A uniform objective system for quality description of Australian fodder products

A report for the Rural Industries Research and Development Corporation

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Foreword

Fodder is defined as the wide range of crop and pasture species grown that are harvested and lightly processed to facilitate both on-farm use and domestic and export trade.

The fodder industry is worth approximately $700 million per annum to the Australian economy, and of the 7 million tonnes of fodder produced annually, some 1.5 million tonnes are traded on the domestic market. In 1996/77, various types of hay, chaff and pellets worth $57 million were exported to Japan alone.

Historically, the fodder industry has been fragmented, without a peak industry body to represent it, and with inadequate quality standards.

While Australian rural industries are typically based more on a competitive spirit than on active cooperation and coordination, there are a number of instances where a particular industry or section of an industry has adopted a successful collaborative approach to the export or domestic marketing of their produce.

Collaborative marketing groups have the potential to be an effective alternative to other marketing arrangements.

This publication considers some of the features of group marketing together with general features of membership groups and how they influence the effectiveness of collaborative marketing groups. It analyses data from case studies and surveys collected from representatives of 13 primary industry groups and other industry representatives in Western Australia.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report, a new addition to RIRDC’s diverse range of over 600 research publications, forms part of our Fodder Crops R&D program, which aims to (To facilitate the development and maintenance of a viable fodder crops industry.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Peter Core
Managing Director
Rural Industries Research and Development Corporation
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Appreciation is also due to representatives of all the fodder testing laboratories who devoted a considerable amount of time to the difficult issue of forging an agreement on uniform testing methodology.

Finally, sincere thanks is due to AFIA Chairman Colin Simpson for his support, together with the professional secretarial assistance of Anne Fleming at various AFIA meetings.
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Executive Summary

The objective of this project was to develop and implement a national uniform objective system for quality description of Australian fodder products, based on the needs and specifications of end-users, in order to facilitate effective marketing.

The fodder industry is worth approximately $700 million to Australia, but the industry is fragmented and has been operating without adequate quality standards. There is an increasing awareness of the need to trade hay and other fodders on the basis of their feeding value, i.e. in terms of animal performance, rather than on subjective criteria such as colour and aroma. Objective measurements of fodder quality are being increasingly used, but not all testing laboratories measure the same feed constituents or use the same testing procedures, which can cause confusion within the industry. Agreement is needed throughout the industry on the most important quality measurements, and uniform analytical procedures are required between laboratories for these measurements.

The first action of this project was to convene a national forum, with delegates representing all sectors of the fodder industry from producers through to end-users. The purpose of this forum was to seek agreement on the criteria needed for a uniform system of objective quality description of fodder. At the initiative of RIRDC, an additional objective of the forum was to decide on the formation of a national body to co-ordinate the activities of the fodder industry.

The major outcomes of the national forum were:

1. An agreement that the principal quality criteria of fodder should be moisture, metabolisable energy and crude protein, with a prediction of voluntary intake to be included at a later date, depending on the development of an appropriate method.
2. An agreement that the fodder testing laboratories represented would work towards the development of uniform procedures for fodder analysis.
3. The formation of a Steering Committee to establish a national fodder industry body, which later led to the formation of the Australian Fodder Industry Association (AFIA), now recognised as the industry’s peak body and currently in its fourth year of operation.

Two subsequent meetings were held between representatives of Australia’s major fodder testing laboratories to decide on detailed procedures for the agreed standard laboratory tests, and to arrange an ongoing quality assurance programme.

A second major part of this project was to produce a set of fodder “standards”, to be used as reference samples for calibration of laboratory techniques to estimate digestibility (and hence metabolisable energy) of fodder on a routine basis. This was achieved by selecting 16 different hays, ranging widely in quality, and feeding them to sheep under controlled conditions in order to measure in vivo digestibility and ad libitum intake.

In vivo dry matter digestibility for the 16 hays, measured at a maintenance level of feeding, was found to range from 49.7% (sorghum) to 68.7% (medic). Ad libitum dry matter intake was found to range from 0.67 kg/head/day (sorghum) to 1.40 kg/head/day (vetch). Agreement has now been obtained between the major fodder testing laboratories on uniform analytical procedures to measure moisture, crude protein and metabolisable energy. Details of these procedures are outlined in the body of this report.
The project also demonstrated the potential of near infrared spectroscopy (NIR), not only for well-established applications such as the estimation of chemical composition of fodder, but also for direct prediction of in vivo digestibility and voluntary intake. Whilst there were not enough samples in this study to derive robust calibrations for these properties, it is evident that future work should focus on the production of additional fodder “standards” using a uniform protocol, so that in time NIR can replace intermediate and often unsatisfactory laboratory measurements.

Meanwhile, the achievement of uniform predictions for digestibility and metabolisable energy between fodder testing laboratories will heavily depend on the acceptance of a mechanism, recommended in this report, which will allow the sale and distribution of the 16 in vivo fodder “standards” to approved laboratories. This mechanism involves the transfer of intellectual property rights for the “standards” from both RIRDC and the Department of Natural Resources and Environment (DNRE) to AFIA, which would manage the distribution. AFIA would also arrange for regular ring tests as part of a quality assurance programme. The revenue raised from these and other activities relating to fodder testing would be utilised for future improvements to the system, in a similar fashion (but on a smaller scale) to that undertaken by the National Forage Testing Association in the USA.

A new grading system for hay and silage quality, based on metabolisable energy and protein content, has also been produced and was adopted by AFIA at its Annual Conference in 1998. The grading system is progressively being implemented nationally and will become part of the AFIA Vendor Declaration Form. It has also been accepted by the National Agricultural Commodities Marketing Association Incorporated (NACMA) as part of its trading standards. A uniform procedure for fodder sampling, which is crucial for accurate analysis, has also been produced and agreed upon by testing laboratories and fodder specialists. This will also be incorporated into the AFIA Vendor Declaration Form.

The fodder “standards” which have been produced in this project will form the basis of a nationally uniform system for laboratory estimation of fodder quality. The agreement obtained among laboratories will result in an effective system of quality description, easily recognised and understood by the industry, and will lead to improved marketing of fodder products, both in Australia and on export markets. Buyers would get better value for money, and sellers would receive bonus payments for a higher quality product.
1. Introduction

The fodder industry is worth approximately $700 million to the Australian economy, but the industry is fragmented and there has been no peak body to represent it and guide its future development. In particular, it has been operating without adequate quality standards, and the prices paid for fodder often do not reflect the likely performance of animals consuming it.

“Fodder” is often considered to be any material fed to animals, but has also been defined as “the wide range of crop and pasture species grown that are harvested and lightly processed to facilitate both on-farm use and domestic and export trade” (AFIA 1998). For the purpose of this report, fodder includes hay, silage and straw, with the emphasis on hay because of its importance as a traded commodity.

The export market for Australian fodder has increased in recent years, particularly to Japan, which bought 7,200 tonnes of lucerne hay, 8,900 tonnes of lucerne meal and pellets and 211,000 tonnes of other hay and chaff (mainly cereal), worth approximately $57 million in 1996/97 (AFIA 1998). However, some 1.5 million tonnes of the 7 million tonnes of fodder produced annually are traded on the domestic market. This growth has been accompanied by an increasing demand from both fodder consumers and producers for quality testing. There is now wide recognition of the need to trade fodder on the basis of its feeding value, rather than on the traditional subjective criteria of colour, aroma, texture and botanical composition, which have been shown to be unreliable indicators of quality. Rapid and efficient testing of fodder and many other agricultural products has become possible with the development of the analytical technique of near infrared spectroscopy (NIR), utilised by numerous testing laboratories such as Agriculture Victoria’s FEEDTEST® service at Hamilton. This has enabled objective measurements to be used as a marketing tool in the fodder industry, with some end-users developing their own pricing mechanisms based on measurements such as metabolisable energy (ME) and crude protein (CP). The quality of fodder can be extremely variable, as shown by the wide range in CP, dry matter digestibility (DMD) and ME in various hays and silages tested during the 1998/99 season (Appendices 9.1 and 9.2).

The major problem is the lack of a uniform product description system across the industry. For example, if a producer in Victoria sells hay to a client in Queensland, both parties need to use a common objective “language” to describe the product. However, not all testing laboratories measure the same fodder constituents or use the same procedures, which results in confusion and reduces confidence in fodder testing. For example, whilst digestibility is recognised as probably the most useful index of nutritive value available at present (Ulyatt 1973), there have been at least 3 different procedures used in Australian laboratories for its estimation. These include an enzymatic technique based on pepsin and fungal cellulase, with analyses adjusted using a linear regression based on feed samples with known in vivo DMD (Clarke et al. 1982); a prediction equation derived in the USA based on the measurement of acid detergent fibre (ADF) (Linn and Martin 1989); and an equation utilising both ADF and nitrogen content (Oddy et al. 1983). This problem is by no means confined to Australia, but in the USA the National Forage Testing Association, a body controlled jointly by producers, traders and laboratories, promulgates recommended procedures and conducts a quality assurance programme among some 150 commercial fodder testing laboratories.

An effective system of quality description, implemented on a national basis, easily recognised and understood by the industry, and above all industry-driven, would greatly enhance fodder
marketing in Australia and assist the competitiveness of the industry in export markets. Buyers would get better value for money, and producers would receive increased returns for a higher quality product.

There has been growing support from industry over some years for the introduction of objective quality description for fodder. A working party was set up in Victoria in 1993 by the Victorian Farmers Federation (VFF) Grains Group, under the leadership of Mr Warwick McClelland, with the aim of establishing a suitable system. However, the working party agreed that any quality system for fodder must be adopted nation-wide for it to be successful, rather than individual States setting up separate procedures. Subsequent discussions were held with representatives of various fodder producers, marketing groups and end-users, and in 1994 RIRDC convened a national workshop in Adelaide to review its research and development programme for fodder crops. A key strategy for the fodder industry emerging from that workshop was the need “to develop a product description language matching buyer specifications as a basis for developing quality standards” (RIRDC 1994). A further meeting of potential researchers in the Fodder Crops Programme was convened by RIRDC in Perth in 1995. This project, together with another proposed by CSIRO Division of Animal Production (WA), was subsequently funded to address the quality description issue.

2. Objective

To develop and implement a national uniform objective system for quality description of Australian fodder products, based on the needs and specifications of end-users, in order to facilitate effective marketing.
3. Methodology

3.1 National Fodder Industry Forum

With the valuable assistance of the VFF Grains Group, and in particular Research Officer Mr Chris Aucote, a national forum was organised and held in Melbourne on 4th and 5th October 1995 with two objectives:

(i) to seek agreement on a national uniform system for the measurement and description of fodder; and
(ii) to determine the extent of industry support for the formation of a national fodder industry body.

Efforts were made to involve representatives of all sectors of the fodder industry. Thirty-eight delegates attended, including producers, processors, traders, exporters, end-users, and representatives of fodder testing laboratories, research organisations and the media. The forum was facilitated by Mr Ian Crook of Crossfield Business Management Group, and commenced with short presentations on prospects for the fodder industry, options for measurement and description systems, nutritional requirements of ruminants, and customer requirements for fodder measurement and description from the viewpoints of a producer, processor, exporter, beef feedlotter, dairy farmer and live sheep exporter. The remainder of the forum consisted of plenary and small group discussions of the following issues:

(i) the definition of fodder and the fodder industry
(ii) the definition of customers and of their requirements for measurement and description of fodder
(iii) the need for a representative body for the fodder industry, and if so, its responsibilities, its membership and the formation of a Steering Committee.

3.2 Fodder testing methodology

Following agreement reached at the National Fodder Industry Forum on the major parameters required for the measurement and description of fodder quality, a discussion paper was circulated to the laboratories represented at the Forum, highlighting the detailed technical issues needing to be addressed on standardisation of fodder analysis procedures (Appendix 9.3).

After comments were received, a set of recommendations on fodder analysis procedures, together with issues still requiring resolution, was presented to a meeting of the AFIA executive committee in May 1996 (Appendix 9.4). With additional support from RIRDC, it was decided to hold a special meeting in Sydney on 11 June 1996 between representatives of the laboratories to seek agreement on the detailed testing procedures in Appendix 9.4.

