecting intake. Any feed residues were dried at 80°C for 24 hours to determine DM content.

During the digestibility runs, trays were placed under the feed boxes to collect any dropped feed. This was done to ensure accurate estimates of digestibility. After the completion of the run, trays were removed and all dropped feed dried to get a total dry weight. This usually meant drying dropped feed for up to 3 days at 80°C, dependant upon how much water had spilled into the trays over the period of the run from the water troughs above.

After the final day of a digestibility run, faeces samples (and any residue samples) were removed from the freezer and allowed to thaw. Following extensive mixing to ensure uniformity of the sample, sub-samples were taken in duplicate and dried (80°C for 24 hours for any residues, 48 hours for faeces) to determine DM content. A portion of the faeces and feed residues was then ground through a 1 mm screen and retained for later analysis. In the case of the maize and sorghum silages a portion of the fresh faeces was also retained to determine whole grain content, a process duplicated on the fresh silage offered.

2. Sample processing and analysis

2.1 Parent forages

The dried parent forage samples were analysed for total N content by a Leco FP 2000® Analyzer and water soluble carbohydrate (WSC) by the alkaline ferricyanide method (Technicon® "Industrial Method No. 302-73A"), after extraction in 0.2% benzoic acid/water solution (shaken for 1 hour).

2.2 Silages

The freshly frozen bulk offered samples collected during the digestibility runs were partially defrosted and then finely chopped in a rotating bowl chopper. Some of this was re-frozen while the majority was used for detailed chemical analysis. The following analyses were conducted on the fresh chopped silage samples:-

Oven DM: As outlined earlier – forced draught oven at 80°C for 24 hours

Total N: By macro-Kjeldahl digestion using a Tecator® Kjeltec

Silage pH: 20 g of fresh silage was mixed with 100 ml of distilled water, and pH was measured after the sample had stood for 30 minutes

True DM: The previously referred to method of Kaiser *et al.* (1995) was used. The water from 2 g fresh silage was extracted in 50mls of methanol for 48 hours. The water in 1 ml of the extract was determined using a Karl Fischer titrator (Mettler Toledo® Karl Fischer moisture titrator).

Ammonia-N: 50gms of the fresh chopped silage was extracted in 250-450 ml (dependant upon silage DM) of 0.3M H_2SO_4, and ammonia-N content of the extract was measured using the Tecator® Kjeltec minus the digestion phase. The result was expressed as a proportion of total N.

The following analyses were conducted on the dried silage samples:-

Total N: Total N were determined by the same method as previously stated for fresh silage samples.

In vitro digestibility: Determined using a modification of the Tilley and Terry (1963)[1] two-stage technique. The method was modified by adding urea (0.156 g/l) and ammonium sulphate (0.156g/l) to the buffer solution. This provided an additional source of N in the buffer solution to compensate for the low N status of some feeds. Digestibility was expressed both on a DM and OM basis.

Pepsin cellulase digestibility: This is based on the method used by Feedtest [2] at Hamilton, Victoria. Samples are digested in acidified pepsin at 40 °C for 24 hours, and then heated to 80 °C for 45 minutes. The final digestion step after pH adjustment with 0.8 ml $NaCO_3$ (1M solution), is with buffered cellulase solution at 40 °C for 24 hours. Digestibility was expressed both on a DM and OM basis.

Ash: This was determined by combusting the sample at 550°C for 6 hours.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF): These were determined sequentially using the filter bag method (Ankom® 200/220 fibre analyser). Analyses were initially based on 0.5 g samples but as this left very small residues for acid insoluble ash analyses (AIA, see below) the sample size was later increased to 0.7 g. Initial comparisons showed that the increase in sample size did not adversely affect the extraction of fibre components (differences not significant, Table 6). It is planned to conduct more direct comparisons in the future.

For NDF analyses the samples were boiled in the detergent solution (amylase included) for 75 minutes, then rinsed three times in boiling water (amylase added to the first two rinses) with a final rinse in acetone. Bags were then left to air dry before drying at 105°C for 2 hours. The NDF residues were then boiled in acid detergent solution for 60 minutes, followed by the same rinsing procedure (without amylase), to determine ADF.

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[1] Tilley, J.M.A. and Terry, R.A. (1963). *Journal of the British Grassland Society* **18**: 104-111.

[2] Feedtest, DPI, Pastoral and Veterinary Institute, Hamilton, Victoria – Method Manual, Method 2.7 Determination of Digestibility using the Pepsin-Cellulase Method.

Acid detergent lignin (ADL): Bags containing the residue after the ADF analysis were suspended in 72% H_2SO_4 for 3 hours. They were then rinsed (4 to 5 times) with boiling water and finally acetone, and air dried before being dried at 105°C for 4 hours.

Acid insoluble ash (AIA): AIA was calculated following ashing of the dried bags from the ADL digestion at 550°C for 6 hours.

Acid detergent insoluble N (ADIN): This is the N content of the residue following the ADF digestion. The N content was determined by the macro-Kjeldahl method (described earlier) and ADIN was expressed both as g/kg DM and g/kg total N.

Table 6. Influence of sample size on the mean analytical results (g/kg DM) from NDF, ADF and ADL analyses*.

Analysis	No. of observations	Sample size	
		0.5 g	0.7 g
NDF	9	656.3	661.6
ADF	12	393.2	392.2
ADL	15	65.2	71.0

* Differences due to sample size not significantly different based on paired t-tests

3. Statistical analyses

3.1 Animal experiments

The three animal experiments were analysed separately using the REML (Restricted Maximum Likelihood Estimation) variance components analysis package within GENSTAT (version 6). Silage, animal species and silage × animal species effects were tested (using a Chi-square test on the Wald statistic) in experiments 1 and 2, and silage in experiment 3. Mean digestibilities for sheep, cattle and combined sheep/cattle were derived for each silage, with two group means/silage in experiments 1 and 2. In experiment 3, mean digestibilities in cattle were derived for each silage with three group means/silage.

The predicted group means for each silage were used in the regression analyses (generalised linear regressions within GENSTAT) comparing sheep and cattle, and for predicting OMD and DOMD from DMD.

3.2 Relationships between in vivo digestibility and laboratory measures

The laboratory analyses provided estimates of the composition of the silages fed to each group (two groups/silage in experiments 1 and 2, and three groups/silage in experiment 3). The predicted group means for *in vivo* digestibility with the matching laboratory measures were used in the regression analyses (generalised linear regressions with GENSTAT), and for generating the correlation matrix for the fibre measures. Various multiple regressions were evaluated to produce the best prediction of *in vivo* digestibility. Non-linear relationships were also investigated using Sigma Plot but did not offer any significant improvement in r^2.

4. Laboratory Studies on Silage DM and Total N Analysis

This component of the work investigated the development of equations to predict true DM content and true N content (determined on fresh silage) from oven DM and analyses conducted on an oven dried sample respectively.

4.1 Silages

The silages used included the 30 used in the animal studies and silage 31 (Table 1), and 17 silages collected from farmers properties in southern NSW. The farmer silages were a diverse set made from various crops and pastures (Table 18).

4.2 Sampling and analyses

Sampling procedures and analytical methods were the same as those outlined earlier for the silage digestibility studies under section 2. The analyses conducted on these silages were oven DM, true DM (Karl Fischer method), pH, ammonia-N, and total N both on fresh silage and oven-dried silage.

4.3 Statistical analyses

The relationships between true DM content and oven DM, pH and ammonia-N were analysed using the generalised linear regressions procedure within GENSTAT, and the regressions later plotted using Sigma Plot. The earlier data of Kaiser *et al.* (1995) provided an additional 13 silages for inclusion in the regressions.

Similar statistical procedures were used to study the relationships between total N determined on fresh silage and total N determined on oven dried silage, pH, DM content and ammonia-N.

Results and Discussion

1. Composition of the Silages in the Feeding Experiments.

A diverse set of silages was produced for this project. It was important that the silages selected covered a broad sample of the forages from which silages are made in Australia. In addition it was important that a wide range in digestibility was obtained, so some of the silages were made from weedy crops, and others were deliberately cut late to produce silages of low digestibility. In one case the sealing of a sweet sorghum silage (silage 10) was delayed by 12 hours to increase respiration losses and produce a silage of lower digestibility. The composition of the crops from which the silages were made is presented in Table 7.

As intended, the silages covered a wide range composition (Tables 8 and 10):-

True DM	233-576 g/kg
Crude protein	33-228 g/kg DM
Ash	58-110 g/kg DM
Neutral detergent fibre	369-671 g/kg DM
Acid detergent fibre	217-418 g/kg DM
Acid detergent lignin	22-82 g/kg DM
Acid insoluble ash	2-23 g/kg DM
Acid detergent insoluble N	47-206 g/kg total N
pH	3.4-5.5
Ammonia-N	51-149 g/kg total N

The fibre and total N results indicate that we were successful in producing a good range in silage quality. Based on the ammonia-N results, seven of the silages had only moderate fermentation quality (100-150 g/kg total N), while the remainder had good fermentation quality. None of the silages could be considered to have a poor fermentation quality (>150 g/kg total N).

Six of the silages showed some evidence of possible heat damage (ADIN > 120g/kg total N) during the ensiling process, with three of these (26, 27 and 28) having ADIN > 150 g/kg total N – usually an indication of extensive heating. However, these silages were forage sorghums which may have higher levels of naturally bound N (perhaps due to tannins binding N) increasing the ADIN content. So they may not have been heat damaged. A prolonged aerobic phase and accompanying heating early in the ensiling process would be expected to elevate ammonia-N levels but this was not the case with these three silages.