An extension to this project, following provision of some additional funding from RIRDC, allowed a further meeting to be held in Melbourne on 18 February 1998, involving laboratory
representatives, together with specialists in animal nutrition and fodder conservation. The purpose of this meeting was to address the following issues:

(i) re-consideration and possible amendment of some aspects of the methodology, particularly relating to silage analysis
(ii) establishment of an agreed procedure for packaging and distribution of “standard” hay samples
(iii) a financially viable mechanism to
   (a) set up a group of laboratories and other interested personnel with expertise in and commitment to standardised fodder testing
   (b) distribute the standards and a set to recommended laboratory methods
   (c) conduct regular ring tests as part of a quality assurance programme
   (d) arrange regular workshops on fodder testing issues.

3.3 Hay digestibility trials

Sixteen different hays were identified from FEEDTEST® analyses and other sources to cover a wide range in species, origin and quality. Quantities of these hays were purchased for feeding to sheep in order to measure in vivo digestibility and voluntary intake for each hay. Two separate trials were conducted, each utilising 8 hays. The first trial commenced on 22 January 1996 and concluded on 18 April 1996. The hays comprised Persian clover, lucerne, medic, vetch/cereal, “good” quality mixed pasture, “poor” quality mixed pasture, “good” quality oaten and “poor” quality oaten hays. All of these represented different types of hays commonly produced and marketed in southern Australia. The second trial commenced on 19 August 1996 and concluded on 14 November 1996. In this case, the 8 hays comprised Balansa clover, lucerne, vetch, mixed pasture, wheaten (frost-damaged) and 2 sorghum hays. The first 5 of these hays originated in southern Australia, and the remaining 3 were obtained from Queensland.

In each trial, the respective 8 hays were fed unchaffed to 32 Merino wether sheep, randomly allocated in a Latin square design (4 periods, 2 feeding levels, 2 sheep per hay per period). The sheep were fed at either ad libitum or restricted (2.3% of bodyweight) levels of feeding. An introductory feeding period of 14 days was used in each trial to allow the animals to adjust to each diet. This was followed by an 8-day measurement period, during which each animal was fitted with a faecal harness containing a plastic bag to collect all faeces excreted. Each day, animals were fed a measured amount of hay and all hay not eaten was collected and dried at 60°C to constant weight. Faeces were also collected daily and similarly treated, except that the drying temperature was 100°C. At the end of each trial, dry matter digestibility (DMD) values were calculated, as the percentage difference between the dry matter of hay eaten and the faecal dry matter excreted, for 8 sheep on each hay, at both levels of feeding. Ad libitum dry matter intake (DMI) values were also calculated for 8 sheep on each hay. Means and standard errors were also calculated for both measurements. Similar calculations were performed to obtain organic matter digestibility (OMD) and organic matter intake (OMI) after ash content was determined in samples of both feeds and faeces.
3.4 Sampling and analysis

During each digestibility trial, samples of each hay were taken daily during the 8-day collection for all 4 periods. Each day a sub-sample of each hay was weighed, dried overnight at 100°C then reweighed to determine dry matter (DM) content. The remaining portions were pooled and mixed to produce 4 samples of all 16 hays. Representative sub-samples of these were ground in a cyclone mill to pass a 1 mm screen and analysed for crude protein (CP) (Kjeldahl nitrogen x 6.25) and ash using standard methods (AOAC 1995). DMD and OMD were estimated on all 64 hay samples using a pepsin-cellulase enzymatic technique (Clarke et al. 1982) with some modifications (Appendix 9.4). Analytical values for pepsin-cellulase dry matter disappearance (PCDMD) were adjusted using a linear regression based on existing “standard” samples of known in vivo DMD or OMD.

All 64 hay samples were also scanned on a model 6500 NIR spectrophotometer (Foss-NIRSystems Inc., Silver Spring, MD, USA) using ISI software (Infrasoft International, Port Matilda, PA, USA). An existing NIR calibration for hay, based on more than 400 samples, was used to predict CP and DMD. The reference values for DMD in this calibration were predicted values, obtained in turn from a linear regression between PCDMD and in vivo DMD for the existing “standards”.

Samples of the dried feed refusals from all sheep throughout both trials were taken, ground and analysed for ash, DMD and OMD in the same manner as for hay samples (excluding NIR analysis). The values for DMD and OMD determined on the refusals were used to adjust the final figures for in vivo DMD and OMD on the 16 hays.

Samples of the dried faeces from each sheep were ground in a hammermill to pass a 1 mm screen and analysed for ash content as above. These values, together with ash values on the hay samples, were used to calculate in vivo OMD for all 16 hays.

When the digestibility trials were completed, the hay samples from each period were bulked, thoroughly mixed and coarsely ground in a hammermill to pass a 4 mm screen. This resulted in quantities of from 4 to 13 kg of the 16 hays, which were then stored in airtight containers in an air-conditioned store pending distribution to other laboratories. Additional quantities of each hay were taken from the original bales and set aside for later processing if required.

Representative sub-samples of the bulked, coarsely hammermilled samples were reground in a cyclone mill to pass a 1 mm screen and analysed for DMD using the pepsin-cellulase technique, this time constructing a new linear regression between in vivo and analytical values and comparing it with the one based on existing in vivo “standards”. The samples were also analysed for ADF and neutral detergent fibre (NDF) by means of an ANKOM fibre analyser (ANKOM Technology Corporation, Fairport, NY, USA), based on the procedures of Goering and Van Soest (1970). DMD predictions were compared with those based on ADF (Linn and Martin 1989) and on ADF and nitrogen (N) (Oddy et al. 1983). All 16 samples were scanned on the NIR instrument as described above, and calibrations for in vivo DMD and DMI, PCDMD, ADF and NDF were derived using the new standards.

Sub-samples of the 16 hays were sent to CSIRO (WA) for measurement of shear energy (Baker et al. 1993). Sub-samples of the 40 hays studied by CSIRO as part of a related project were sent to Hamilton and scanned on the NIR instrument as described above.
Additional NIR calibrations were derived for shear energy on the 16 Hamilton hays only, for \textit{in vivo} DMD and DMI, PCDMD, ADF, NDF and shear energy on the CSIRO hays only, and also on a combined population including both the Hamilton and CSIRO hays.

Linear regressions relating either DMD or DMI to ADF, NDF, PCDMD and shear energy respectively were compared with direct calibrations for DMD and DMI against NIR spectra.
4. Results

4.1 Decisions from National Forum

The National Fodder Industry Forum was judged to be very successful, with both of its objectives being achieved. It was agreed that the basic objective measurements needed for fodder were dry matter, metabolisable energy and crude protein, and that it would be desirable at a later date to include a measurement to predict voluntary intake, if available. After much discussion, it was decided not to adopt a system widely used in the USA which involves the estimation of DMD from ADF and DMI from NDF. The agreed measurements should form the basis of a national quality description system, but laboratories would be free to include other measurements as well, depending on the specific requirements of their clients. Consultations between the fodder testing laboratories were now expected to occur in order to obtain agreement on appropriate analytical methods, prediction equations and a quality assurance programme which would lead to the analysis of a given sample of fodder being the same (within acceptable error limits) from all laboratories.

There was also unanimous support for the formation of a national fodder industry body, and a steering committee was set up for this purpose. This subsequently led to the formation of the Australian Fodder Industry Association (AFIA), now in its fourth year of operation.

A full account of the proceedings of the Fodder Forum was produced by the VFF Grains Group (VFF 1995).

4.2 Agreed methods for fodder sampling and testing

Following exhaustive debate at meetings between laboratory representatives in June 1996 and February 1998, agreement was reached on procedures for sampling, sample preparation, dry matter, crude protein, digestibility and estimated metabolisable energy of fodder samples. With the assistance of AFIA Vice-Chairman Mr Bill Fell, a standard protocol for sampling fodder has now been finalised, and is shown in Appendix 9.5. This is expected to be included in an updated version of the AFIA Vendor Declaration Form.

4.2.1 Sample preparation and dry matter

Fodder samples (150-250 g) containing less than 85% DM will be dried at 60°C in a forced draught oven for a maximum of 24 hours, so that DM is no lower than 90% after that period. This DM measurement is to be known as “partial dry matter”.

The partially dried samples (or samples as received, if DM exceeds 85%) are to be ground, either firstly through a coarse (4 mm) screen then through a 1 mm screen, or using a 1 mm screen only, depending on the size of sample and capacity of the mill. A cyclone mill is preferred for fine grinding.
The residual DM of the ground sample is to be measured by drying it in a forced draught oven at 135°C for 2 hours. This DM measurement is to be known as “laboratory dry matter”, and the total DM of the sample will be partial DM (if required) x laboratory DM.

Alternative procedures for determining DM (e.g. microwave drying) can be used, but it is the responsibility of the laboratory to ensure that results obtained agree with the standard method.

Special attention is required for silage samples. It was recognised that the loss of volatiles during oven drying can result in underestimation of silage DM, but that more rigorous DM methods are unrealistic in commercial laboratories. It was therefore resolved that, pending further research, silage DM be corrected as follows, using an equation recommended by Kaiser et al. (1995):

\[
CDM\% = 3.96 + 0.94 \times ODM\%
\]

where CDM is corrected dry matter and ODM is oven dry matter. This equation is to be used for values of ODM up to and including 50%. Alternatively, where ODM exceeds 50%, one percentage unit is to be added to the ODM value to obtain CDM, provided no silage additives have been used.

All fodder analyses are to be expressed on a 100% DM basis, with clients able to calculate values on an as fed basis using the measured total DM value.

### 4.2.2 Crude protein

The reference method to measure CP will be the Kjeldahl method. The digestion step will utilise a block digestor and either copper/titanium oxide or selenium as a catalyst, followed by the boric acid modification of the distillation process. Total nitrogen thus determined will be converted to CP using a factor of 6.25.

It was acknowledged that the Dumas oxygen combustion method is rapidly superseding the Kjeldahl method because of safety and environmental issues. It was agreed to undertake a collaborative effort to determine the relationship between the 2 methods for a comprehensive range of fodder samples, leading to the adoption of the Dumas method as the industry standard within 2 years.

Because CP values on oven-dried silage samples are underestimated due to losses in volatile nitrogen compounds, it was agreed that ammonia nitrogen should be measured separately on all silages, using a fresh portion of the sample. The steam distillation method used in the UK will be adopted. Total nitrogen will therefore be obtained by the sum of the nitrogen content of the oven-dried sample and the ammonia nitrogen content (DM basis) of the fresh sample. Ammonia nitrogen will also be used as an indication of fermentation quality of silages.
4.2.3 Digestibility and metabolisable energy

The pepsin-cellulase enzymatic procedure currently used at Hamilton (Appendix 4), with the addition of amylase, will be the standard method to estimate digestibility of fodder samples. The 16 hay samples with measured *in vivo* digestibility (derived as part of this project) will be used to calibrate the pepsin-cellulase method and will be available to fodder testing laboratories through AFIA. *In vivo* values for both DMD and OMD will be available on the 16 “standards”, but it was decided to standardise on DMD for routine measurements.

Because of the need to correct DM values for silage samples, DMD values for silage will also have to be corrected, as follows:

\[
\text{Corrected DMD} = 100 - \frac{\{(100 - \text{DMD}) \times \text{ODM}\}}{\text{CDM}}
\]

Prediction of metabolisable energy (ME) will be accomplished using the following equation:

\[
\text{ME (MJ/kg DM)} = 0.168 \times (\text{DOMD} + \text{EE}) - 1.19 \quad \text{(Freer et al. 1997)}
\]

where DOMD is digestible organic matter in the dry matter and EE is ether extract.

DOMD is to be calculated from DMD using the equation:

\[
\text{DOMD} = 0.95 \times \text{DMD} - 0.9 \quad \text{(SCA 1990)}
\]

For fodder samples, a value of 2% will be assumed for EE in all cases.

The above prediction equation for ME is to be used for all fodder samples except maize silage and sorghum silage, which contain lower levels of ash than that assumed in the SCA equation to convert DMD to DOMD. For maize and sorghum silages, OMD is to be measured and DOMD calculated using the equation:

\[
\text{DOMD} = \frac{\text{OMD} \times \text{OM}}{100}
\]

where OM is organic matter (100 – Ash%).

In these cases, the DOMD value is used directly in the ME prediction equation (Freer et al. 1997).

4.3 *In vivo* digestibility and intake measurements

The mean (± SE) values for *in vivo* DMD and OMD at both maintenance and *ad libitum* feeding levels, and for DMI and OMI, for the 16 hays, are shown in Table 1.
Table 1. Mean *in vivo* digestibility and intake values for the 16 hays fed to sheep (8 sheep per hay)

<table>
<thead>
<tr>
<th>Hay</th>
<th>Code</th>
<th>DMD% (SE) Maintenance</th>
<th>DMD% (SE) Ad libitum</th>
<th>OMD% (SE) Maintenance</th>
<th>OMD% (SE) Ad libitum</th>
<th>DMI g/hd/d (SE)</th>
<th>OMI g/hd/d (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne</td>
<td>LUC R1</td>
<td>61.9 (0.4)</td>
<td>62.6 (0.4)</td>
<td>62.6 (0.4)</td>
<td>63.3 (0.4)</td>
<td>1174 (73)</td>
<td>1097 (67)</td>
</tr>
<tr>
<td>Medic</td>
<td>MED R1</td>
<td>68.7 (0.4)</td>
<td>68.6 (1.0)</td>
<td>69.4 (0.4)</td>
<td>68.6 (1.0)</td>
<td>1259 (82)</td>
<td>1132 (74)</td>
</tr>
<tr>
<td>Oaten, good</td>
<td>OAG R1</td>
<td>58.0 (1.1)</td>
<td>56.6 (0.8)</td>
<td>59.6 (1.0)</td>
<td>58.1 (0.9)</td>
<td>841 (41)</td>
<td>799 (38)</td>
</tr>
<tr>
<td>Oaten, poor</td>
<td>OAS R1</td>
<td>51.5 (0.7)</td>
<td>47.6 (1.2)</td>
<td>53.4 (0.7)</td>
<td>49.3 (1.2)</td>
<td>765 (30)</td>
<td>723 (30)</td>
</tr>
<tr>
<td>Pasture, good</td>
<td>PAG R1</td>
<td>68.6 (0.5)</td>
<td>68.5 (0.5)</td>
<td>70.2 (0.4)</td>
<td>69.5 (0.5)</td>
<td>1070 (63)</td>
<td>953 (57)</td>
</tr>
<tr>
<td>Pasture, poor</td>
<td>PAP R1</td>
<td>53.6 (1.2)</td>
<td>53.3 (1.5)</td>
<td>55.9 (1.2)</td>
<td>55.6 (1.4)</td>
<td>965 (47)</td>
<td>892 (43)</td>
</tr>
<tr>
<td>Persian clover</td>
<td>PER R1</td>
<td>67.7 (0.5)</td>
<td>67.8 (0.7)</td>
<td>68.6 (0.5)</td>
<td>68.2 (0.7)</td>
<td>1128 (52)</td>
<td>1029 (48)</td>
</tr>
<tr>
<td>Vetch/cereal</td>
<td>VET R1</td>
<td>55.8 (0.8)</td>
<td>53.1 (1.1)</td>
<td>57.6 (0.7)</td>
<td>55.3 (0.9)</td>
<td>999 (58)</td>
<td>920 (56)</td>
</tr>
<tr>
<td>Balansa clover</td>
<td>BAL R2</td>
<td>67.1 (0.4)</td>
<td>66.2 (0.9)</td>
<td>68.1 (0.4)</td>
<td>66.8 (1.0)</td>
<td>1131 (60)</td>
<td>1030 (55)</td>
</tr>
<tr>
<td>Barley</td>
<td>BH R2</td>
<td>62.2 (0.9)</td>
<td>63.1 (1.1)</td>
<td>63.4 (0.9)</td>
<td>64.2 (1.1)</td>
<td>803 (48)</td>
<td>759 (44)</td>
</tr>
<tr>
<td>Lucerne</td>
<td>LUC R2</td>
<td>58.7 (0.9)</td>
<td>60.4 (0.7)</td>
<td>60.8 (1.0)</td>
<td>62.1 (0.7)</td>
<td>1288 (64)</td>
<td>1214 (61)</td>
</tr>
<tr>
<td>Pasture</td>
<td>PAS R2</td>
<td>61.0 (0.3)</td>
<td>60.5 (0.8)</td>
<td>62.7 (0.3)</td>
<td>62.0 (0.8)</td>
<td>927 (45)</td>
<td>851 (40)</td>
</tr>
<tr>
<td>Sorghum 1</td>
<td>SO1 R2</td>
<td>50.2 (0.9)</td>
<td>50.8 (0.8)</td>
<td>52.9 (1.0)</td>
<td>53.4 (0.9)</td>
<td>670 (54)</td>
<td>609 (49)</td>
</tr>
<tr>
<td>Sorghum 2</td>
<td>SO2 R2</td>
<td>49.7 (0.8)</td>
<td>50.3 (1.6)</td>
<td>53.2 (0.9)</td>
<td>53.6 (1.5)</td>
<td>715 (43)</td>
<td>652 (40)</td>
</tr>
<tr>
<td>Vetch</td>
<td>VET R2</td>
<td>66.7 (0.5)</td>
<td>65.8 (0.3)</td>
<td>68.7 (0.6)</td>
<td>67.3 (0.2)</td>
<td>1397 (53)</td>
<td>1286 (48)</td>
</tr>
<tr>
<td>Wheaten, frosted</td>
<td>WHT R2</td>
<td>55.7 (0.5)</td>
<td>*</td>
<td>60.5 (0.7)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

DMD = dry matter digestibility
OMD = organic matter digestibility
DMI = dry matter intake
OMI = organic matter intake

* No *ad libitum* values were obtained for this diet due to very low intake levels
4.4 Chemical and NIR measurements

Table 2 shows the mean chemical values for CP, ash, DMD and OMD from all 4 periods of the digestibility trials for each of the 16 hays. DMD and OMD values were obtained using the pepsin-cellulase method, with analytical values (pepsin-cellulase dry matter disappearance, PCDMD) adjusted using a linear regression based on existing *in vivo* “standards”. The table also includes predicted CP and DMD values from existing NIR calibrations.

**Table 2. Mean values (dry matter basis) for ash, protein and digestibility (chemical and NIR-predicted) on samples from all 4 periods of the digestibility trials for each of the 16 hays**

<table>
<thead>
<tr>
<th>Hay</th>
<th>Code</th>
<th>Ash %</th>
<th>CP % chemical</th>
<th>CP % NIR</th>
<th>DMD % Chemical</th>
<th>DMD % NIR</th>
<th>OMD % Chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne</td>
<td>LUC R1</td>
<td>6.5</td>
<td>15.8</td>
<td>15.7</td>
<td>64.3</td>
<td>62.9</td>
<td>65.5</td>
</tr>
<tr>
<td>Medic</td>
<td>MED R1</td>
<td>10.1</td>
<td>19.8</td>
<td>19.4</td>
<td>71.8</td>
<td>72.8</td>
<td>72.9</td>
</tr>
<tr>
<td>Oaten, good</td>
<td>OAG R1</td>
<td>5.0</td>
<td>5.5</td>
<td>4.6</td>
<td>64.8</td>
<td>63.6</td>
<td>66.8</td>
</tr>
<tr>
<td>Oaten, poor</td>
<td>OAS R1</td>
<td>5.6</td>
<td>5.8</td>
<td>4.8</td>
<td>54.4</td>
<td>54.7</td>
<td>55.8</td>
</tr>
<tr>
<td>Pasture, good</td>
<td>PAG R1</td>
<td>10.9</td>
<td>19.7</td>
<td>19.5</td>
<td>71.7</td>
<td>68.7</td>
<td>72.5</td>
</tr>
<tr>
<td>Pasture, poor</td>
<td>PAP R1</td>
<td>7.6</td>
<td>8.4</td>
<td>6.8</td>
<td>57.7</td>
<td>53.7</td>
<td>60.4</td>
</tr>
<tr>
<td>Persian clover</td>
<td>PER R1</td>
<td>8.8</td>
<td>13.9</td>
<td>13.2</td>
<td>71.7</td>
<td>69.2</td>
<td>73.6</td>
</tr>
<tr>
<td>Vetch/cereal</td>
<td>VET R1</td>
<td>8.0</td>
<td>16.6</td>
<td>15.8</td>
<td>59.1</td>
<td>59.2</td>
<td>61.2</td>
</tr>
<tr>
<td>Balansa cereal</td>
<td>BAL R2</td>
<td>8.9</td>
<td>13.0</td>
<td>13.6</td>
<td>68.0</td>
<td>66.7</td>
<td>68.5</td>
</tr>
<tr>
<td>Barley</td>
<td>BH R2</td>
<td>5.5</td>
<td>7.1</td>
<td>6.6</td>
<td>64.3</td>
<td>62.7</td>
<td>65.6</td>
</tr>
<tr>
<td>Lucerne</td>
<td>LUC R2</td>
<td>5.8</td>
<td>13.3</td>
<td>13.5</td>
<td>58.6</td>
<td>58.4</td>
<td>59.7</td>
</tr>
<tr>
<td>Pasture</td>
<td>PAS R2</td>
<td>8.2</td>
<td>10.6</td>
<td>10.4</td>
<td>62.5</td>
<td>61.9</td>
<td>63.6</td>
</tr>
<tr>
<td>Sorghum 1</td>
<td>SO1 R2</td>
<td>9.1</td>
<td>8.1</td>
<td>8.6</td>
<td>47.8</td>
<td>53.4</td>
<td>49.7</td>
</tr>
<tr>
<td>Sorghum 2</td>
<td>SO2 R2</td>
<td>8.8</td>
<td>7.1</td>
<td>7.5</td>
<td>46.8</td>
<td>53.9</td>
<td>49.4</td>
</tr>
<tr>
<td>Vetch</td>
<td>VET R2</td>
<td>7.9</td>
<td>19.4</td>
<td>19.6</td>
<td>70.4</td>
<td>70.5</td>
<td>71.3</td>
</tr>
<tr>
<td>Wheaten, frosted</td>
<td>WHT R2</td>
<td>9.8</td>
<td>10.4</td>
<td>10.7</td>
<td>57.7</td>
<td>61.0</td>
<td>62.3</td>
</tr>
</tbody>
</table>

CP = crude protein  
DMD = dry matter digestibility, predicted from pepsin-cellulase DM disappearance  
OMD = organic matter digestibility, predicted from pepsin-cellulase OM disappearance

NIR-predicted values (Table 2) for CP and DMD were compared to chemical values using the method of Jorgensen (1985), which evaluates precision and bias. For CP, there was a high correlation (r = 0.99), no difference in precision (t = 0.98, not significant, P>0.1) and no bias (t = 1.68, not significant, P>0.1) between NIR and chemical values. For DMD, there was also a high correlation (r = 0.94), negligible bias (t = -0.19, not significant, P>0.1) but a difference in precision (t = -2.58, significant, P<0.05). When the 2 sorghum hays were omitted, there was no difference in precision. This is not surprising, as this type of hay was poorly represented in the existing NIR calibration.
Table 3. Chemical values for digestibility and fibre fractions (dry matter basis), and shear energy values on bulked hay samples at the end of the trials, together with comparisons of various digestibility predictions