The fibre data presented in Table 10 and summarised above have been determined in the sequence NDF→ADF→ADL→AIA. In other words each analysis is determined on the residue remaining after the previous analysis. This is particularly important with the analysis of ADF because the NDF procedure removes some components that are not removed by the ADF solution – pectin is the main source of the difference and is only partially hydrolysed in the low pH ADF solution. To highlight this issue we analysed the ADF of our silage samples both directly and sequentially after the NDF digestion. The results in Table 9 show a clear difference between the two procedures, and this difference was similar over the various

Table 7. Composition of the parent forages used for the experimental silages

Silage No.	**1**	**2**	**3**	**4**	**5**	**6**	7	**8**	**9**	**10**	**11**	**12**
DM (g/kg)	435	369	318	399	376	283	448	300	261	272	345	382
WSC content (g/kg)	-	-	144	106	208	142	159	138	265	265	157	97.3
N content (g/kg DM)	-	6.6	10.6	7.55	23.9	11.9	22.5	13.4	5.9	5.9	9.2	9.75
CP(g/kg DM)	-	41	66	47.2	144	98.3	151	12.6	37.2	36.9	57.5	60.9

Silage No.	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
DM (g/kg)	530	383	521	574	369	324	410	262	381	361	460	350
WSC content (g/kg)	184	227	189	189	130	257	284	95	101	85.4	214	185
N content (g/kg DM)	11.6	10.5	10.3	14.3	26	16	10.7	28.3	11.8	33.4	14.4	12.6
CP(g/kg DM)	72.5	65.2	87.7	89.1	162	99.8	73.7	187	73.5	231	89.9	79

Silage No.	**25**	**26**	**27**	**28**	**29**	**30**	**31**
DM (g/kg)	383	205	227	196	177	358	561
WSC content (g/kg)	207	145	144	111	85.7	101	-
N content (g/kg DM)	8.2	8.1	5.2	7.9	8.3	8	26.8
CP(g/kg DM)	51.5	50.7	32.3	49.5	52	50.3	167.5

Table 8. Dry matter, organic matter, total N, pH and ammonia-N content of the silages fed in the digestibility studies (true DM basis)

Experiment 1

Silage No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Oven DM (g/kg)	330.1	367.6	314.7	374.4	331.6	250.4	405.3	303.5	269.0	259.5	339.7	357.6
True DM (g/kg)	343.7	404.8	335.3	397.6	368.1	274.0	447.2	342.4	279.2	267.7	351.6	382.3
Total N (g/kg DM)	28.4	14.6	9.8	9.1	24.5	17.4	24.4	18.9	5.7	5.6	10.5	10.6
pH	4.58	4.27	3.88	3.88	4.36	4.04	4.66	4.23	3.87	3.8	4.00	4.15
Ammonia-N (g/kg total N)	105.8	148.5	71.8	61.1	84.6	104.4	71.0	104.0	83.6	66.5	75.2	57.0
Organic matter (g/kg DM)	896.7	932.6	949.2	942.3	907.4	923.5	890.5	918.7	928.1	925.0	926.2	929.1

Experiment 2

Silage No.	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Oven DM (g/kg)	510.5	380.6	480.1	521.1	353.4	291.8	356.0	260.2	339.5	325.9	398.5	304.7
True DM (g/kg)	513.4	393.7	523.0	543.9	400.4	331.9	381.5	291.7	350.8	336.2	441.0	331.5
Total N (g/kg DM)	12.1	12.5	16.1	16.8	27.2	15.1	11.4	29.3	13.6	36.5	16.5	13.5
pH	4.84	4.64	4.83	5.49	4.32	4.26	4.37	4.19	3.67	4.51	4.59	4.13
Ammonia-N (g/kg total N)	82.4	119.5	91.0	83.1	67.4	51.6	90.0	83.7	106.6	91.1	78.8	94.0
Organic matter (g/kg DM)	937.5	928.4	921.5	936.0	907.0	915.0	922.9	901.8	940.3	892.2	930.7	936.9

Table 8. *(continued)*
Experiment 3

Silage No.	25	26	27	28	29	30	31
Oven DM (g/kg)	344.2	202.9	221.9	206.9	186.1	299.3	558.8
True DM (g/kg)	383.6	233.9	255.1	244.6	232.6	311.5	576.3
Total N (g/kg DM)	8.0	8.5	5.3	7.8	7.5	9.0	26.1
pH	3.64	3.41	3.86	3.65	3.93	3.62	5.0
Ammonia-N (g/kg total N)	100.0	57.9	56.1	50.6	68.7	64.4	55.1
Organic matter (g/kg DM)	918.3	922.8	925.8	912.8	917.0	941.3	910.1

groups of silages. Overall, when ADF was determined directly the values were on average 49 g/kg DM higher than ADF determined after the NDF step. However the range was 21.6 to 77.4, so the error can vary considerably for individual silages. The highest difference was with the white clover silage (20).

Table 9. Effect of determining ADF directly or sequentially after an NDF analysis on the analytical result obtained with 70 silage samples.

Species group	No. of observations	Sequential ADF analysis	Direct ADF analysis	Difference
Grass dominant	16	325.4	375.1	49.7
Legume dominant	12	285.5	334.9	49.4
Cereal dominant	13	358.7	405.3	46.6
Maize	9	259.2	300.8	41.6
Summer forage crops	20	373.7	427.4	53.7
All samples	*70*	*330.0*	*379.2*	*49.2*

The results of the two biological assays – *in vitro* digestibility and pepsin-cellulase digestibility – are presented in Table 11. They are "raw" unadjusted values (i.e. for standards of known digestibility). The mean values presented are from four *in vitro* digestibility runs and four pepsin-cellulase (three at Wagga Wagga and one at Hamilton) digestibility runs. In addition to the 30 silage samples used in this project, we also obtained hay standards from DPI, Hamilton (Peter Flinn's earlier RIRDC project). The *in vivo* digestibility of the 16 hays was determined with sheep at close to the maintenance level of feeding, and the data are therefore comparable to our silage digestibility data (with sheep). The objective here was to determine whether the relationships between these assays and *in vivo* digestibility were similar for silage and hay.

It was important to generate samples with a wide range in digestibility and this was achieved. The values for *in vitro* digestibility (OMD) varied from 0.547 to 0.817, a little larger than the

Table 10. Fibre and bound N (acid detergent insoluble N, ADIN) for the silages fed in the digestibility studies (true DM basis). ADF, NDF and ADL on an ash included basis.

Experiment 1

Silage No.	1	2	3	4	5	6	7	8	9	10	11	12
NDF (g/kg TDM)	408.8	596.0	409.0	459.0	422.2	560.8	390.4	470.0	548.6	593.0	438.5	438.3
ADF (g/kg TDM)	268.8	339.7	218.9	244.0	241.8	340.8	249.5	303.3	303.0	325.1	236.5	228.4
ADIN (g/kg total N)	119.48	72.18	58.61	56.55	49.59	54.95	89.89	126.92	95.15	98.49	90.99	106.64
ADL (g/kg TDM)	61.61	74.82	50.38	28.71	32.06	54.18	54.66	46.96	45.08	40.66	39.96	46.18
AIA (g/kg TDM)	8.80	22.65	16.65	12.8	15.82	14.1	15.52	6.95	17.27	9.97	17.27	17.1

Experiment 2

Silage No.	13	14	15	16	17	18	19	20	21	22	23	24
NDF (g/kg TDM)	620.2	564.9	542.4	600.8	368.6	451.8	494.8	315.4	450.1	385.9	507.8	622.6
ADF (g/kg TDM)	355.2	325.4	338.8	364.8	235.3	259.2	286.3	217.4	240.0	237.8	289.8	342.0
ADIN (g/kg total N)	65.12	70.90	78.50	80.97	62.61	82.59	86.18	78.14	46.57	60.13	53.75	71.80
ADL (g/kg TDM)	59.06	58.21	68.63	67.86	37.88	22.43	68.41	33.51	32.23	51.38	46.13	42.81
AIA (g/kg TDM)	12.75	18.6	19.37	12.12	8.1	5.62	14.45	1.8	12.67	11.3	11.87	11.52

Table 10. *(continued)*
Experiment 3

Silage No.	25	26	27	28	29	30	31
NDF (g/kg TDM)	562.9	605.4	671.2	584.2	574.7	495.7	486.7
ADF (g/kg TDM)	337.0	357.2	418.3	345.3	342.0	273.1	331.9
ADIN (g/kg total N)	121.28	158.95	206.0	166.03	144.11	106.44	109.54
ADL (g/kg TDM)	63.07	39.98	57.08	46.45	39.27	37.73	82.20
AIA (g/kg TDM)	19.78	5.73	7.53	8.53	10.49	11.36	4.74

range for the hays (0.532 to 0.733). The range in pepsin-cellulase OM digestibility for the silages ranged from 0.435 to 0.797, the range again a little larger than that for the hays (0.395 to 0.683).

2. *In vivo* Digestibility – Cattle *vs* Sheep

The *in vivo* digestibility results are presented in Tables 12, 13 and 14. As was expected with such a diverse set of silages, there was a wide range in digestibility (eg. 0.550 to 0.810 for OMD in cattle). The estimated metabolisable energy (ME) content of each of the silages can be determined using the AFRC (1993)[1] equation -

$$\text{Silage ME (MJ/kg DM)} = 0.16 \times \text{DOMD\%}$$

with both ME and DOMD being expressed on a true DM basis. Note that the value of 0.16 has already been reduced by 5% to allow for the possible loss of volatiles from silages during feeding out.

Based on the cattle DOMD data the ME content of the 31 silages ranged from 8.10 to 12.18 MJ/kg DM. This would cover the range of ME normally observed on farms, although it is possible that some farmers may produce very low quality silages with ME's as low as 7 MJ/kg DM.

In Experiment 1 there was a tendency over the 12 silages for cattle to have a higher DMD than sheep ($P<0.086$), and the DOMD of silages 1 and 5 was higher in cattle (interaction significant $P<0.005$). Silage OMD was higher in sheep than cattle in the second experiment ($P<0.05$) and a similar trend was observed in the DOMD data ($P<0.065$). The greatest difference in digestibility was with silage 21 and for DOMD the silage × species interaction approached statistical significance ($P<0.105$).

Although some statistically significant species differences in digestibility were detected, the overall difference between sheep and cattle was not large. Compared to sheep, the rankings (DOMD) of silages 1, 7 and 10 were a little higher in cattle while that of silage 21 was a little

[1] AFRC (1993). *Energy and Protein Requirements of Ruminants.* An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients, (CAB International: Wallingford, UK).

lower. The relationships between sheep and cattle digestibility were determined by regression analysis, and the relationship for OMD is illustrated in Fig. 3.

Table 11. *In vitro* digestibility and pepsin cellulase digestibility of the experimental silages, and hays from a previous project (RIRDC) at Hamilton, Victoria. The data are "raw" unadjusted digestibility values.

Silage No.	*In vitro* (TDM)			Pepsin cellulase (TDM)	
	DMD	OMD	DOMD	DMD	OMD
1	0.741	0.686	0.612	0.688	0.598
2	0.657	0.580	0.537	0.625	0.547
3	0.719	0.721	0.682	0.756	0.703
4	0.681	0.685	0.643	0.721	0.674
5	0.835	0.817	0.733	0.848	0.797
6	0.650	0.653	0.599	0.606	0.554
7	0.740	0.699	0.614	0.769	0.686
8	0.734	0.665	0.603	0.666	0.590
9	0.707	0.703	0.650	0.657	0.603
10	0.675	0.658	0.609	0.633	0.589
11	0.644	0.639	0.590	0.717	0.686
12	0.649	0.659	0.610	0.709	0.664
13	0.595	0.551	0.517	0.599	0.530
14	0.604	0.608	0.563	0.626	0.573
15	0.612	0.593	0.543	0.617	0.569
16	0.571	0.547	0.511	0.608	0.537
17	0.835	0.803	0.718	0.780	0.724
18	0.761	0.767	0.693	0.826	0.771
19	0.709	0.689	0.632	0.744	0.669
20	0.851	0.821	0.731	0.755	0.689
21	0.691	0.665	0.624	0.715	0.656
22	0.746	0.727	0.646	0.754	0.683
23	0.649	0.649	0.599	0.649	0.600
24	0.614	0.631	0.587	0.639	0.560
25	0.649	0.652	0.593	0.606	0.570
26	0.698	0.678	0.618	0.647	0.594
27	0.628	0.612	0.560	0.497	0.435
28	0.677	0.670	0.601	0.643	0.584
29	0.758	0.809	0.713	0.660	0.602
30	0.692	0.693	0.651	0.699	0.654
31	0.680	0.638	0.581	0.682	0.610
Hays from Hamilton					
Balansa clover (R2)	0.728	0.665	0.593	0.676	0.589
Barley (R2)	0.640	0.645	0.610	0.625	0.569
Lucerne (R1)	0.619	0.599	0.560	0.578	0.529
Lucerne (R2)	0.611	0.532	0.496	0.590	0.508
Medic (R1)	0.730	0.733	0.662	0.751	0.683
Oaten, good (R1)	0.677	0.688	0.652	0.641	0.588
Oaten, poor (R1)	0.592	0.579	0.549	0.524	0.469
Pasture, good (R1)	0.747	0.724	0.645	0.730	0.650
Pasture, poor (R1)	0.593	0.584	0.539	0.522	0.492
Pasture (R2)	0.623	0.618	0.568	0.556	0.489
Persian clover (R1)	0.725	0.705	0.635	0.665	0.614
Sorghum 2 (R2)	0.641	0.555	0.504	0.523	0.451
Sorghum 1(R2)	0.571	0.564	0.512	0.454	0.395
Vetch (R1)	0.610	0.597	0.548	0.468	0.429
Vetch (R2)	0.741	0.709	0.641	0.748	0.679
Frosted wheat (R2)	0.586	0.636	0.556	0.610	0.560

Table12. Digestibility of silages by cattle and sheep – Experiment 1, silages 1-12*.

Silage No.	DMD		OMD		DOMD	
	Cattle	Sheep	Cattle	Sheep	Cattle	Sheep
1	0.685	0.643	0.708	0.669	0.631†	0.589
2	0.651	0.632	0.668	0.655	0.603	0.600
3	0.714	0.708	0.731	0.733	0.674	0.686
4	0.664	0.672	0.686	0.707	0.642	0.675
5	0.772	0.749	0.810	0.786	0.761†	0.702
6	0.679	0.666	0.689	0.687	0.613	0.627
7	0.713	0.698	0.745	0.734	0.681	0.656
8	0.685	0.660	0.704	0.679	0.630	0.620
9	0.665	0.661	0.691	0.689	0.626	0.635
10	0.684	0.648	0.708	0.676	0.646	0.621
11	0.652	0.666	0.681	0.700	0.623	0.644
12	0.594	0.602	0.621	0.638	0.572	0.590
Mean	0.680	0.667	0.703	0.696	0.642	0.637
Significance of:-						
Silage	P<0.001		P<0.001		P< 0.001	
Species	P<0.086 sed = 0.007458		ns sed = 0.007338		ns sed = 0.007131	
Silage × species	ns sed = 0.01959		ns sed = 0.01926		P<0.005 sed = 0.02022	

* All data presented on a true DM basis
† Digestibility in cattle higher than that in sheep (P<0.05)

Table 13. Digestibility of silages by cattle and sheep – Experiment 2, silages 13-24*.

Silage No.	DMD		OMD		DOMD	
	Cattle	Sheep	Cattle	Sheep	Cattle	Sheep
13	0.552	0.567	0.565	0.587	0.521	0.542
14	0.612	0.610	0.635	0.636	0.581	0.579
15	0.613	0.599	0.647	0.638	0.592	0.583
16	0.536	0.550	0.550	0.584	0.506	0.537
17	0.717	0.725	0.745	0.756	0.676	0.671
18	0.705	0.723	0.738	0.762	0.660	0.686
19	0.607	0.621	0.638	0.662	0.577	0.602
20	0.689	0.710	0.718	0.741	0.646	0.658
21	0.617	0.647	0.645	0.691	0.589†	0.640
22	0.714	0.728	0.743	0.755	0.674	0.667
23	0.634	0.637	0.653	0.660	0.591	0.604
24	0.650	0.658	0.681	0.701	0.622	0.644
Mean	0.637	0.648	0.663	0.681	0.603	0.618
Significance of:-						
Silage	P<0.001		P<0.001		P< 0.001	
Species	ns sed = 0.008238		P<0.03 sed = 0.008416		P< 0.065 sed = 0.008042	
Silage × species	ns sed = 0.01717		ns sed = 0.01781		P<0.105 sed = 0.01764	

* All data presented on a true DM basis
† Digestibility in sheep higher than that in cattle (P<0.05)

Table 14. Digestibility of silages by cattle – Experiment 3, silages 25-30*.

Silage No.	DMD	OMD	DOMD
25	0.590	0.643	0.583
26	0.664	0.696	0.634
27	0.616	0.650	0.594
28	0.651	0.684	0.616
29	0.685	0.724	0.655
30	0.679	0.713	0.662
Significance of silage	P<0.001	P<0.001	P< 0.001

* All data presented on a true DM basis

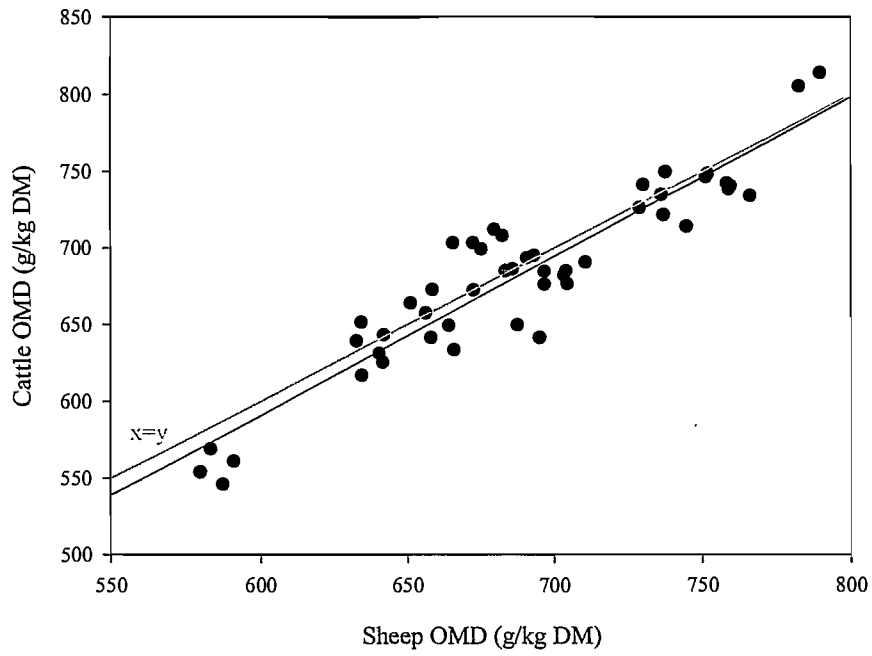
The regression analyses (see also Fig. 3) show that there is little difference between sheep and cattle and that the former could be used to estimate digestibility at close to the maintenance level of feeding in cattle. The regressions (digestibility expressed as g/kg) were as follows:-

$$DMD_{cattle} = (1.016 \times DMD_{sheep}) - 9.7 \qquad (r^2 = 0.88;\ s.e. = 19.3)$$

$$OMD_{cattle} = (1.032 \times OMD_{sheep}) - 27.5 \qquad (r^2 = 0.85;\ s.e. = 22.2)$$

$$DOMD_{cattle} = (1.076 \times DOMD_{sheep}) - 52.6 \qquad (r^2 = 0.78;\ s.e. = 25.5)$$

Figure 3. Relationship between the organic matter digestibility (OMD) of silages in cattle and sheep.



3. Predicting OMD and DOMD from DMD (Using *In vivo* Data)

For the estimation of ME content, digestibility data are usually first converted to DOMD and ME is then calculated. Equations for estimating OMD or DOMD from DMD data are provided in some feeding standards and are based on *in vitro* digestibility data (eg. MAAF (1975)[1] and SCA (1990)[2]). This project provides an opportunity to derive regressions for silages using *in vivo* data, obtaining means for each silage from both periods – these are listed below. The individual regressions are based on the period means of 30 silages for cattle and 24 for sheep. The combined equation is based on the period means of the 30 silages (species meaned within period).

Prediction of OMD from DMD (g/kg):

Combined cattle and sheep	OMD = 33.6 + (0.995 × DMD)	(r^2 = 0.97; s.e. = 8.19)
Cattle	OMD = -2.9 + (1.046 × DMD)	(r^2 = 0.98; s.e. = 7.16)
Sheep	OMD = 23.2 + (1.012 × DMD)	(r^2 = 0.98; s.e. = 7.53)

Prediction of DOMD from DMD (g/kg):

Combined cattle and sheep	DOMD = 54.6 + (0.870 × DMD)	(r^2 = 0.94; s.e. = 10.9)
Cattle	DOMD = 43.6 + (0.883 × DMD)	(r^2 = 0.95; s.e. = 9.74)
Sheep	DOMD = 90.8 + (0.814 × DMD)	(r^2 = 0.93; s.e. = 11.8)

Prediction of DOMD from OMD (g/kg):

Combined cattle and sheep	DOMD = 27.3 + (0.870 × OMD)	(r^2 = 0.97; s.e. = 7.26)
Cattle	DOMD = -3.7 + (0.916 × OMD)	(r^2 = 0.97; s.e. = 8.59)
Sheep	DOMD = 96.3 + (0.770 × OMD)	(r^2 = 0.97; s.e. = 6.62)

It is preferable to directly measure OMD or DOMD to account for variations in ash content and to avoid an additional calculation step (and a potential source of error) when predicting DOMD from DMD. However some Australian feed testing laboratories determine DMD and incorporate a DOMD calculation step (using the SCA equations) into their ME predictions. A comparison of the predicted DOMD values using the SCA equation and the combined equation above is provided in Table 15.

Table 15. Prediction of DOMD from DMD (g/kg true DM) using the SCA (1990) equation* and the combined cattle/sheep regression from this project.

DOMD prediction equation	Silage DMD (g/kg true DM)					
	500	**550**	**600**	**650**	**700**	**750**
SCA (1990)	466	514	561	609	656	704
Combined regression (this project)	490	533	577	620	664	707

* DOMD% = 0.95 DMD% - 0.9

[1] MAAF (1975). "Energy Allowances and Feeding Systems for Ruminants", Technical Bulletin 33 (HMSO: London, UK).

[2] SCA (1990). "Feeding Standards for Australian Livestock. Ruminants." (CSIRO: Melbourne, Australia).

When silage digestibility was low our equation gave higher predicted values for DOMD than the SCA equation. The difference (equivalent to 0.3 MJ of ME/kg DM) declined with increasing digestibility and at a DMD of 750 g/kg there was virtually no difference.

SCA (1990) emphasised that their equation should be confined to forages with ash contents in the range 90-120 g/kg DM. The ash content of our silages varied from 51 to 110 g/kg DM (Table 8). It is common for crops such as maize to have low ash contents, and use of the SCA equation for these forages would result in an underestimation of DOMD and hence ME. For our cattle equation we divided the silages into low (<930 g/kg) and high (>930 g/kg) ash categories to determine whether there was a need to develop separate regressions – this did not prove necessary. Although our animals were fed a mineral supplement, this only marginally increased the ash content of the whole diet offered to cattle from 51-110 g/kg in the silages to 53-124 g/kg whole diet, so would not effect the conclusions drawn above.