<table>
<thead>
<tr>
<th>Hay</th>
<th>Code</th>
<th>ADF%</th>
<th>NDF%</th>
<th>Predicted DMD% ¹ (USA eqn)</th>
<th>Predicted DMD% ² (Oddy eqn)</th>
<th>PCDMD%</th>
<th>Predicted DMD% from PCDMD ³</th>
<th>Predicted DMD% (NIR) ⁴</th>
<th>Shear Energy KJ/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne</td>
<td>LUC R1</td>
<td>35.0</td>
<td>45.4</td>
<td>61.6</td>
<td>61.4</td>
<td>64.9</td>
<td>63.8</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>Medic</td>
<td>MED R1</td>
<td>28.8</td>
<td>38.7</td>
<td>66.5</td>
<td>68.2</td>
<td>70.2</td>
<td>72.1</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Oaten, good</td>
<td>OAG R1</td>
<td>32.0</td>
<td>54.6</td>
<td>64.0</td>
<td>59.5</td>
<td>60.6</td>
<td>64.3</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Oaten, poor</td>
<td>OAS R1</td>
<td>43.3</td>
<td>70.1</td>
<td>55.2</td>
<td>50.3</td>
<td>45.5</td>
<td>52.0</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>Pasture, good</td>
<td>PAG R1</td>
<td>30.2</td>
<td>41.9</td>
<td>65.4</td>
<td>67.0</td>
<td>67.6</td>
<td>70.0</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Pasture, poor</td>
<td>PAP R1</td>
<td>39.9</td>
<td>63.1</td>
<td>57.8</td>
<td>54.2</td>
<td>51.6</td>
<td>57.0</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>Persian clover</td>
<td>PER R1</td>
<td>28.7</td>
<td>39.3</td>
<td>66.5</td>
<td>65.8</td>
<td>69.1</td>
<td>71.2</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>Vetch/cereal</td>
<td>VET R1</td>
<td>40.4</td>
<td>60.2</td>
<td>57.4</td>
<td>57.3</td>
<td>50.3</td>
<td>55.9</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Balansa clover</td>
<td>BAL R2</td>
<td>36.2</td>
<td>42.9</td>
<td>60.7</td>
<td>59.2</td>
<td>61.9</td>
<td>65.3</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>BH R2</td>
<td>35.7</td>
<td>60.4</td>
<td>61.1</td>
<td>57.1</td>
<td>58.6</td>
<td>62.6</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Lucerne</td>
<td>LUC R2</td>
<td>43.7</td>
<td>55.1</td>
<td>54.9</td>
<td>53.2</td>
<td>50.1</td>
<td>55.8</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>Pasture</td>
<td>PAS R2</td>
<td>40.6</td>
<td>66.1</td>
<td>57.3</td>
<td>54.6</td>
<td>55.0</td>
<td>59.7</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>Sorghum 1</td>
<td>SO1 R2</td>
<td>49.8</td>
<td>72.5</td>
<td>50.1</td>
<td>45.9</td>
<td>40.0</td>
<td>47.5</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>Sorghum 2</td>
<td>SO2 R2</td>
<td>48.3</td>
<td>71.9</td>
<td>51.3</td>
<td>46.8</td>
<td>39.8</td>
<td>47.3</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>Vetch</td>
<td>VET R2</td>
<td>34.2</td>
<td>44.2</td>
<td>62.3</td>
<td>63.6</td>
<td>66.5</td>
<td>69.1</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Wheaten, frosted</td>
<td>WHT R2</td>
<td>34.6</td>
<td>60.3</td>
<td>61.9</td>
<td>59.4</td>
<td>52.2</td>
<td>57.4</td>
<td>11.1</td>
<td></td>
</tr>
</tbody>
</table>

ADF = Acid detergent fibre
DMD = Dry matter digestibility
NDF = Neutral detergent fibre
PCDMD = Pepsin-cellulase dry matter disappearance

¹DMD% = 88.9 – (0.779 ADF%) (Linn and Martin 1989)
²DMD% = 83.58 – 0.824 ADF% + 2.626 N% (Oddy et al. 1983)
³Based on existing in vivo “standards”
⁴Derived from PCDMD values on a large population of hay samples, but in turn adjusted to DMD based on existing in vivo standards
Table 3 shows chemical values for PCDMD, ADF and NDF on sub-samples from each bulked hay sample obtained at the end of the trials. Values for shear energy, measured by CSIRO (WA), are also included. NIR-predicted values for DMD (based on the existing NIR calibration) and predicted DMD values from both the USA equation (Linn and Martin 1989) and the equation of Oddy et al. (1983) are included for comparison. It should be noted that PCDMD is not used alone; a linear regression between PCDMD and in vivo DMD for the in vivo “standards” is derived for each batch of samples, with PCDMD values for unknown samples converted to predicted in vivo DMD using the regression.

The method of Jorgensen (1985) was again used to compare precision and bias between the determined values for in vivo DMD (at maintenance) and those values predicted respectively from the “USA equation” (Linn and Martin 1989), the “Oddy equation” (Oddy et al. 1983), a regression between PCDMD and in vivo DMD of the existing “standards”, and the NIR calibration based on DMD predicted in turn from PCDMD. The results of these statistical comparisons are shown in Table 4.

Table 4. Statistical comparisons between measured in vivo dry matter digestibility and predicted values using various methods

<table>
<thead>
<tr>
<th>Prediction method</th>
<th>Correlation (r)</th>
<th>t-value (precision test)</th>
<th>t-value (bias test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA equation¹</td>
<td>0.83</td>
<td>1.89 (s., P&lt;0.1)</td>
<td>-0.20 (n.s., P&gt;0.1)</td>
</tr>
<tr>
<td>Oddy equation²</td>
<td>0.89</td>
<td>-0.03 (n.s., P&gt;0.1)</td>
<td>-2.64 (s., P&lt;0.05)</td>
</tr>
<tr>
<td>PCDMD³</td>
<td>0.95</td>
<td>-2.10 (s., P&lt;0.1)</td>
<td>1.40 (n.s., P&gt;0.1)</td>
</tr>
<tr>
<td>NIR⁴</td>
<td>0.95</td>
<td>0.04 (n.s., P&gt;0.1)</td>
<td>4.40 (s., P&lt;0.01)</td>
</tr>
</tbody>
</table>

¹,²,³,⁴As for Table 3
s. = significant
n.s. = not significant

It can be deduced from the correlation values between in vivo DMD and the prediction methods (Table 4) that measurement variance was small relative to product variance, with correlations higher for the pepsin-cellulase method (and the NIR calibration based on it) than for predictions based on fibre fractions. Results varied between methods in terms of precision and bias. Predictions using the USA equation and the pepsin-cellulase method differed in precision but not bias compared with in vivo DMD, but the opposite was the case using the Oddy equation and the NIR calibration derived from PCDMD. When the 2 sorghum hays were omitted, the pepsin-cellulase method no longer differed in precision (t = -0.91, not significant, P>0.01), but this time there was a bias (t = 2.17, significant, P<0.05).
The linear relationships between *in vivo* DMD and PCDMD for both the existing *in vivo* “standards” used at Hamilton and the new hay “standards” produced in this project are compared in Figure 1. For this particular batch, the respective relationships were as follows:

(1) Existing standards:  
\[ \text{in vivo DMD} = 16.21 + 0.790 \text{ PCDMD} \]  
\[ R^2 = 0.97 \]  
\[ \text{RSD} = 2.08 \]  
\[ N = 6 \]

(2) New hay standards:  
\[ \text{in vivo DMD} = 23.42 + 0.647 \text{ PCDMD} \]  
\[ R^2 = 0.90 \]  
\[ \text{RSD} = 2.19 \]  
\[ N = 16 \]

In each case, \( R^2 \) is the coefficient of determination, RSD is the residual standard deviation and \( N \) is the number of samples.
Figure 1. Linear regressions between \textit{in vivo} dry matter digestibility and pepsin-cellulase dry matter disappearance for both the existing \textit{in vivo} “standards” used at Hamilton (\(\nu\) – \(\chi\)) and the 16 new hay “standards” (\(\sigma\) – \(\varphi\)).

It can be seen from Figure 1 that the separate regression lines relating \textit{in vivo} DMD and PCDMD for the old and new “standards” are quite close, although the 2 lines differ somewhat in slope. This resulted in the regression based on the new “standards” giving higher DMD predictions at the low end of the range and lower predictions at the high end of the range. Such differences are to be expected, given that the old “standards” included both hay and harvested pasture, some were fed to sheep and some to cattle, and different feeding levels were used (either maintenance or \textit{ad libitum}). The new “standards” were produced using a common protocol throughout, i.e. they were fed to sheep at maintenance.

Statistics for the NIR calibrations derived between NIR spectra of the 16 new hay “standards” and values for \textit{in vivo} DMD, \textit{in vivo} DMI, ADF, NDF and shear energy respectively (from Table 3) are shown in Table 5.

NIR calibration statistics for the CSIRO hays and for a combined population of the Hamilton and CSIRO hays are shown in Tables 6 and 7 respectively. The reference data used for these calibrations was taken from the CSIRO final report on RIRDC project CSJ-1A, with the exception of PCDMD, which was determined at Hamilton.
Table 5. NIR calibration statistics for digestibility, intake, fibre fractions and shear energy on the 16 Hamilton hay “standards”

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
<th>SECV</th>
<th>R²</th>
<th>SECV/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vivo</em> DMD%</td>
<td>59.8</td>
<td>49.7-68.7</td>
<td>6.7</td>
<td>1.97</td>
<td>0.92</td>
<td>0.29</td>
</tr>
<tr>
<td>PCDMD%</td>
<td>56.3</td>
<td>39.8-70.2</td>
<td>9.8</td>
<td>3.33</td>
<td>0.90</td>
<td>0.34</td>
</tr>
<tr>
<td><em>In vivo</em> DMI g/hd/d</td>
<td>1009</td>
<td>670-1397</td>
<td>222</td>
<td>95.1</td>
<td>0.83</td>
<td>0.43</td>
</tr>
<tr>
<td>ADF % of DM</td>
<td>37.6</td>
<td>28.7-49.8</td>
<td>6.5</td>
<td>2.34</td>
<td>0.88</td>
<td>0.36</td>
</tr>
<tr>
<td>NDF % of DM</td>
<td>55.4</td>
<td>38.7-72.5</td>
<td>11.9</td>
<td>3.19</td>
<td>0.94</td>
<td>0.27</td>
</tr>
<tr>
<td>Shear Energy kJ/m²</td>
<td>11.9</td>
<td>8.3-15.7</td>
<td>2.1</td>
<td>0.99</td>
<td>0.80</td>
<td>0.47</td>
</tr>
</tbody>
</table>

SD = standard deviation of values across population
SECV = standard error of cross validation
R² = coefficient of determination
DMD = dry matter digestibility
DMI = dry matter intake
ADF = acid detergent fibre
NDF = neutral detergent fibre

Table 6. NIR calibration statistics for digestibility, intake, fibre fractions and shear energy on the 40 CSIRO hays

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
<th>SECV</th>
<th>R²</th>
<th>SECV/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vivo</em> DMD%</td>
<td>59.1</td>
<td>48.8-67.7</td>
<td>4.2</td>
<td>2.53</td>
<td>0.63</td>
<td>0.60</td>
</tr>
<tr>
<td>PCDMD%</td>
<td>55.9</td>
<td>42.9-68.6</td>
<td>5.7</td>
<td>2.21</td>
<td>0.85</td>
<td>0.39</td>
</tr>
<tr>
<td><em>In vivo</em> DMI g/hd/d</td>
<td>1263</td>
<td>845-1769</td>
<td>220</td>
<td>111</td>
<td>0.75</td>
<td>0.50</td>
</tr>
<tr>
<td>ADF % of DM</td>
<td>34.7</td>
<td>26.9-43.4</td>
<td>4.1</td>
<td>1.43</td>
<td>0.88</td>
<td>0.35</td>
</tr>
<tr>
<td>NDF % of DM</td>
<td>59.4</td>
<td>53.0-68.9</td>
<td>4.4</td>
<td>1.89</td>
<td>0.82</td>
<td>0.43</td>
</tr>
<tr>
<td>Shear Energy kJ/m²</td>
<td>14.5</td>
<td>11.3-19.2</td>
<td>2.1</td>
<td>1.14</td>
<td>0.73</td>
<td>0.54</td>
</tr>
</tbody>
</table>

SD = standard deviation of values across population
SECV = standard error of cross validation
R² = coefficient of determination
DMD = dry matter digestibility
DMI = dry matter intake
ADF = acid detergent fibre
NDF = neutral detergent fibre

Table 7. NIR calibration statistics for digestibility, intake, fibre fractions and shear energy on a combined population of the 16 Hamilton hays and the 40 CSIRO hays