4. Using Laboratory Methods to Predict Digestibility (*in vivo*)

4.1 *In vitro* digestibility

4.1.1 Silage regressions

The relationships between *in vivo* and *in vitro* (IV) digestibility on both a true and oven DM basis are listed below. The individual regressions are based on the period means of 30 silages for cattle and 24 for sheep. The combined equation is based on the period means of the 30 silages (species meaned within period).

True DM basis (g/kg):

Combined DMD = 252.2 + (0.583 × IVDMD)	(r^2 = 0.67; s.e. = 27.6)
Cattle DMD = 247.0 + (0.591 × IVDMD)	(r^2 = 0.63; s.e. = 30.8)
Sheep DMD = 268.9 + (0.559 × IVDMD)	(r^2 = 0.69; s.e. = 28.6)
Combined OMD = 214.0 + (0.702 × IVOMD)	(r^2 = 0.77; s.e. = 23.4)
Cattle OMD = 284.8 + (0.588 × IVOMD)	(r^2 = 0.64; s.e. = 31.0)
Sheep OMD = 269.9 + (0.620 × IVOMD)	(r^2 = 0.78; s.e. = 24.6)
Combined DOMD = 180.0 + (0.723 × IVDOMD)	(r^2 = 0.74; s.e. = 22.8)
Cattle DOMD = 222.5 + (0.646 × IVDOMD)	(r^2 = 0.60; s.e. = 30.5)
Sheep DOMD = 253.9 + (0.604 × IVDOMD)	(r^2 = 0.70; s.e. = 24.2)

The above sheep regressions are illustrated in Fig. 4, 5 and 6 in section 4.1.2.

Oven DM basis (g/kg):

Combined DMD = 168.1 + (0.720 × IVDMD)	(r^2 = 0.68; s.e. = 29.0)
Cattle DMD = 160.1 + (0.734 × IVDMD)	(r^2 = 0.62; s.e. = 33.8)
Sheep DMD = 203.8 + (0.665 × IVDMD)	(r^2 = 0.65; s.e. = 29.3)

Combined OMD = 186.0 + 0.756 × IVOMD) ($r^2 = 0.73$; s.e. = 26.7)
Cattle OMD = 184.5 + (0.757 × IVOMD) ($r^2 = 0.64$; s.e. = 33.0)
Sheep OMD = 184.6 + (0.762 × IVOMD) ($r^2 = 0.76$; s.e. = 25.8)

Combined DOMD = 152.9 + (0.772 × IVDOMD) ($r^2 = 0.70$; s.e. = 25.7)
Cattle DOMD = 136.4 + (0.800 × IVDOMD) ($r^2 = 0.59$; s.e. = 34.1)
Sheep DOMD = 182.6 + (0.724 × IVDOMD) ($r^2 = 0.70$; s.e. = 23.9)

Given the diversity of the silages fed and the relatively small number (30), the precision of these relationships is good compared to other published studies. With grass silages in the UK *in vitro* DOMD accounted for 74% of the variation in *in vivo* DOMD (Barber *et al.* 1990[1]; Givens *et al.* 1989[2]), a result similar to the combined regression above (true DM basis, 73%). These UK studies were both based on more than 120 grass silages. In an earlier study with a total of 56 grass and maize silages *in vitro* OMD and DOMD accounted for 73 and 58% of the variation in *in vivo* digestibility respectively (Aerts *et al.* 1977)[3]. There was no evidence in our data of different regressions for different classes of silages (using categories similar to those in Table 9).

Generally the precision of the OMD and DOMD regressions was better than that for DMD, and better for sheep than for cattle. The latter may reflect the greater number of animals used to determine digestibility in sheep than in cattle. With the exception of DMD, the combined regressions based on true DM gave higher precision than those based on oven DM.

We investigated the inclusion of silage N and the various fibre fractions in multiple regressions with *in vitro* digestibility but there was no improvement in the precision of *in vivo* prediction.

4.1.2 Silage vs hay

Using the sheep data a comparison was made of the *in vivo* / *in vitro* relationships for the silages from this project and the hays from the project at Hamilton. The results are presented in Fig. 4, 5 and 6 below.

In vitro digestibility accounted for less of the variation in *in vivo* OMD and DOMD of the hays than of the silages, but the variance accounted for was similar for DMD. In all case the standard errors were higher for the hay regressions. An interesting feature of the results was that the regressions were different – although *in vivo* digestibility of hays and silages were similar when *in vitro* digestibility was high, *in vivo* digestibility of hay was lower than that of silage when *in vitro* digestibility was low. Such a result is surprising but may reflect the experimental protocol used in the *in vivo* studies at Hamilton. Although the N content of some of the hays was low (see Table 16), no supplementary N was provided to ensure that dietary rumen degradable N was adequate for normal rumen function. Hence digestibility may have been depressed on some hays. This would not have been a problem in the silage experiments as supplementary urea was provided if silage N content was low.

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[1] Barber, G.D., Givens, D.I., Kridis, M.S., Offer, N.W. and Murray, I. (1990). *Animal Feed Science and Technology* **28**: 115-128.

[2] Givens, D.I., Everington, J.M. and Adamson, A.H. (1989). *Animal Feed Science and Technology* **24**: 27-43.

[3] Aerts, J.V., De Brabander, D.L., Cottyn, B.G. and Buysse, F.X. (1977). *Animal Feed Science and Technology* **2**: 337-349.

Figure 4. Relationships between *in vivo* and *in vitro* DMD's (g/kg DM) for silages and hays.

Silage: DMD = 268.9 + (0.559 × IVDMD) ($r^2 = 0.69$; s.e. = 28.6)
Hay: DMD = 3.2 + (0.912 × IVDMD) ($r^2 = 0.71$; s.e. = 36.1)

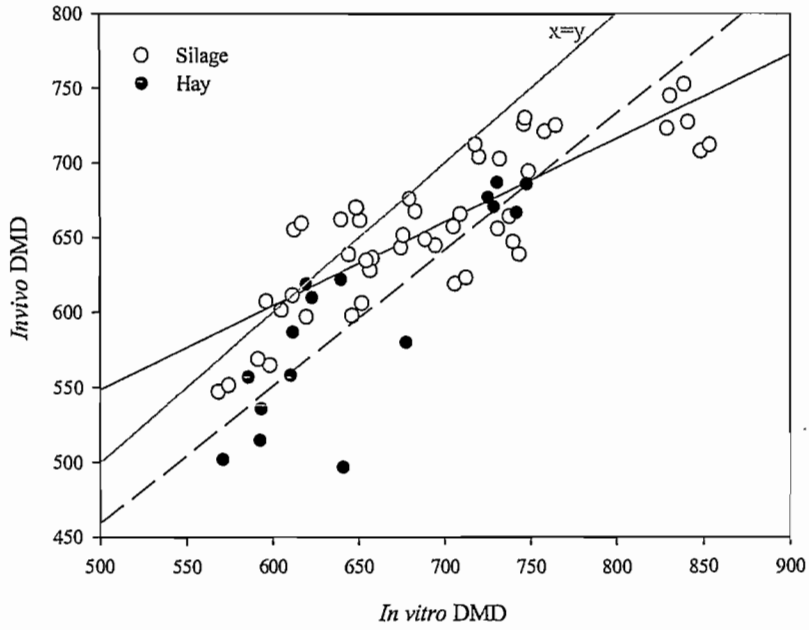


Figure 5. Relationships between *in vivo* and *in vitro* OMD's (g/kg OM) for silages and hays.

Silage: OMD = 269.9 + (0.620 × IVOMD) ($r^2 = 0.78$; s.e. = 24.6)
Hay: OMD = 122.6 + (0.781 × IVOMD) ($r^2 = 0.67$; s.e. = 34.7)

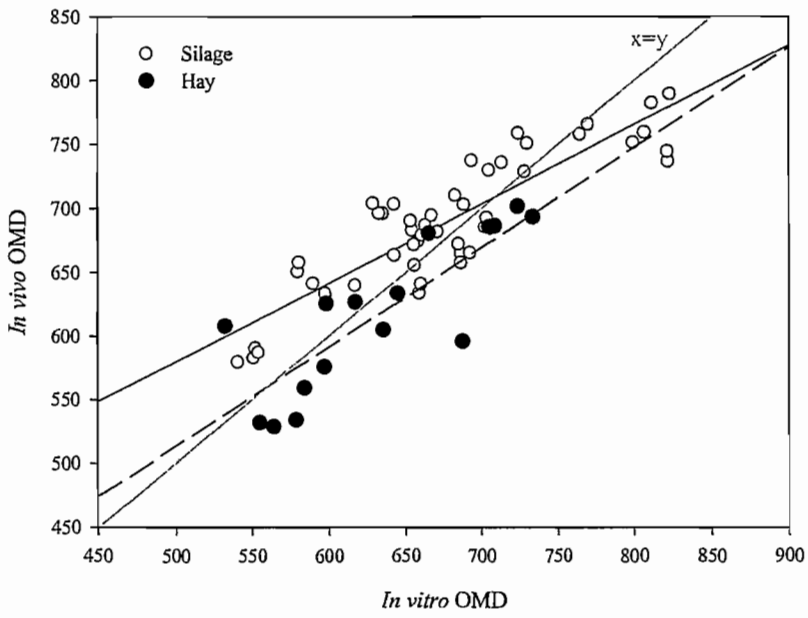


Figure 6. Relationships between *in vivo* and *in vitro* DOMD's (g/kg DM) for silages and hays.

Silage: DOMD = 253.9 + (0.604 × IVDOMD) (r^2 = 0.70; s.e. = 24.2)
Hay: DOMD = 136.5 + (0.740 × IVDOMD) (r^2 = 0.62; s.e. = 31.8)

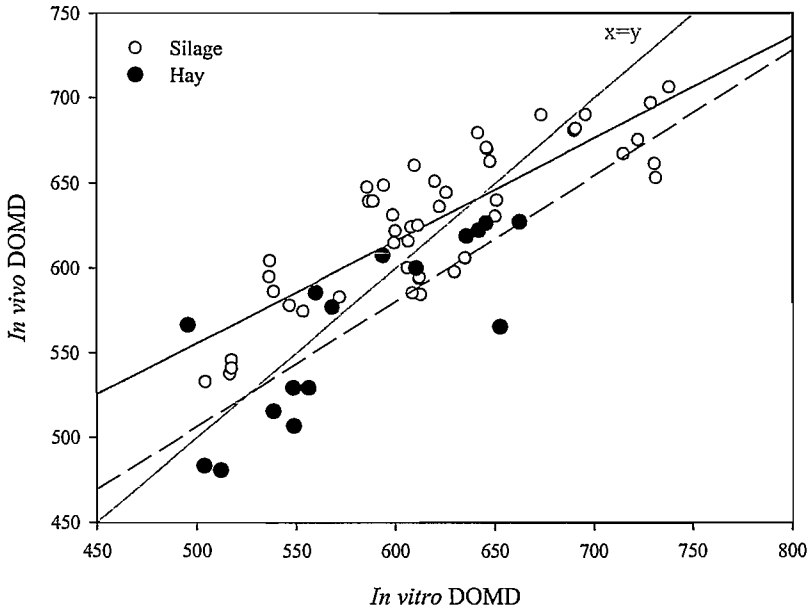


Table 16. The N content and *in vivo* and *in vitro* DOMD of the standard hays from Hamilton.

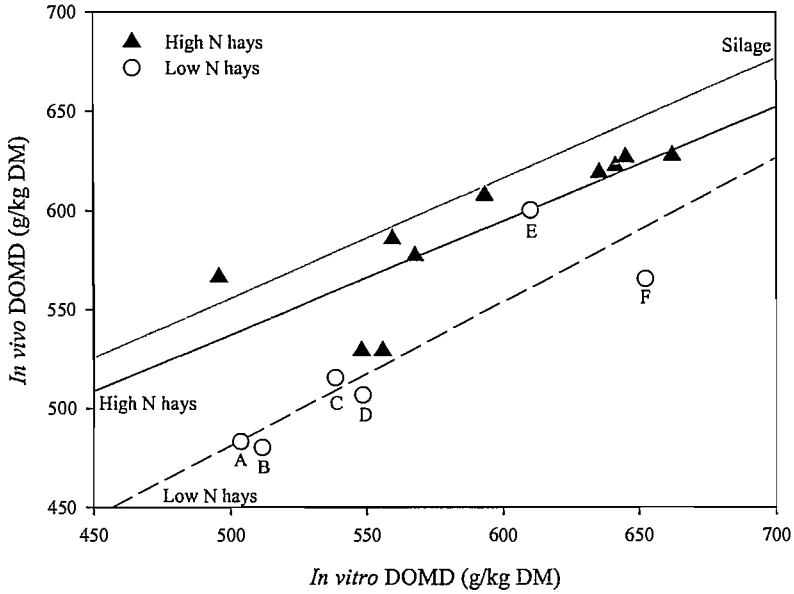
Hay	Total N (g/kg DM)*	DOMD *in vivo* (g/kg DM)	DOMD *in vitro* (g/kg DM)
Balansa clover (BAL R2)	20.8	607.6	593.4
Barley (BH R2) E	11.4	600.1	610.1
Lucerne (LUC R1)	25.3	585.6	559.7
Lucerne (LUC R2)	21.3	566.8	495.7
Medic (MED R1)	31.7	627.5	662.1
Oaten, good (OAG R1) F	8.8	565.6	652.4
Oaten, poor (OAS R1) D	9.3	506.8	548.7
Pasture, good (PAG R1)	31.5	626.7	645.1
Pasture, poor (PAP R1) C	13.4	515.6	538.7
Pasture (PAS R2)	17.0	577.2	568.1
Persian clover (PER R1)	22.2	619.0	635.4
Sorghum 2 (SO2 R2) A	11.4	483.3	503.7
Sorghum 1 (SO1 R2) B	13.0	480.7	511.7
Vetch (VET R1)	26.6	529.5	548.4
Vetch (VET R2)	31.0	622.4	641.4
Frosted wheat (WHT R2)	16.6	529.5	556.1

* For low N hays (< 14 g/kg DM), hays A to F, mean N content = 11.2 g/kg DM. Letters identified above are also shown on Fig. 7.

Supporting evidence for an effect of hay N content on *in vivo* digestibility is provided in Fig. 7. Here the hays have been divided into low (< 14 g/kg DM) and high N (> 14 g/kg DM)

groups and regressions fitted. Apart from two outliers (WHT and VET RI) the higher N hays are on a similar relationship to the silages ($r^2 = 0.66$), while the low N hays ($r^2 = 0.79$), with the exception of E (BH R2), have lower *in vivo* digestibilities.

Figure 7. Partitioning of the Hamilton hays into low and high N categories – a comparison of the relationships between *in vivo* and *in vitro* DOMD's for silages and the two categories of hays (for identity of low N hays see Table 16).



4.2 Pepsin cellulase digestibility

4.2.1 Silage regressions

The relationships between *in vivo* and pepsin cellulase (PC) digestibility on both a true and oven DM basis are listed below. The individual regressions are based on the period means of 30 silages for cattle and 24 for sheep. The combined equation is based on the period means of the 30 silages (species meaned within a period).

True DM basis (g/kg):

Combined DMD = 301.2 + (0.520 × PCDMD)	($r^2 = 0.51$; s.e. = 34.2)
Cattle DMD = 367.9 + (0.426 × PCDMD)	($r^2 = 0.40$; s.e. = 39.1)
Sheep DMD = 267.1+ (0.562 × PCDMD)	($r^2 = 0.61$; s.e. = 31.7)
Combined OMD = 358.8 + (0.524 × PCOMD)	($r^2 = 0.52$; s.e. = 34.1)
Cattle OMD = 425.3 + (0.418 × PCOMD)	($r^2 = 0.38$; s.e. = 40.8)
Sheep OMD = 323.7 + (0.574 × PCOMD)	($r^2 = 0.66$; s.e. = 30.2)

The above sheep regressions are illustrated in Fig. 8 and 9 in section 4.2.2. .

Oven DM basis (g/kg):

Combined DMD = 325.9 + (0.481 × PCDMD) (r^2 = 0.52; s.e. = 35.5)
Cattle DMD = 331.1 + (0.475 × IPCDMD) (r^2 = 0.45; s.e. = 40.9)
Sheep DMD = 240.8 + (0.607 × PCDMD) (r^2 = 0.54; s.e. = 33.6)

Combined OMD = 380.4 + 0.483 × PCOMD) (r^2 = 0.52; s.e. = 35.7)
Cattle OMD = 388.5 + (0.467 × PCOMD) (r^2 = 0.42; s.e. = 41.9)
Sheep OMD = 278.6 + (0.651 × PCOMD) (r^2 = 0.61; s.e. = 32.8)

Our results show that the pepsin cellulase method does not predict *in vivo* digestibility of silages with the same precision of the *in vitro* digestibility method. A similar result was observed by Barber *et al.* (1990) with 122 grass silages in the UK, although their r^2 was a little higher (0.55). Better precision (r^2 = 0.68) was obtained by Givens *et al.* (1989) in a study with 124 grass silages. The magnitude of the difference between the two methods was greater than expected and may reflect the very diverse set of silages used in the study – certainly more diverse than would be the case with hays.

As was the case with the *in vitro* digestibility data, we divided the silages into various silage type categories (see Table 9), but there was no clear evidence of different pepsin cellulase / *in vivo* relationships for different silage types. However, the summer forage crops gave variable results, and if dropped from the OMD regressions, the r^2 values were improved to 0.69, 0.59 and 0.74 for the combined, cattle and sheep regressions respectively. There was no evidence of a different regression for the silages containing starch (maize, sorghum and perhaps some whole crop cereal silages), so it would appear that an amylase step in the digestion process (as is the case in the recommended AFIA method) may not be needed.

The precision of the sheep regressions was better than that for the cattle regressions, possibly due to the greater number of animals per observation (period mean) for the sheep data.

We also investigated the inclusion of silage N and the various fibre fractions in multiple regressions with pepsin cellulase digestibility but there was no improvement in the precision of *in vivo* prediction.

4.2.2 Silage vs hay

Using the sheep data a comparison was made of the *in vivo* / pepsin cellulase relationships for the silages from this project and the hays from the project at Hamilton. The results are presented in Fig. 8 and 9 below.

As expected, there was a good relationship between pepsin cellulase digestibility and *in vivo* DMD and OMD for the hays. This result is consistent with other studies, including those at Hamilton. Although the variance accounted for was less with the silages, the hay and silage regressions were generally similar. This contrasts with the results from *in vitro / in vivo* digestibility relationships discussed earlier where they were quite different. We suggested that the low N hays may have had depressed *in vivo* digestibilities and for this reason they lay on a separate *in vitro / in vivo* relationship to the silages and high N hays. In Fig. 10 there is evidence of a similar trend in the pepsin cellulase / *in vivo* relationships – the low N hay regression (r^2 = 0.79) has a similar slope, but at any pepsin cellulase value had a corresponding *in vivo* value 40-50 g/kg lower than the high N hays (r^2 = 0.83) and silages. It should be noted that these comparisons are based on small numbers of hays.

Figure 8. Relationships between *in vivo* and pepsin cellulase DMD's (g/kg DM) for silages and hays.

Silage: DMD = 267.1 + (0.562 × PCDMD) ($r^2 = 0.61$; s.e. = 31.7)
Hay: DMD = 222.3 + (0.623 × PCDMD) ($r^2 = 0.75$; s.e. = 33.3)

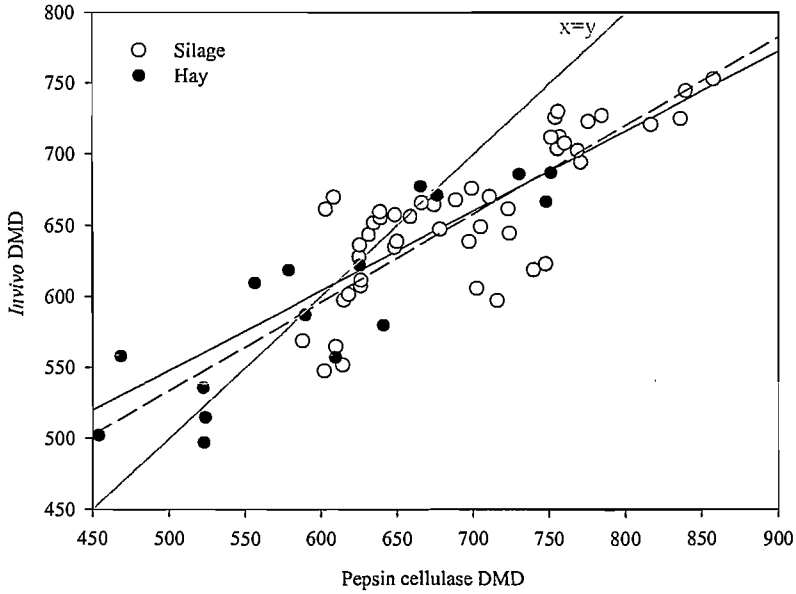


Figure 9. Relationships between *in vivo* and pepsin cellulase OMD's (g/kg OM) for silages and hays.