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
<th>SECV</th>
<th>R²</th>
<th>SECV/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vivo</em> DMD%</td>
<td>61.5</td>
<td>48.7-72.0</td>
<td>6.1</td>
<td>1.98</td>
<td>0.92</td>
<td>0.27</td>
</tr>
<tr>
<td>PCDMD%</td>
<td>56.9</td>
<td>39.8-70.0</td>
<td>9.8</td>
<td>3.33</td>
<td>0.90</td>
<td>0.34</td>
</tr>
<tr>
<td><em>In vivo</em> DMI g/hd/d</td>
<td>1025</td>
<td>670-1397</td>
<td>222</td>
<td>95.2</td>
<td>0.83</td>
<td>0.43</td>
</tr>
<tr>
<td>ADF % of DM</td>
<td>37.4</td>
<td>28.7-49.7</td>
<td>6.5</td>
<td>2.34</td>
<td>0.88</td>
<td>0.36</td>
</tr>
<tr>
<td>NDF % of DM</td>
<td>55.3</td>
<td>38.7-72.5</td>
<td>11.9</td>
<td>3.19</td>
<td>0.94</td>
<td>0.27</td>
</tr>
<tr>
<td>Shear Energy kJ/m²</td>
<td>11.9</td>
<td>8.3-15.8</td>
<td>2.1</td>
<td>0.99</td>
<td>0.80</td>
<td>0.47</td>
</tr>
</tbody>
</table>

SD = standard deviation of values across population
SECV = standard error of cross validation
R² = coefficient of determination
DMD = dry matter digestibility
DMI = dry matter intake
ADF = acid detergent fibre
NDF = neutral detergent fibre
### Measurement Mean Range SD SECV $R^2$ SECV/SD

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
<th>SECV</th>
<th>$R^2$</th>
<th>SECV/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo DMD%</td>
<td>59.3</td>
<td>48.8-68.7</td>
<td>5.0</td>
<td>2.76</td>
<td>0.70</td>
<td>0.55</td>
</tr>
<tr>
<td>PCDMD%</td>
<td>56.3</td>
<td>39.8-70.2</td>
<td>9.8</td>
<td>3.45</td>
<td>0.88</td>
<td>0.35</td>
</tr>
<tr>
<td>In vivo DMI g/hd/d</td>
<td>1191</td>
<td>670-1769</td>
<td>247</td>
<td>156</td>
<td>0.60</td>
<td>0.63</td>
</tr>
<tr>
<td>ADF % of DM</td>
<td>35.6</td>
<td>26.9-49.8</td>
<td>5.0</td>
<td>1.57</td>
<td>0.90</td>
<td>0.31</td>
</tr>
<tr>
<td>NDF % of DM</td>
<td>58.3</td>
<td>38.7-72.5</td>
<td>7.5</td>
<td>1.85</td>
<td>0.94</td>
<td>0.25</td>
</tr>
<tr>
<td>Shear Energy kJ/m²</td>
<td>13.7</td>
<td>8.3-19.2</td>
<td>2.4</td>
<td>1.32</td>
<td>0.71</td>
<td>0.55</td>
</tr>
</tbody>
</table>

SD = standard deviation of values across population  
SECV = standard error of cross validation  
$R^2$ = coefficient of determination  
ADF = acid detergent fibre  
NDF = neutral detergent fibre

Statistics which compare the ability of various laboratory measurements to predict in vivo DMD and DMI are shown in Tables 8 and 9 respectively.

**Table 8. Comparison of various laboratory methods in their prediction of in vivo dry matter digestibility (DMD%) of the hays studied**

<table>
<thead>
<tr>
<th>Hay population</th>
<th>Measurement</th>
<th>R²</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton (n = 16)</td>
<td>ADF (% DM)</td>
<td>0.68</td>
<td>3.89</td>
</tr>
<tr>
<td></td>
<td>NDF (% DM)</td>
<td>0.84</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>PCDMD (%)</td>
<td>0.90</td>
<td>2.19</td>
</tr>
<tr>
<td></td>
<td>Shear Energy (kJ/m²)</td>
<td>0.77</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>NIR</td>
<td>0.92</td>
<td>1.97</td>
</tr>
<tr>
<td>CSIRO (n = 38)</td>
<td>ADF (% DM)</td>
<td>0.56</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>NDF (% DM)</td>
<td>0.45</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>PCDMD (%)</td>
<td>0.60</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>Shear Energy (kJ/m²)</td>
<td>0.39</td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td>NIR</td>
<td>0.63</td>
<td>2.53</td>
</tr>
<tr>
<td>Combined (n = 54)</td>
<td>ADF (% DM)</td>
<td>0.54</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td>NDF (% DM)</td>
<td>0.63</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>PCDMD (%)</td>
<td>0.75</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>Shear Energy (kJ/m²)</td>
<td>0.43</td>
<td>3.79</td>
</tr>
<tr>
<td></td>
<td>NIR</td>
<td>0.70</td>
<td>2.76</td>
</tr>
</tbody>
</table>

R² = coefficient of determination (linear regression)  
ADF = acid detergent fibre  
NDF = neutral detergent fibre  
PCDMD = pepsin-cellulase DM  
SD = standard deviation of values across population  
SECV = standard error of cross validation (SECV)  
RSD = residual standard deviation (linear regression)  
1 For NIR, R² is for modified partial least squares regression  
2 For NIR, this statistic is standard error of cross validation (SECV)  
3 For NIR, this statistic is standard error of cross validation (SECV)
Table 9. Comparison of various laboratory methods in their prediction of *in vivo* dry matter intake (DMI g/hd/d) of the hays studied

<table>
<thead>
<tr>
<th>Hay population</th>
<th>Measurement</th>
<th>$R^2$</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton (n = 15)</td>
<td>ADF (% DM)</td>
<td>0.32</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>NDF (% DM)</td>
<td>0.66</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>PCDMD (%)</td>
<td>0.48</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Shear Energy</td>
<td>0.67</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>(kJ/m$^2$)</td>
<td>0.83$^1$</td>
<td>95$^2$</td>
</tr>
<tr>
<td></td>
<td>NIR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSIRO (n = 38)</td>
<td>ADF (% DM)</td>
<td>0.25</td>
<td>194</td>
</tr>
<tr>
<td></td>
<td>NDF (% DM)</td>
<td>0.12</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>PCDMD (%)</td>
<td>0.25</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>Shear Energy</td>
<td>0.22</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>(kJ/m$^2$)</td>
<td>0.75$^1$</td>
<td>111$^2$</td>
</tr>
<tr>
<td></td>
<td>NIR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined (n = 53)</td>
<td>ADF (% DM)</td>
<td>0.32</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>NDF (% DM)</td>
<td>0.10</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td>PCDMD (%)</td>
<td>0.23</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>Shear Energy</td>
<td>0.05</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>(kJ/m$^2$)</td>
<td>0.60$^1$</td>
<td>156$^2$</td>
</tr>
<tr>
<td></td>
<td>NIR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2$ = coefficient of determination (linear regression)  
ADF = acid detergent fibre  
RSD = residual standard deviation (linear regression)  
NDF = neutral detergent fibre  
$^1$ For NIR, $R^2$ is for modified partial least squares regression  
PCDMD = pepsin-cellulase DM  
$^2$ For NIR, this statistic is standard error of cross validation (SECV)  
Disappearance

Despite the small number of samples, the statistics in Tables 5 and 6 confirm many published studies which indicate that NIR is capable of accurately predicting ADF, NDF and PCDMD in hay. Table 5 shows that, even with a very small number of diverse hays, it was possible to obtain a standard error as low as 1.97% for a direct NIR calibration against *in vivo* DMD, without any intermediate laboratory technique. This was an unexpected result, given the normally higher error associated with animal measurements than with laboratory measurements. Reasonable calibrations appeared possible for shear energy, although it was interesting to note (Tables 5 and 6) that in both sample populations, NIR calibration accuracy was marginally better for *in vivo* DMI than for shear energy. As well as SECV and $R^2$, another useful statistic for assessing calibration accuracy is the ratio of SECV to SD. If the ratio is 0.3 or less, considerable confidence is justified. On the other hand, if SECV is close to SD, the result is meaningless. The SECV/SD ratio was close to 0.3 for most of the measurements in Table 5, with a reasonable value obtained even for *in vivo* DMI.

Some caution is warranted when examining Table 7, which shows the NIR calibration statistics for the combined hay populations. This is due to the reference measurements (with the exception of PCDMD and shear energy) being made in two different centres. Whilst there
should be only minor differences in technique for ADF and NDF, the protocols for the *in vivo* measurements differed in some respects, particularly in terms of the type and liveweight of sheep used. Intake values on cereal hays for the CSIRO sheep appeared considerably higher than for the Hamilton sheep.

Excluding NIR, PCDMD appeared the to be the “wet” laboratory technique most closely related to *in vivo* DMD (Table 8), which is consistent with many other studies. However, for both sample populations, NIR was as good as or better than other techniques in its relationship with DMD. This also agrees with other published work, for example Barber *et al.* (1990).

In the case of *in vivo* DMI (Table 9), NIR was considerably better than any other technique.
5. Discussion

Some major steps have been taken towards a national uniform system for quality description of Australian fodder as a result of this project. An additional major development, which grew out of this project (with additional support from RIRDC), was the formation of AFIA, which is now recognised as the peak body representing the important and growing Australian fodder industry.

The origin of these two developments was the National Fodder Industry Forum, funded from this project. It was most important to determine the requirements of the end-users of fodder as regards the most useful measurements for assessment of quality, rather than telling them what they will get. This consultative process resulted in agreement that the basic objective measurements needed for fodder were DM, ME and CP, but this by no means precludes fodder testing laboratories from providing additional measurements requested by their clients such as fibre fractions and minerals.

Following exhaustive debate among the major fodder testing laboratories, the agreement in principle on uniform procedures for testing DM, ME and CP, and also on a standard protocol for fodder sampling and sample preparation, was an historic achievement. Previously, each laboratory had independently developed their own methods, and differences in measurements, procedures and prediction equations for ME had been a particular problem. A major focus of this project was to address the need for a set of standard fodder samples which could be used by all participating laboratories to calibrate the agreed laboratory method to estimate digestibility, which in turn would be used to predict ME. The second vital factor to achieve this objective was the agreement on a single ME prediction equation (Freer et al. 1997), rather than the several equations which had been used up to this point.

The 16 hays selected to be in vivo DMD “standards” comprised a wide range in quality, species and origin. DMD was found to range from 49.7% for sorghum hay to 68.7% for medic hay when sheep were fed at a maintenance level. Probably for the first time in Australia, the chosen protocol has resulted in sufficient quantities being retained of 16 hay samples with known in vivo DMD so that a number of appropriate laboratories can have access to them for the purpose of standardising laboratory estimates of DMD.

It was somewhat difficult to obtain a clear picture from the statistical comparisons between measured in vivo DMD of the 16 hays and predicted values using various laboratory methods (Table 4). This was possibly due to the techniques not being directly comparable. The USA equation (utilising ADF) was originally derived in the 1970’s for legume and grass hays (mainly lucerne) in the USA. The Oddy equation (utilising ADF and N), dating from the early 1980’s, was based on a variety of feedstuffs including both forages and grains. The regression utilising PCDMD was derived at the time of measuring PCDMD on the 16 hays, but was based on 6 in vivo “standards” including different forages (hay and harvested pasture), and produced using different methods (fed at either maintenance or ad libitum levels and to either sheep or cattle). Finally the NIR calibration used was derived from predicted DMD values on hundreds of hay samples, based in turn on PCDMD measurements adjusted using separate regressions (for the respective batch) against in vivo DMD values on the above 6 (and sometimes additional) “standards”. Despite this, more confidence can be placed in the
pepsin-cellulase technique or an NIR calibration based upon it, because of the reliance on a regression against \textit{in vivo} DMD derived with each batch of unknown samples and hence a greater control of error. The limitation in the past has been the lack of sufficient numbers, quantity and range of \textit{in vivo} “standards” produced according to a common protocol. This could explain why the omission of the 2 sorghum hays from the comparison removed the difference in precision between the pepsin-cellulase and \textit{in vivo} methods.

In one sense, the above statistical comparisons are of academic interest only, because it has been agreed that (1) the pepsin-cellulase method will be used as the reference method to estimate DMD, and (2) the 16 new hay samples will be used as the \textit{in vivo} “standards” needed to calibrate the method for use across the fodder industry.

An important point needs to be made regarding the results of NIR calibrations based on the 16 hay “standards” and on the CSIRO hays. Whilst the accuracy of the direct calibrations for \textit{in vivo} DMD and DMI appear remarkably promising, the set of samples used in this study is too small to form the basis of an adequate calibration for routine use. It is widely recognised that a robust NIR calibration for any agricultural product must be based on an absolute minimum of 50 samples and preferably several hundred. A calibration also needs to be validated on a separate and independent set of samples before it is implemented. This presents a major problem for an \textit{in vivo} versus a laboratory measurement, because of the labour, time and expense required to undertake \textit{in vivo} measurements. Hence for the forseeable future, an intermediate laboratory measurement (recommended to be PCDMD) will be necessary for the accurate routine estimation of DMD.

There were insufficient samples in this study upon which to draw a firm conclusion on the usefulness of shear energy as a reliable means of estimating DMI. Whilst shear energy appeared to be as good as or better than NDF for this purpose, over time the direct use of NIR would seem to be the preferred approach.

It cannot be expected that the 16 “standards” produced in this project will adequately cover all types of Australian fodder into the future. This is only a beginning; there is a definite need to undertake further \textit{in vivo} trials in order to obtain a larger and more diverse set of “standards”. This is particularly important for silage, which was not included at all in this study.
6. Implications

The impact of the outcomes of this project on the Australian fodder industry will be considerable. Already AFIA is recognised as the peak body and voice for the industry. It has organised 4 successful annual conferences, produces a regular newsletter, now employs a part-time executive officer, is involved in fodder evaluation trials, has submitted a comprehensive case for an industry R&D levy to the Federal Government, and has attracted significant private sponsorship.

Major achievements have been made in terms of agreement across the industry and among fodder testing laboratories on the major objective measurements required for quality description and the development of uniform procedures for these measurements. If every laboratory in Australia involved in fodder testing eventually adopts the agreed procedures, this will be a major advance for the industry. Producers, traders and end-users will have confidence that the analysis of a given sample will be the same (within recognised error limits) from any reputable participating laboratory. Once these procedures have been implemented throughout the country, it is expected that marketing of fodder products on the domestic market will be greatly enhanced, with a better correlation between quality and price. It could also be expected that a uniform and widely accepted quality description system will assist in giving the fodder industry a competitive edge on export markets.

An additional achievement of AFIA, again originating from this project, is a new grading system for hay and silage quality, based on ME and CP content (Appendix 9.6). This was adopted by AFIA at its Annual Conference in 1998. The grading system has also been accepted by NACMA, and included in its trading standards (reference numbers CSF-1 and CSF-2).

Successful implementation of the agreed system for quality description, with the resulting benefits to the industry, will heavily depend on the recommendations in this report relating to distribution of the hay “standards” being accepted and adopted. Success will also rely on an ongoing and co-ordinated program of “ring tests” among laboratories, to ensure test results on a given sample continue to remain within acceptable limits.

A major implication of this work is the need for additional in vivo measurements of DMD and DMI on a range of fodders. The power and convenience of the NIR technique will not be fully realised in this field until there are sufficient numbers of samples in Australia with in vivo values produced using a standard protocol, thus allowing the development of robust direct NIR calibrations without the need for intermediate laboratory measurements.
7. Recommendations

The respective shares of the intellectual property and project income from this project are 39% RIRDC and 61% DNRE (Agriculture Victoria).

The major source of intellectual property produced in this project is the set of 16 hay “standards”. The clear expectation of the fodder industry is that these “standards” are to be used by all appropriate fodder testing laboratories in Australia as the basis of calibration of the agreed reference method for estimation of digestibility, and hence ME of fodder samples.

It is therefore strongly recommended that the 16 hay “standards” be made the property of the Australian Fodder Industry Association (AFIA), and that as a consequence both RIRDC and DNRE relinquish their intellectual property rights over the “standards” in favour of AFIA. AFIA would then assume responsibility for the distribution of the “standards” to approved laboratories. These laboratories may be either commercial laboratories or those which conduct fodder analysis as part of research projects in the public sector. In either case, a quantity of 500 grams of each hay would be made available to laboratories which:

(a) affiliate with AFIA
(b) adopt the agreed uniform fodder testing procedures
(c) participate in regular ring tests (to be organised by AFIA)
(d) pay AFIA $1,600 for the set of 16 “standards” ($100 per “standard”)

This process was agreed to in principle at a meeting of representatives of the major fodder testing laboratories, and was later ratified by the AFIA executive, but of course cannot take place until approved by both RIRDC and DNRE.

It is further recommended that a detailed manual of fodder testing methods, based on those outlined in this report, be produced as soon as possible.

It must be emphasised that neither the 16 hay “standards” nor the recommended uniform fodder testing procedures can be considered as final. There must be sufficient flexibility to allow appropriate modifications or additions to the methods as results of new research and development in the fodder industry become available, for example in the prediction of voluntary intake.

Finally, it is recommended that additional in vivo digestibility trials be conducted on other Australian fodder, including silage, and also with animals other than ruminants, particularly horses, where there is an urgent need to establish a sound basis for accurate prediction of digestible energy of horse rations. It is expected that the funds raised by AFIA through the sale of the 16 hay “standards” will assist in supporting the conduct of further digestibility trials.
8. Appendices

Appendix 8.1. Variation in quality of hay samples tested during the 1998/99 season
(Source: FEEDTEST®)

<table>
<thead>
<tr>
<th>Description</th>
<th>No. of samples</th>
<th>CP (%)</th>
<th>DMD (%)</th>
<th>ME (MJ/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay, Legume</td>
<td>721</td>
<td>Mean</td>
<td>18.1</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>6.2-28.2</td>
<td>42.5-76.5</td>
</tr>
<tr>
<td>Hay, Legume/Grass</td>
<td>671</td>
<td>Mean</td>
<td>14.7</td>
<td>62.9</td>
</tr>
<tr>
<td>(Legume dominant)</td>
<td></td>
<td>Range</td>
<td>5.7-23.5</td>
<td>50.8-72.5</td>
</tr>
<tr>
<td>Hay, Grass/Legume</td>
<td>661</td>
<td>Mean</td>
<td>11.2</td>
<td>61.9</td>
</tr>
<tr>
<td>(Grass dominant)</td>
<td></td>
<td>Range</td>
<td>3.7-21.1</td>
<td>47.5-71.5</td>
</tr>
<tr>
<td>Hay, Grass</td>
<td>52</td>
<td>Mean</td>
<td>8.5</td>
<td>58.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>4.4-17.7</td>
<td>45.2-66.7</td>
</tr>
<tr>
<td>Hay, Cereal</td>
<td>1073</td>
<td>Mean</td>
<td>7.6</td>
<td>61.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>1.8-18.1</td>
<td>32.9-76.6</td>
</tr>
<tr>
<td>Hay, Cereal/Legume</td>
<td>239</td>
<td>Mean</td>
<td>9.9</td>
<td>62.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>4.7-19.6</td>
<td>41.9-72.9</td>
</tr>
</tbody>
</table>

Appendix 8.2. Variation in quality of silage samples tested during the 1998/99 season
(Source: FEEDTEST®)

<table>
<thead>
<tr>
<th>Description</th>
<th>No. of Samples</th>
<th>CP (%)</th>
<th>DMD (%)</th>
<th>ME (MJ/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silage, Legume</td>
<td>73</td>
<td>Mean</td>
<td>19.5</td>
<td>66.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>14.6-27.1</td>
<td>52.2-76.3</td>
</tr>
<tr>
<td>Silage, Legume/Grass</td>
<td>210</td>
<td>Mean</td>
<td>16.2</td>
<td>65.3</td>
</tr>
<tr>
<td>(Legume dominant)</td>
<td></td>
<td>Range</td>
<td>8.6-24.7</td>
<td>42.9-77.1</td>
</tr>
<tr>
<td>Silage, Grass/Legume</td>
<td>743</td>
<td>Mean</td>
<td>14.1</td>
<td>65.6</td>
</tr>
<tr>
<td>(Grass dominant)</td>
<td></td>
<td>Range</td>
<td>6.2-25.0</td>
<td>51.0-77.8</td>
</tr>
<tr>
<td>Silage, Grass</td>
<td>69</td>
<td>Mean</td>
<td>12.9</td>
<td>65.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>5.2-20.5</td>
<td>57.4-72.4</td>
</tr>
<tr>
<td>Silage, Cereal</td>
<td>105</td>
<td>Mean</td>
<td>9.9</td>
<td>62.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>4.0-20.5</td>
<td>51.9-76.7</td>
</tr>
<tr>
<td>Silage, Maize</td>
<td>75</td>
<td>Mean</td>
<td>7.3</td>
<td>68.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>4.7-11.7</td>
<td>57.8-76.7</td>
</tr>
<tr>
<td>Silage, Cereal/Legume</td>
<td>47</td>
<td>Mean</td>
<td>11.1</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>6.6-16.8</td>
<td>53.8-73.7</td>
</tr>
</tbody>
</table>
Introduction

At the National Fodder Industry Forum held in Melbourne in October 1995, agreement was reached on the parameters required for measurement and description of fodder. The key measurements are dry matter (DM), metabolisable energy (ME), crude protein (CP) and intake (if available). Other measurements were also listed as optional, but these will not be considered in this paper at this time.

Clearly, the industry's main concern is that the analysis of a given sample of fodder should be "the same" (i.e. within appropriate error limits) from all laboratories. This represents a challenge to us all, as each laboratory has established its own methods over time, and there has traditionally been little if any liaison between laboratories on fodder testing procedures.

The primary aim of any analysis of fodder is to predict animal performance, i.e. what the fodder will "do" rather than merely its composition. This results in a "double-barrelled" problem for the laboratory: (a) what measurements should be made, and (b) which prediction equation(s) should be used. An additional problem is that many fodder analyses are "operationally defined", i.e. the answer you get depends on the method you use.

This paper outlines the technical issues which require consideration and action in order to ensure the successful implementation of a uniform national system for objective quality description of fodder. Success will depend heavily on agreement between laboratories on these issues.

The ball is in our court!

Sampling and sample preparation

We all know that an analysis is of very little value unless the sample tested is representative of the product. This is of particular importance in the case of hay or silage, which can be highly variable. Each laboratory has an obligation to advise clients of proper sampling procedures. Hay (and if possible silage) should always be sampled using a coring device. FEEDTEST recommends that each sample submitted should consist of cores taken at random from at least 10 conventional square bales, with each core taken from the "butt" end of a bale. Alternatively, one core should be taken from each side of at least five large round or square bales. If it is not possible to sample silage with a corer, a freshly cut face of a silage stack should be sampled by hand from 10-20 spots, the material mixed thoroughly and sub-sampled to yield a total amount of about 500 grams.
Care is needed to ensure that silage samples are packaged to exclude air and arrive in the laboratory as soon as possible after sampling, particularly during hot weather. Some debate exists on the desirability of freezing silage samples before despatching to the laboratory.

Sample preparation normally involves drying and/or grinding prior to analysis. There can be considerable divergence in drying temperature and time, and in mill screen size. At Hamilton, all fodder samples are ground through a 1 mm screen in a Tecator Cyclotec mill, and if need be they are dried at 60°C overnight in a forced draught oven prior to grinding. Procedures for sample preparation and dry matter determination are usually closely linked.

**Dry Matter (DM)**

DM analysis is usually given little thought as it is regarded as such a simple procedure. However there are several different ways of measuring DM, and it is (or should be) used to express all other analyses on a DM basis. Many in the fodder industry use moisture meters to measure DM in hay, and there are sometimes disputes with laboratories about DM content. This can be due to changes in DM between sampling and arrival in the laboratory, and also to faulty or improperly calibrated moisture meters. Hence great care and consistency is needed for accurate measurement of DM, particularly if deriving an NIR calibration for DM.