Silage: OMD = 323.7 + (0.574 × PCOMD) ($r^2 = 0.66$; s.e. = 30.2)
Hay: OMD = 283.8 + (0.614 × PCOMD) ($r^2 = 0.78$; s.e. = 28.6)

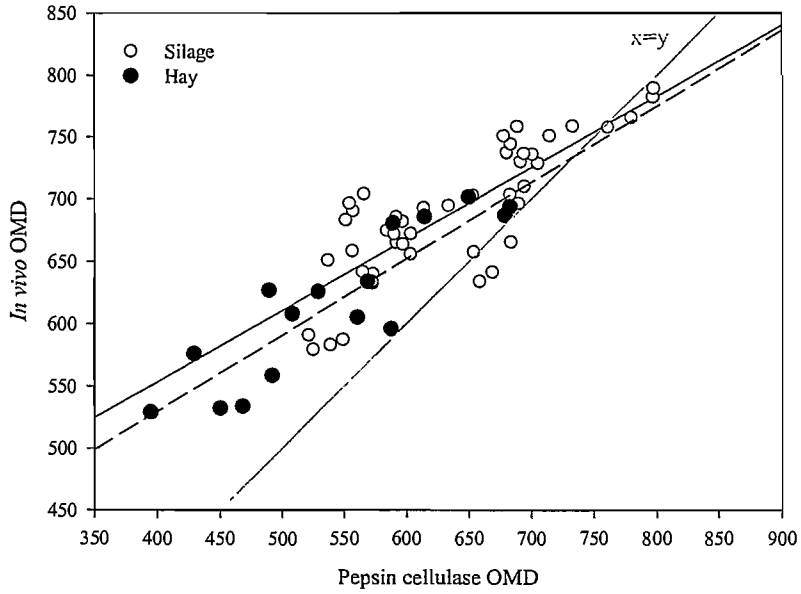
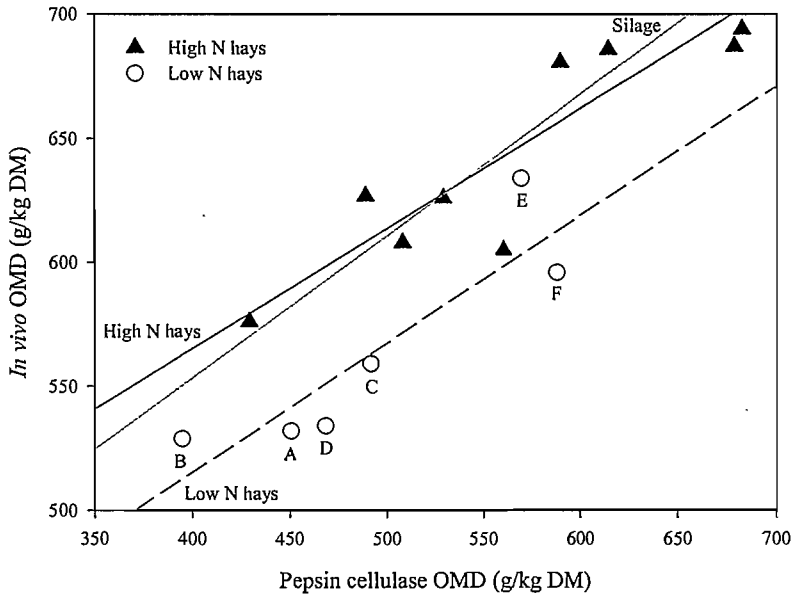


Figure 10. Partitioning of the Hamilton hays into low and high N categories – a comparison of the relationships between *in vivo* and pepsin cellulase OMD's for silages and the two categories of hays (for identity of low N hays see Table 16).



4.3 Fibre analyses

The correlations between the three *in vivo* digestibility determinations (combined sheep and cattle) and the fibre components and total N content (fresh basis) are presented in Table 17. Negative correlations were observed between digestibility and NDF, ADF, ADL and AIA, but only the correlations with NDF and ADF exceeded 0.5. Presenting the fibre data on an ash free basis had little effect on the correlations, nor did analysing ADF sequentially (after NDF) or directly.

The lack of high correlations between digestibility and the various fibre measures indicated that they would have limited value in predicting digestibility, explaining no more than 33% of the variation (NDF). Other studies have reported r^2 values in the range 0.44 to 0.66 for NDF, 0.32 to 0.65 for ADF, and 0.22 to 0.78 for lignin. The range in silage types was smaller, and the number of silages larger in these European studies.

A number of multiple regressions were evaluated, examining combinations of fibre analyses with and without total N content. Previously, some Australian feed testing laboratories used a prediction based on ADF and N. As can be seen from Fig. 11 only 28% of the variation in *in vivo* DOMD (combined sheep and cattle) is explained by this relationship, and it is therefore of little value for predicting the digestibility of silages.

The best relationship we examined was little better. It was based on silage NDF, ADL and N and accounted for a little under half of the variation in digestibility (Fig. 12). Our conclusion is that for the diverse set of silages examined, prediction equations based on fibre analyses are likely to be of limited value for predicting digestibility *in vivo*. While some studies in Europe have had more success in developing prediction equations based on fibre analyses, these have invariably proved inferior to prediction based on digestibility assays – either the rumen fluid or pepsin-cellulase methods.

Table 17. Correlations between *in vivo* digestibility (DMD, OMD and DOMD) fibre components and N content*.

NDF-ash	0.998								
ADF	0.945	0.95							
ADF-ash	0.929	0.94	0.996						
ADF direct	0.913	0.920	0.985	0.984					
ADL	0.205	0.187	0.287	0.254	0.265				
ADL-ash	0.195	0.193	0.317	0.309	0.303	0.962			
AIA	0.029	-0.01	-0.044	-0.106	-0.090	0.741	0.586		
Total N	-0.674	-0.665	-0.493	-0.471	-0.460	0.013	0.071	-0.125	
DMD	-0.578	-0.561	-0.546	-0.51	-0.552	-0.395	-0.321	-0.204	0.423
OMD	-0.564	-0.548	-0.533	-0.499	-0.543	-0.397	-0.331	-0.187	0.379
DOMD	-0.543	-0.53	-0.555	-0.526	-0.578	-0.417	-0.367	-0.144	0.285
	NDF	NDF-ash	ADF	ADF-ash	ADF direct	ADL	ADL-ash	AIA	Total N

* All data expressed on a true DM basis. Total N determined on fresh silage. NDF, ADF and ADL determined sequentially, and presented as analysed or on an ash free basis (AIA subtracted). ADF also determined directly.

Figure 11. Relationship between OMD predicted from a regression based on silage ADF and N and actual (*in vivo*) OMD. All data expressed as g/kg and on a true DM basis.

In vivo OMD = 793.6 – (0.405 × ADF) + (0.960 × N) ($r^2 = 0.28$; s.e. = 41.4)

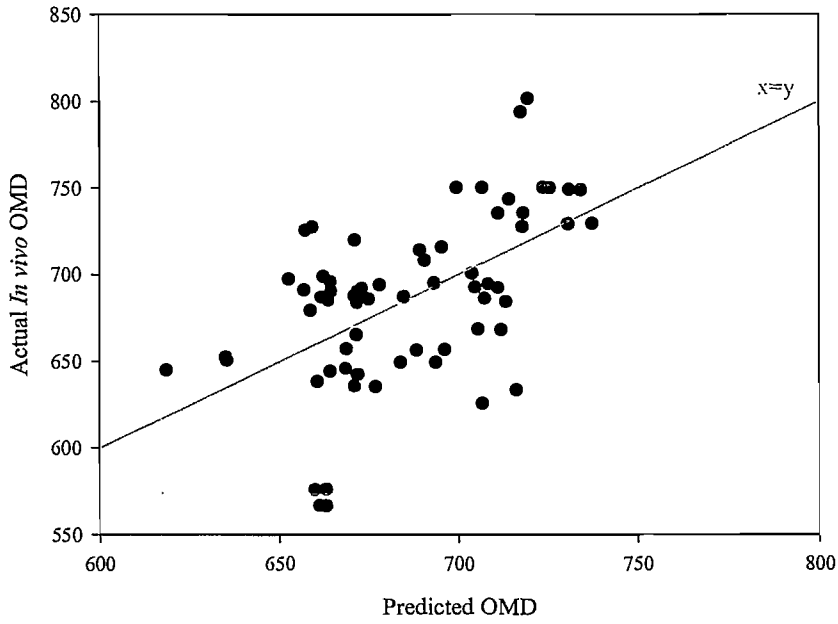
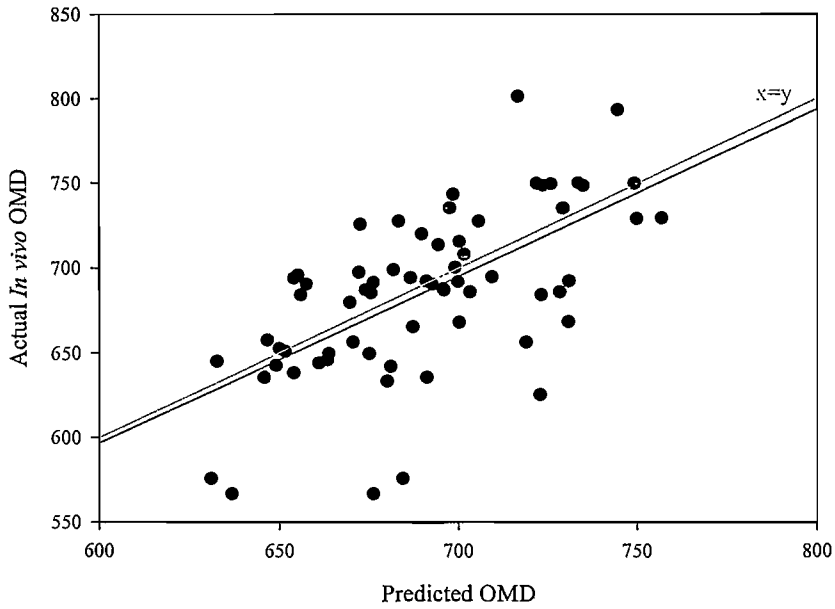


Figure 12. Relationship between OMD predicted from a regression based on silage NDF, ADL and N and actual (*in vivo*) OMD. All data expressed as g/kg and on a true DM basis.

In vivo OMD = 815.4 – (0.176 × NDF) – (1.025 × ADL) + (0.917 × N)
($r^2 = 0.47$; s.e. = 32.4)



5. Predicting the True DM Content of Silages

Many feed testing laboratories in Australia determine the DM content of silages by on oven drying, leading to a loss of silage volatiles and an underestimation of true DM content. Earlier we developed an equation for predicting true DM content of silages from oven DM determined at 80°C (Kaiser *et al.* 1995). While this has been adopted by some laboratories, a more robust equation based on a larger number of more diverse silages is required. This was done using the silage data presented in Tables 8 and 18.

The equation presented in Fig. 13 is based on the data presented in this report and our earlier data (Kaiser *et al.* 1995). It covers 60 silages with true DM contents in the range 180-728 g/kg. Data collected in the future will be added to this set – the regression will be updated if necessary, and circulated to feed testing laboratories.

The error due to underestimation of DM content can have important implications when appraising silage quality. Chemical analysis results (eg. fibre) will generally be overestimated when expressed on an oven DM rather than true DM basis. Exceptions are digestibility and N content as the volatile compounds (= organic matter) lost on oven drying are completely digestible and contain some N (see section 6 below). As a result, silage digestibility and N content will be underestimated. The impact on silage digestibility and estimated ME content for the silages fed in this project is illustrated in Table 19. With this set of silages, failure to take account of silage volatiles resulted in an underestimation of ME between 0.1 and 1.3 MJ/kg DM (mean 0.5 MJ/kg DM).