Additional complications are involved in the case of silage, as oven-drying can drive off volatile compounds which contribute to nutritive value. Dr Mike Freer (ex CSIRO Canberra and a designer of the GRAZFEED model) has suggested that oven DM of silage samples be corrected to take account of this, using an equation derived by Alan Kaiser and colleagues at ARI Wagga. This would also have an effect on digestibility estimates of silage.

The US National Forage Testing Association (NFTA) has compiled a manual of recommended "Forage Analyses Procedures", and it is interesting to note that of its 150 pages, 50 pages are devoted to DM determination! Common drying techniques include overnight at 105°C, 2 hours at 135°C, and microwave drying.

Where samples are too wet to grind immediately, our procedure is to split the sample in half, dry one half at 105°C overnight to determine DM, and the other half overnight at 60°C prior to grinding. Residual DM is determined in the ground sample to correct other analyses to a 100% DM basis.

**Crude Protein (CP)**

There should hopefully not be too many problems in obtaining agreement between laboratories on CP content of fodder samples. At Hamilton, we use the Kjeldahl procedure (Tecator Kjeltec equipment) to obtain total nitrogen and convert to CP with the factor 6.25. In practice, we use NIR for most samples, with calibrations based on the Kjeltec procedure.

The most frequent cause of differences in CP analyses between laboratories is that some express CP on an "as is" basis rather than on a DM basis.
It is likely that, over time, many laboratories will change from Kjeldahl to the combustion method (e.g. Leco). This may result in slight changes to CP figures for some samples, particularly those containing appreciable levels of nitrate-nitrogen. Hence NIR calibrations may have to be modified.

**Metabolisable Energy (ME)**

ME, the proportion of total feed energy which can be utilised by an animal, is arguably the most important property of a feedstuff, but presents the most problems as it cannot be measured in the laboratory, only predicted from some other measurement(s). There can be serious differences between laboratories in ME estimation, due to different measurements and different prediction equations. In addition, digestible energy (DE) is sometimes quoted instead of ME, and mistakenly considered the same as ME. (DE is always around 20% higher than ME). All this causes confusion in the industry, and the problem must be fixed if a uniform quality description system is to have any chance of success.

The preferred parameter from which to predict ME is digestibility, the percentage difference between feed consumed and faeces excreted. Although digestibility is much easier to measure than ME, it is still a laborious *in vivo* measurement. One complication is that digestibility can be expressed in 3 different ways, as follows:

\[
\text{Dry Matter Digestibility (DMD\%)} = \frac{\text{Feed DM} - \text{Faeces DM}}{\text{Feed DM}}
\]

\[
\text{Organic Matter Digestibility (OMD\%)} = \frac{\text{Feed OM} - \text{Faeces OM}}{\text{Feed OM}}
\]

\[
\text{Digestible Organic Matter in the Dry Matter (DOMD\%)} = \frac{\text{Feed OM} - \text{Faeces OM}}{\text{Feed DM}}
\]

DOMD is used commonly in the UK, and the original UK equation to predict ME was based on DOMD. In Australia, DMD has probably been most commonly used. This is also the case in the USA, and is because the extra step of determining ash in feed and faeces samples (required for OMD or DOMD) is not necessary.

There is usually an appreciable difference between DOMD and either DMD or OMD. If the ash content of a sample is high (e.g. due to soil contamination), DMD can be much lower than OMD. There is a strong case for OMD to be measured in all future *in vivo* trials.

Digestibility can be estimated in the laboratory in a number of ways, and there are at least 3 different methods currently used by Australian laboratories. Two of these utilise prediction equations involving acid detergent fibre (ADF), which is negatively correlated with DMD, but the scientific literature has shown that this correlation is often very poor.

It is well-recognised that two-stage *in vitro* digestion techniques, either using rumen fluid/pepsin or pepsin/cellulase, are much more accurate than ADF in estimating digestibility. ADF has been popular in the past because it takes much less time to complete an ADF test than an *in vitro* test (a day vs. several days). However, the advent of NIR has made such considerations irrelevant. NIR calibrations for *in vitro* DMD can be established with equal or better accuracy compared to ADF.
At Hamilton, we have been using the pepsin-cellulase technique successfully for 18 years, and it forms the basis of our NIR calibrations for DMD. However, there are two important requirements for success: (a) a set of *in vivo* "standards" to calibrate the lab method (i.e. feeds which are similar to unknown samples being tested, with known *in vivo* digestibility, and included with each batch of unknowns); and (b) a supply of the appropriate cellulase enzyme, which must be obtained direct from Japan.

Because we have animal house facilities, we have acquired a range of feed standards over the years. *In vivo* trials are an ongoing requirement, in order to maintain a supply of an appropriate range of standards. This procedure is slow, labour-intensive and costly, but vitally important to ensure the integrity of the *in vitro*/*in vivo* relationship.

It is recognised that not all Australian fodder testing laboratories have access to these facilities. If the pepsin-cellulase technique is to be adopted as the method of choice to predict digestibility, all laboratories will need to use the same set of fodder samples with known *in vivo* digestibility in order to standardise the laboratory method. If suitable arrangements can be made, FEEDTEST would be prepared to provide other laboratories with a set of these samples as required.

Having obtained digestibility, the second issue which must be addressed is the appropriate equation to predict ME. The equation adopted by MAFF in the UK in 1984 was:

\[
\text{ME (MJ/kg DM)} = a \times (\text{DOMD} \%) 
\]

where \(a\) can range from 0.14 to 0.16 depending on the feedstuff (0.15 is frequently used).

To avoid the problem of varying coefficients, Corbett (1990) recommended a simplified equation (Feeding standards for Australian livestock: ruminants, Standing Committee on Agriculture, p.9, equation 1.13A):

\[
\text{ME (MJ/kg DM)} = 0.18 \times \text{DOMD}\% - 1.8 
\]

If only DMD\% is available, Corbett recommended the equation:

\[
\text{ME (MJ/kg DM)} = 0.17 \times \text{DMD}\% - 2.0 
\]

with the proviso that the ash content of the sample should be between 9 and 12\%.

The latter equation has been used by FEEDTEST for all fodder samples since 1991.

Dr Mike Freer has suggested an equation which incorporates both DMD and ether extract (EE), which should be taken into account for ME prediction of certain grains and mixed feeds containing appreciable levels of fat. The equation is:

\[
\text{ME (MJ/kg DM)} = 0.164 \times (\text{DMD}\% + \text{EE}\%) - 1.6 
\]

It is questionable whether it is appropriate to use this equation for hay and silage, where the EE level is low. However, Freer has incorporated the equation into GRAZFEED, and suggests using an EE figure of 2\% (for silage) if it is not measured.
Different prediction equations are used in some other laboratories. Considerable discussion is required among laboratories (and with nutritionists) before agreement can be reached on one national equation for fodder.

**Intake**

Although neutral detergent fibre (NDF) is widely used in the USA to predict dry matter intake for certain forages, there is considerable doubt in many quarters about its value for this purpose. Laboratory prediction of intake has been a research objective for many years, but is a difficult task. Dr Barrie Purser's group at CSIRO, Perth is developing a physical (rather than chemical) technique for predicting intake of fodder, and sufficient flexibility should be allowed in the proposed national quality description system to include this test if and when it proves successful as a result of his RIRDC-funded project. Meanwhile, there is probably no point in attempting to predict intake from any other measurement.

**Quality assurance and certification of laboratories**

There must be a mechanism to ensure ongoing repeatability and accuracy of fodder testing among laboratories. In the USA, this is accomplished by the National Forage Testing Association (NFTA). The NFTA Board has representation from growers, traders and commercial laboratories, and oversees a voluntary laboratory certification programme, based on a number of check samples sent regularly to all participating laboratories. Each laboratory pays an annual fee of US$100, and NFTA conducts an annual workshop on feed quality testing issues. In 1993, approximately 150 laboratories took part in the NFTA programme.

Because there are so few fodder testing laboratories in Australia compared to the USA, it is probably unnecessary to set up an organisation like NFTA. However the task could be undertaken by a sub-committee of the new national fodder industry body. Contact should be made with all laboratories which undertake fodder analysis, inviting them to join a quality assurance programme. Laboratories would be surveyed to ascertain their current procedures, and following agreement by the sub-committee, a set of recommended procedures would be compiled and circulated. Check samples would be circulated regularly, and the results statistically analysed.

The NFTA model is worth investigating in detail, and it may be a good idea to invite a key person from the USA to visit Australia and participate in a national workshop of fodder testing laboratories (provided funding could somehow be obtained).

**Quality grading system**

There was fairly general agreement at the National Fodder Industry Forum that a table of fodder grades should be introduced, although it is recognised that not all sectors of the industry may use it. A grade could be allocated to each fodder sample based on its test, and included in the analysis report.
The following table of grades was suggested at the Forum last October, and can be considered as a starting point for discussion.

<table>
<thead>
<tr>
<th>Grade</th>
<th>DMD %</th>
<th>ME (MJ/kg DM)</th>
<th>CP % (dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>&gt; 70</td>
<td>&gt; 10</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>BB</td>
<td>65-70</td>
<td>9-10</td>
<td>15-20</td>
</tr>
<tr>
<td>CC</td>
<td>60-64</td>
<td>8.2-8.9</td>
<td>12-14</td>
</tr>
<tr>
<td>DD</td>
<td>55-59</td>
<td>7.4-8.1</td>
<td>8-11</td>
</tr>
<tr>
<td>EE</td>
<td>&lt; 55</td>
<td>&lt; 7.4</td>
<td>&lt; 8</td>
</tr>
</tbody>
</table>

E.g. DMD = 62% (ME = 8.5), CP = 16%  Grade = CB

A variation of this table could be an alphanumeric system, as follows:

<table>
<thead>
<tr>
<th>ME Grade</th>
<th>DMD %</th>
<th>ME (MJ/kg DM)</th>
<th>CP Grade</th>
<th>CP % (dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&gt; 70</td>
<td>&gt; 10</td>
<td>1</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>B</td>
<td>65-70</td>
<td>9-10</td>
<td>2</td>
<td>15-20</td>
</tr>
<tr>
<td>C</td>
<td>60-64</td>
<td>8.2-8.9</td>
<td>3</td>
<td>12-14</td>
</tr>
<tr>
<td>D</td>
<td>55-59</td>
<td>7.4-8.1</td>
<td>4</td>
<td>8-11</td>
</tr>
<tr>
<td>E</td>
<td>&lt; 55</td>
<td>&lt; 7.4</td>
<td>5</td>
<td>&lt; 8</td>
</tr>
</tbody>
</table>

E.g. DMD = 62% (ME = 8.5), CP = 16%  Grade = C2

A third variation would be to grade fodder only on the basis of ME (or DMD), from A to E as above, and to lower the grade if CP was below a certain limit. For example, if a sample was graded A for ME, but had a CP level below 15%, the grade would be lowered to B. Similarly, if a sample was graded B for ME, but had a CP level below 12%, the grade would be lowered to C. Finally, if a sample was graded C for ME, but had a CP level below 8%, the grade would be lowered to D.

**Inclusion of other feedstuffs in a quality description system**

A recent approach has been made from the Stockfeed Manufacturers of Victoria on the issue of uniform procedures between laboratories for ME prediction of compound feeds. They are particularly interested in an agreed equation for this purpose.

Similarly, the GRDC recently commissioned a study on feed grains, and the issue also arose of uniform procedures for testing feed grain quality.
There is a strong case for the new fodder industry body to take the initiative and include these feedstuffs in a set of recommended procedures for fodder quality evaluation.
Appendix 8.4. Recommendations on fodder analysis procedures presented to AFIA and a special meeting of fodder testing laboratories

STANDARDISATION OF FODDER ANALYSIS - WHERE ARE WE AT?

Report to AFIA Steering Committee Meeting 27-28 May 1996

Peter Flinn and Don Law

Background

Peter Flinn prepared a discussion paper on fodder analysis procedures in January 1996, outlining the methods used at Hamilton and highlighting the issues which need to be addressed in the process towards inter-laboratory agreement. He circulated it to the other labs represented at the 1995 National Fodder Industry Forum, seeking comments and also details on current procedures used. Written responses were received from John Milton (Independent Lab Services & Uni of WA), Roy Kellaway (Uni of Sydney) and Helena Warren (EMAI, NSW Ag), and a verbal response from Don Law (Agritech, Qld). Brief comments were also received from Alan Kaiser (fodder conservation specialist, NSW Ag, Wagga) and nutritionists from Kyabram Dairy Centre (Ag Vic).