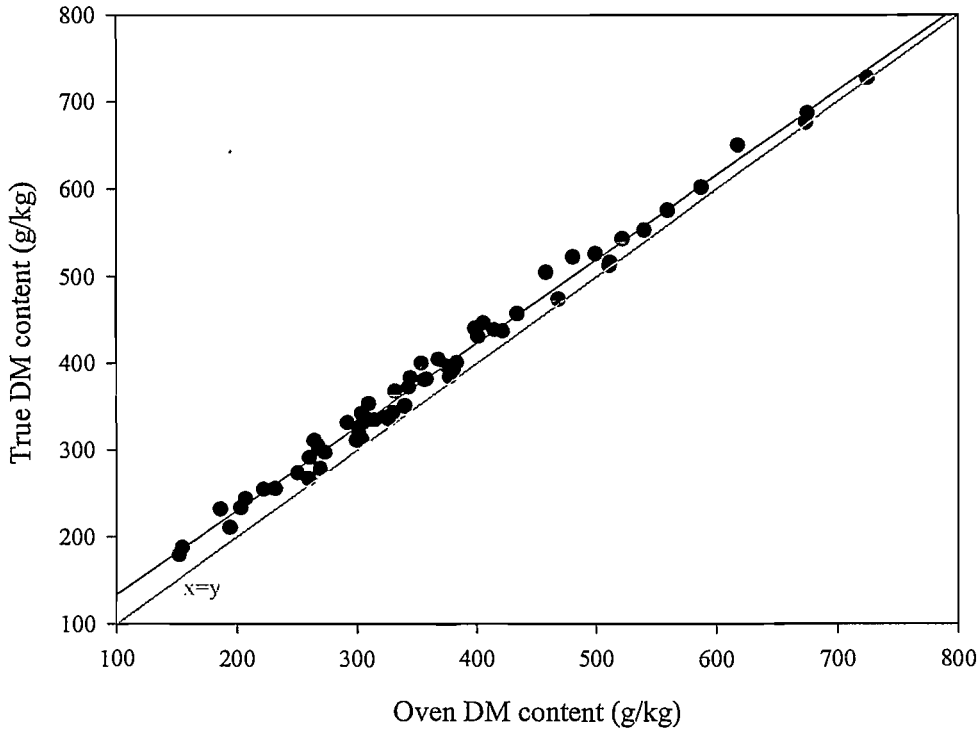
Table 18. Additional silages collected on farms in southern NSW for the silage DM and total N studies

Silage No.	Description of silage	Oven DM (g/kg)	True DM (g/kg)	pH	Ammonia-N (g/kg total N)	N content in fresh sample (g/kg TDM)
32	Oat	674.8	687.4	5.1	31.9	13.6
33	Unspecified (pasture)	303.7	312.8	3.8	110.9	21.9
34	Lucerne	400.9	431.8	4.6	70.2	16.79
35	Oat	264.0	311.1	3.9	87.5	11.89
36	Forage sorghum	252.5	287.0	8.4*	270.0	13.76
37	Pasture	342.9	373.0	4.0	85.6	23.72
38	Lucerne	310.4	335.2	3.9	74.4	26.85
39	Unspecified (pasture)	421.3	438.0	4.9	86.1	28.2
40	Unspecified (pasture)	587.0	602.2	5.3	49.8	15.94
41	Pasture	539.2	553.6	4.6	67.7	21.23
42	Clover	673.5	676.8	5.4	42.0	22.98
43	Clover / ryegrass	617.5	650.4	5.3	38.0	11.99
44	Lucerne	511.0	517.0	5.4	52.7	22.24
45	Unspecified (pasture)	724.4	727.5	5.1	35.2	10.93
46	Unspecified (pasture)	457.8	505.6	4.3	84.9	22.35
47	Lucerne	498.9	526.5	4.5	113.4	27.26
48	Triticale / lucerne	309.6	353.7	3.7	61.3	12.02

* This sample is clearly spoiled, given the high pH, and has been dropped from the data set

Figure 13. Relationship between true DM (as determined by Karl Fischer titration) and oven DM content of silages. DM expressed as g/kg.

True DM = 38.46 + (0.96 × oven DM) ($r^2 = 0.99$; s.e. = 12.8)



6. Error in Total N Content of Silages Resulting from Oven Drying

Because some of the N fraction in silage is volatile, conducting N analyses on oven dried silage samples will result in an underestimation of silage N. There is the additional problem of taking into account true DM content when expressing the results of N analyses. The two sources of error can to some extent cancel one another out as can be seen in Fig. 14(a) where N content of the oven dried silage sample is expressed as g/kg oven DM. Where the results of N analyses on fresh and oven dried samples are both expressed on a true DM basis (Fig. 14(b)), the losses due to oven drying become more evident.

Attempts to predict, with any precision, the **difference** between N analyses on the fresh and dried samples using the silage DM, pH and ammonia-N were not successful. These measures are more likely to be available to feed testing laboratories than more sophisticated analyses on the N fraction in silages. The volatility of N will vary from silage to silage and in order to predict this, more detailed information will be required on silage fermentation products, particularly the N fraction.

Table 19. The effect of calculating digestibility on an oven DM or true DM basis on the error in DOMD and ME estimates in the 30 silages fed in this project.

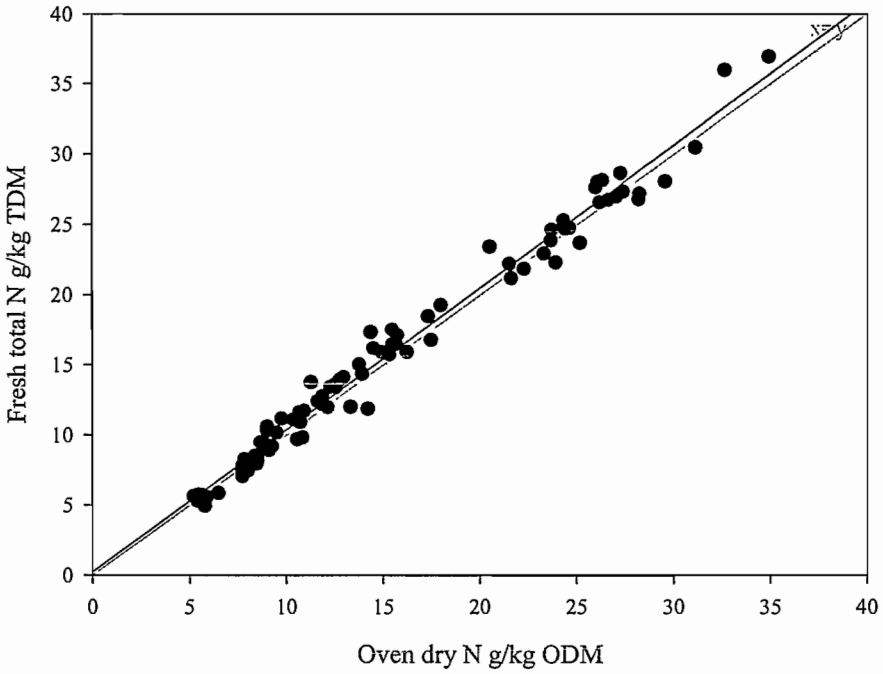
Silage No.	DOMD (g/kg DM)		Error in DOMD (g/kg DM)	Error in ME (MJ/kg DM)
	Oven DM basis	True DM basis		
1	595.9	609.9	14.0	0.2
2	560.2	601.6	41.4	0.7
3	658.3	679.9	21.6	0.3
4	629.1	658.3	29.2	0.5
5	717.9	731.5	13.6	0.2
6	581.0	619.9	38.9	0.6
7	635.2	668.3	33.1	0.5
8	576.5	624.8	48.3	0.8
9	622.8	630.7	7.9	0.1
10	619.5	633.4	13.9	0.2
11	620.9	633.6	12.7	0.2
12	550.4	580.8	30.4	0.5
13	528.2	531.6	3.4	0.1
14	573.1	580.1	7.0	0.1
15	551.9	587.3	35.4	0.6
16	493.8	521.3	27.5	0.4
17	630.8	673.6	42.8	0.7
18	630.2	673.1	42.9	0.7
19	560.5	589.5	29.0	0.5
20	611.2	651.6	40.4	0.6
21	601.9	614.6	12.7	0.2
22	660.8	670.2	9.4	0.2
23	556.5	597.8	41.3	0.7
24	599.9	632.7	32.8	0.5
25	537.7	583.0	45.3	0.7
26	579.1	634.0	54.9	0.9
27	535.3	593.6	58.3	0.9
28	547.9	615.8	67.9	1.1
29	573.4	655.4	82.0	1.3
30	643.0	661.8	18.8	0.3

It is not surprising that there is a strong relationship between N in fresh silage and that in an oven dried sample of the same silage. The regressions in Fig. 14 provide Australian feed testing laboratories working with dried silage samples with an objective basis for correcting their analytical results and predicting N in the fresh silage.

Figure 14. Prediction of silage N (g/kg true DM) determined on a fresh silage sample from N determined on an oven dried sample.

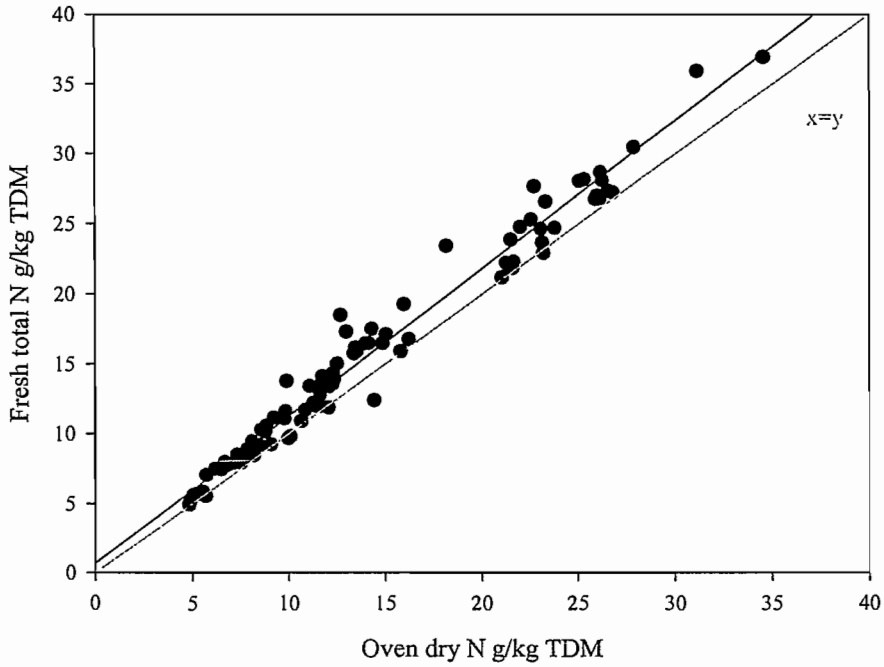
(**a**) N on oven dried sample expressed as g/kg oven DM

$N_{true} = 0.291 + (1.004 \times N_{oven\ dried})$ ($r^2 = 0.99$; s.e. = 0.883)



(**b**) N on oven dried sample expressed as g/kg true DM

$N_{true} = 0.653 + (1.051 \times N_{oven\ dried})$ ($r^2 = 0.98$; s.e. = 1.000)



Milestones Reached

Milestone	Date	Achievement
1. Funding and methodology finalised.	31/12/00	*Achievement Criteria:* Final budget available provided to NSW Agriculture by DRDC. Budget, revised methodology and new milestones added to this application and returned to DRDC. Contract varied if necessary. *Outcome:* At the time the contract was signed it was unclear whether there would be sufficient funds to complete the research planned. MLA had not decided on their level of investment in the project. The funds were subsequently provided and no change to the methodology was required, and we were able to expand the number of silages fed to both cattle and sheep to 24. RIRDC requested that the hay standards developed at Hamilton be included in our laboratory evaluation to determine whether similar prediction equations applied to both silages and hays. None of the above changes required a revision of the milestones or a variation of the contract.
2. Complete *in vivo* digestibilities with cattle and sheep for a range of silages	30/06/03	A progress report in RIRDC format was submitted on 30/11/02 providing the DMD, OMD and DOMD of the 30 silages in both cattle and sheep.
3. Complete prediction equations for estimating true DM and true N from oven dried silage samples.	30/06/03	These equations were presented at RIRDC's Fodder R & D Workshop on 13 August 2003, along with other data from the project. However presentation of these and all other results was held over to this report. While there was some delay in procurement of enzyme and hay samples, the main issue was the surprisingly poor relationship between pepsin-cellulase and *in vivo* digestibilities. Given that the pepsin-cellulase method is widely used by feed testing laboratories it was considered, after discussions with DRDC and RIRDC, that additional analyses were required. This additional work was conducted at Wagga Wagga and Hamilton and strengthens the conclusions on the use of pepsin-cellulase for assessing silage quality.

Industry Implications

TopFodder Silage is encouraging farmers to have their silage tested on a routine basis, as a quality control measure, so that they can identify opportunities to improve their silage management, and for diet formulation purposes. Trading of silage, or crops for silage, should also reflect the quality of the product. A chapter in the "Successful Silage" manual has been devoted to silage testing and the interpretation of silage tests. Given that important management decisions are, and will be, based on laboratory measures of silage quality it is essential that these analyses are accurate and that farmers have confidence in the results.

As well as encouraging farmers to have feed tests conducted on each batch of silage, we are also working with the feed testing laboratories to advise them on more accurate methods for assessing silage quality, and the adoption of new tests that provide a measure of silage fermentation quality. We are also assisting the feed testing laboratories on how to interpret a silage analysis.

Within the context of the whole TopFodder Silage project, it is not possible to separate out the contribution of improved laboratory tests for silages to the overall financial impact (the project has a high benefit cost ratio) on industry. However laboratory testing is a key tool driving practice change so its contribution to project outcomes will be significant. Some of the specific outcomes are:-

Re-evaluation of typical silage ME content:
It is now accepted that the ME content of silages has been systematically underestimated by the use of generalised prediction equations applied over all forages. The AFRC (1993) equation specifically applied to silages, and based on the measurement of DOMD and ME *in vivo* in the UK, was presented earlier (section 2 of Results and Discussion). It is the recommended equation for silages, has been adopted in "Successful Silage", and we are recommending its adoption by AFIA. The impact of the choice of equation on the predicted ME of silages varying in DOMD is as follows:-

Prediction equation used to estimate silage ME (MJ/kg DM)	Silage DOMD (g/kg DM)			
	550	600	650	700
1. AFRC (1993) silage	8.8	9.6	10.4	11.2
2. AFRC (1993) other forages	8.6	9.4	10.2	11.0
3. SCA (1990)	8.1	9.0	9.9	10.8
4. AFIA Laboratory Methods Manual (2003)	8.4	9.2	10.1	11.0

The AFIA equation is based on DMD, and this was estimated from the DOMD values above using the equation in SCA (1990). Note that the general equations 2, 3 and 4 above, covering a range of feeds, each give lower predicted ME's than the recommended AFRC silage equation.

Error due to volatile DM loss:
Direct measurement of the true DM content of silages requires specialised equipment (Karl Fischer titrator) that is unlikely to be purchased by laboratories for routine feed analyses. The project has provided an equation for predicting true DM from oven DM. AFIA has decided to adopt this equation. In the future feed testing laboratories will now be able to present their results on a true DM basis. There are special implications for the determination of digestibility and the subsequent estimation of ME. Our results have shown that with the 30

silages used in the feeding experiments, failure to account for volatile DM could result in an underestimation of silage ME by up to 1.3 MJ/kg DM (see Table 19). Even larger errors would occur for silages with a higher content of volatile DM than observed here.

Error in calculating DOMD from DMD:
Some laboratories measure DMD and subsequently estimate DOMD prior to calculating ME. The DMD / DOMD conversion is based on, for example, equations in SCA (1990) referred to earlier (see Results and Discussion, section 3) or may actually be incorporated into an ME prediction equation. We used our *in vivo* data to derive a prediction of DOMD from DMD for silages. For lower digestibility silages this equation gave higher DOMD predictions than the SCA equation. For silages with DMD in the range 500-600 g/kg the underestimation of ME would be equivalent to 0.3-0.4 MJ/kg DM.

Error in N analyses conducted on oven dried samples:
Conducting N analyses on oven dried silage samples results in losses of volatile N and an underestimation of the true N content (analysis conducted on a fresh silage sample). The error varies from 0.9 (CP = 5.7) to 2.4 (CP = 15.0) g/kg true DM on low and high N silages respectively (see Fig. 14b). The project has provided feed testing laboratories with a correction equation for estimating true N content from an analysis on an oven dried sample. AFIA has decided to adopt this equation.

The most accurate laboratory method for estimating digestibility in vivo:
Our results have shown that for the diverse set of silages evaluated in this project no useful prediction equations could be developed using fibre components. In the past some laboratories have used a generalised equation based on ADF and N, but for our silages this accounted for only a small proportion of the variation – applying this equation to silages would result in significant errors in DOMD and subsequently ME prediction.

The pepsin-cellulase method, used by a number of feed testing laboratories in Australia, did not offer the same level of precision in predicting *in vivo* digestibility for silages as has been observed with hays. Studies with hays have often observed r^2 values of 0.75-0.85, and this was the case here with the Hamilton hays (sheep data). The combined sheep/cattle silage regressions here had r^2 values of only 0.51 and 0.52 for DMD and OMD respectively, while the sheep only regressions were a little better. The summer forage crops appeared to be a particular problem for the pepsin cellulase method and r^2 values improved if these silages were dropped from the regressions.

The pepsin cellulase results contrast with those from the *in vitro* digestibility method, which provided best precision, and r^2 values of 0.67 and 0.77 for DMD and OMD respectively (combined sheep/cattle regressions). Although the *in vitro* digestibility method is the most accurate for predicting *in vivo* digestibility of silages, this method is not likely to be adopted by feed testing laboratories. Providing a large number of standards of known predicted *in vivo* digestibility (using our *in vitro / in vivo* relationship) to provide an NIRS calibration may be the best strategy as it would take considerable time and investment to develop a direct NIRS calibration using *in vivo* data.

Estimating silage fermentation quality:
Although methods for assessing silage fermentation quality were not part of this laboratory methods project, they are an important issue for the TopFodder Silage project. A measure of silage fermentation quality is critical to silage evaluation – if fermentation quality is poor

silage intake and the utilisation of silage N will be depressed. AFIA has agreed to include silage pH and ammonia-N in the recommended list of analyses for silages.

Future Research Needs and Recommendations

- It is recommended that the equations developed here for predicting true DM content and true N content, and predicting DOMD from DMD, be adopted for by Australian laboratories.

- Development of a direct NIRS calibration for *in vivo* digestibility of silage would clearly be the best outcome. However the number of samples required is large (>100). This current project has generated 66 samples (30 silage × digestibility period). We can access 33 maize silage samples (silage × digestibility period) from earlier studies. It is recommended that additional *in vivo* studies be conducted to generate sufficient sample for a robust calibration.

- It is highly unlikely that the *in vitro* digestibility procedure will be used by commercial feed testing laboratories. However, given that a useful *in vitro / in vivo* relationship has been developed using the silages in this project, there is an opportunity to use this relationship to provide predicted *in vivo* digestibility values for a larger set of silages (say 150). These data could then be used to develop an NIRS calibration for use by feed testing laboratories. It is recommended that this work be conducted as soon as possible and that the silage samples be drawn from the TopFodder Silage project.

- There is debate amongst feed testing laboratories on the need to add an amylase digestion step to the pepsin-cellulase method. As some silages contain starch the need for amylase should be investigated.

- UK research has shown that the inclusion of gross energy in equations predicting digestibility may improve the % variance accounted for. This should be investigated using the silages from this project.

- The original version of this project allowed for the development of laboratory procedures to estimate losses during the ensiling process. However this component of the project had to be cut from the final application. Given the importance of silage losses in the TopFodder Silage, a laboratory based procedure for estimating losses remains high priority.

Intellectual Property

One of the key goals was to release the findings of the project and have them adopted by feed testing laboratories (both commercial and research) as quickly as possible. Having access to accurate silage tests is important to the TopFodder Silage program and will give farmers greater confidence in using feed testing laboratories for silage analyses.

The principal intellectual property generated by this project is the set of silage "standards" of known *in vivo* digestibility. Based on biometrical advice, individual period (digestibility run) samples for each silage have been kept separate rather than bulking.

Specific reference was made to the handling of the silage standards in the project application:-

"Bulk samples of the silages will be retained at either Wagga Wagga or by the Australian Fodder Industry Association (subject to an agreement). Samples will be distributed free to research laboratories on the understanding that they pay freight, processing and packaging, do not pass the standards on to a third party, use the samples only for research purposes, and provide co-authorship to the source laboratory if the samples are used to generate a new published method or calibration. If this new method/calibration has a commercial value, then the IP will be shared with the source laboratory and the RIRF's. The standards will be sold to commercial feed testing laboratories for a nominal sum (say $200-300/standard, but yet to be determined) on the understanding that they do not pass them on, or any calibration equation based on them, to a third party. Proceeds from the sale of standards will be re-invested into the project."

This policy will need to be re-visited by the stakeholders (Dairy Australia, NSW Agriculture, RIRDC and MLA) prior to its implementation.

Communication and Publications

Communication to date has concentrated on discussing the project results with feed testing laboratories. Apart from one-to-one discussion with a number of laboratories, the main activities to date have been the presentation of a summary of the results to RIRDC's Fodder R & D Technical Meeting (12-13 August 2003) and to the members of AFIA's Quality Evaluation Committee (QEC, 13 August 2003). The latter meeting was particularly important, as it included representatives from most of the feed testing laboratories, and the QEC determines AFIA policy on recommended laboratory methods for forage analyses. These methods are included in the AFIA Laboratory Methods Manual. Our true DM and N correction calibrations will be adopted by AFIA, and we have been asked to provide recommendations on methods to estimate the ME content of silages.

The results of this project will be distributed more widely to feed testing laboratories and nutritionists once this report is accepted by the funding agencies. The results will also be distributed to all trainers in the TopFodder Silage program. We also plan to publish the results in scientific journals and conference proceedings.