There was insufficient time to fully discuss the issue at the AFIA committee meeting in Melbourne in February (refer minutes). Peter Flinn and Don Law met at Yanco, NSW on 21st March, and had a useful and wide-ranging discussion. Much of this centred on a recommended procedure to determine digestibility of fodder, and hence metabolisable energy (ME).

As a result, a number of recommendations have been put forward, firstly for consideration by the AFIA Steering Committee, and if approved, for immediate circulation to fodder testing laboratories.

The recommendations are listed under the same general headings as in the earlier discussion paper.

Sampling and sample preparation

There was little comment received on this. It seems there is general agreement on the procedures outlined. Core sampling is strongly recommended for hay samples. Particular care is needed in the collection and despatch of silage samples.

RECOMMENDATION: That laboratories advise their clients to freeze silage samples prior to sending, unless the samples can arrive in the laboratory on the same day as they were collected.

Dry Matter (DM)
There appears to be a number of different procedures in use, either to determine DM itself or to dry samples prior to analysis. (This is in line with overseas experience). One lab uses microwave drying, others oven-dry at 60°C, and at ARI Wagga samples are dried at 80°C. The problem of loss of volatiles due to oven-drying of silage samples is widely recognised. Mike Freer (CSIRO Plant Industry) has suggested a correction be applied to DM (and also to digestibility estimates) to take account of these losses.

RECOMMENDATIONS:

(1) That total DM be determined by oven-drying for 16 hours at 105°C (forced draught oven).

(2) That, where samples require drying prior to analysis, they be oven-dried overnight at 60°C (forced draught oven).

(3) That where microwave drying is used, it is the responsibility of the laboratory to ensure that results obtained agree with the standard method.

(4) That silage DM determined by oven or microwave drying (ODM) be adjusted for loss of volatiles by using Alan Kaiser's recommended equation (Corrected DM% = 3.96 + 0.94 ODM%), but that further discussion take place on this issue (involving Alan Kaiser and Mike Freer).

(5) That all fodder analyses be expressed on a 100% DM basis.

(6) That all dried fodder samples be ground to pass a 1 mm screen, preferably in a cyclone mill, prior to analysis.

***COMMENTS ON THE ABOVE***

Alan Kaiser: prefers wet forage samples to be dried at 80°C as he considers drying at 60°C is too slow and that respiration losses will occur.

Don Law: recommends the following steps for a standard moisture method:

(1) Dry at 60°C in forced draught oven until moisture is less than 10%

(2) Grind sample to pass through 2 mm sieve

(3) Use AOAC method for moisture (2 hours at 135°C)

THIS ITEM NEEDS FURTHER DISCUSSION AT TUESDAY’S MEETING.

Crude Protein (CP)

RECOMMENDATIONS:

(1) That CP be determined by measuring total nitrogen using either the Kjeldahl or the Dumas oxygen combustion method (calibrated against the Kjeldahl method) and multiplying by the factor 6.25.

***COMMENTS ON THE ABOVE***
Don Law: recommends that the standard Kjeldahl method using copper sulphate/titanium oxide catalyst and boric acid modification be used. It is anticipated that the Kjeldahl method will eventually be replaced by the Dumas method, in which case it will be necessary to calibrate it against chemical standards.

(2) That CP values on dried silage samples be adjusted to account for loss of volatiles (further discussion required as above).

***COMMENTS ON THE ABOVE***

Alan Kaiser: considers that it is impossible at this stage to correct CP values on dried silage samples for volatile nitrogen losses, and that the only option at present for silages is to conduct nitrogen analyses on fresh (“wet”) samples.

Digestibility (and hence ME)

Digestibility is the major issue requiring resolution, as it is one of the most important indicators of fodder quality and is the preferred parameter used to predict metabolisable energy (ME). Currently, some labs use a pepsin-cellulase procedure (with their own in vivo fodder standards) to determine digestibility, while others calculate it either from ADF (using a US equation) or from an equation utilising ADF and nitrogen (N). The respective equations are:

\[
\begin{align*}
\text{DMD\%} &= 88.9 - (0.779 \text{ADF\%}) \\
\text{DMD\%} &= 83.58 - 0.824 \text{ADF\%} + 2.626 \text{N\%}
\end{align*}
\]

where DMD = dry matter digestibility.

There is general agreement that the use of an in vitro procedure is the more accurate and preferred method to determine digestibility, provided that (a) a common procedure is used; (b) adequate and on-going supplies of the required enzymes can be obtained; and (c) a common set of in vivo fodder standards is available to each laboratory, covering the range of digestibility and type of fodder encountered in practice, and with adequate quantities assured.

At present, some labs express digestibility as DMD (because their in vivo standards have had DMD determined only), but most labs agree that digestibility should be expressed as organic matter digestibility (OMD) or digestible organic matter in the dry matter (DOMD). This is only possible where the in vivo standards used also have known OMD and DOMD.

There is provision in Peter Flinn's current RIRDC project for in vivo trials (with sheep) to produce 16 new in vivo hay standards, covering a wide range of hay type and quality. The animal measurements for 8 of these have just been completed, and the other 8 will be completed by the end of 1996. (NOTE: EFFORTS ARE BEING MADE TO INCLUDE AS WIDE A VARIETY OF HAYS AS POSSIBLE IN THESE SECOND 8 TRIALS). It is proposed that samples of these 16 hays be made available to the AFIA for distribution to laboratories as required, for a fee. Funding would have to be available in the future for additional in vivo trials as required, to maintain the supply of appropriate standards.

RECOMMENDATIONS:

(1) That the pepsin-cellulase technique be adopted as the standard method for estimating digestibility of fodder samples (refer to attached method currently used at Hamilton).
NOTE: the details of this method are open for discussion and may warrant minor modifications.

(2) That a bulk purchase of Onozuka FA cellulase from Japan be organised, to enable each participating laboratory to obtain their desired quantity.

(3) That an audit be taken among participating laboratories of what (if any) in vivo fodder standards are currently held, and details be provided on type of fodder, digestibility value, method of measurement, method of sample preparation, and quantity on hand. NOTE: it is essential that a common set of in vivo standards be used across labs, all having been measured the same way (i.e. same animal type, same feeding level, etc.).

(3) That the 16 Hamilton hay standards be made available, through the AFIA, to each participating laboratory, provided that the laboratory (a) is affiliated with the AFIA; (b) pays a fee (to be negotiated) to the AFIA for the supply of the standards; and (c) agrees to participate in ongoing ring tests.

(4) That digestibility be determined and expressed as organic matter digestibility (OMD). NOTE: Data supplied with the standards would also include DMD, and DOMD can easily be calculated from OMD knowing the ash content.

(5) That a correction be applied to digestibility estimates of silage samples to account for loss of volatiles, according to Mike Freer's recommendation (further discussion required as above).

***COMMENTS ON THE ABOVE***

Don Law:
(1) need to specify the application of the method relative to the 16 standards
(2) suggests the addition of a heat stable amylase prior to the 80°C step (refer to Hamilton method)

Prediction of metabolisable energy (ME)

A variety of prediction equations are currently used, as follows:

Lab 1: ME = 0.17 DMD% - 2.0 (hay, pasture, pasture silage)  
       ME = 0.18 DOMD% - 1.8 (maize silage)

Lab 2: ME = 0.17 DMD% - 2.0 (hay, dry forage)  
       ME = 0.156 DMD% - 0.535 (silage)

Lab 3: ME = 0.15 DMD% (except where high oil content)

Lab 4: Net energy calculated from ADF using US equations, then ME converted from NE using NRC equation.

Alan Kaiser (Wagga) uses the equation ME = 0.1565 DOMD%.
Mike Freer has recommended the following equation:

\[ \text{ME} = 0.164 \times (\text{DMD}\% + \text{EE}\%) - 1.6 \]  
(where EE = ether extract, i.e. oil content)

This equation has been incorporated into the GRAZFEED model, with the aim of accounting for high fat feeds. For forages, Mike assumes an EE content of 2%.

Clearly we need to agree on one ME prediction equation for fodder!!

**RECOMMENDATION:** That the following ME prediction equation be used for fodder samples:

\[ \text{ME} = 0.18 \times \text{DOMD}\% - 1.8 \]  
(requires further discussion).

***COMMENTS ON THE ABOVE***

Alan Kaiser: recommends a wider discussion with nutritionists to debate the merits of the alternative equations.

**Intake**

Only a few comments were received on this issue. There is probably no point in attempting to predict intake (say from NDF) until results are available from Barrie Purser's project. In any case, there is some reservation on the applicability of an intake prediction derived on a few hays across all fodder types (e.g. silages).

**RECOMMENDATION:** That no attempt be made at this stage to predict intake from lab measurements, pending results from Barrie Purser's RIRDC project.

**GENERAL NOTE ON ALL ANALYSES**

1. No mention has been made specifically of NIR methodology. This is deliberate: NIR is a secondary method, calibrated against a reference method. It is the reference methods which require standardisation at this stage. Not all labs have access to NIR equipment, and those which do often have different types of instruments and software. It is entirely up to the individual laboratory to use or not use NIR for a given analysis, and each laboratory is responsible for the calibrations it uses. In time, it may be possible to standardise NIR instruments used by fodder testing labs, but there are both technical and commercial issues involved here, and this is not a priority at present.

2. Clearly, no lab is restricted to providing only the above analyses. Additional measurements may be very relevant in particular situations and industries, and other measurements may become an integral part of fodder analysis in the future. **It is the parameters listed above where standardisation of methodology is the most vital.**

**Quality assurance and certification of laboratories**

Some labs indicated clearly that they would be willing to participate in a quality assurance scheme through circulation of check samples. It is assumed that all the labs contacted would also agree to participate. In the USA, the NFTA actually certifies labs which perform adequately in regular ring tests, and makes public a list of such labs. There is some reservation about this
approach, and it is felt that labs which are affiliated with the AFIA, follow the recommended procedures, and take part in the ring tests should automatically be regarded as providing accurate and reliable analysis on fodder samples. (If a given lab was found to have a problem, it would discover this quickly, and confidentially, from the ring test and it would obviously be in the lab's interests to immediately fix the problem). As a totally separate issue, it would be advisable for labs to be accredited with NATA. However, that is totally up to the lab concerned, and NATA registration should not be a pre-requisite for participation in the fodder analysis quality assurance scheme.

Some thought needs to be given on a formal structure to implement a quality assurance scheme for fodder testing laboratories. The US model involves a separate organisation (the National Forage Testing Association, NFTA), the Board of which has 9 elected members - 3 each from the respective growers and traders organisations, and 3 from commercial laboratories. The NFTA runs regular ring tests, produces a methods manual and runs an annual workshop on fodder testing issues. Each participating lab (there are about 150) pays US$100 per year to NFTA.

In Australia, we now have AFIA (with currently 2 labs represented on the Steering Committee), and far fewer fodder testing labs than in the USA. The question is, do we form a separate organisation or sub-committee for laboratories, and should labs have to affiliate separately to this group, or should affiliation to the AFIA itself be sufficient? Remember that labs which test fodder samples also test other feedstuffs (e.g. grains and mixed stockfeeds), and uniformity of methods is also currently an issue in these industries, where there are additional complications in predicting digestibility and ME due to issues such as processing method and oil content.

**RECOMMENDATION:** That an appropriate structure be set up, either as part of the AFIA or as a separate body, to oversee a quality assurance scheme for fodder analysis. Specifically, this group would have the following functions:
* establish a database of all laboratories in Australia known to be conducting fodder analysis, and encourage them to join the group
* manage the distribution of in vivo fodder standards to participating labs
* conduct regular ring tests
* produce a comprehensive fodder analysis manual of recommended methods
* conduct an annual conference/workshop on fodder testing issues
* other tasks as appropriate

**What happens next??**

An agreement among fodder testing laboratories on methodology for the major parameters identified at the 1995 Fodder Forum is an urgent and vital requirement for the Australian fodder industry, if uniform objective quality criteria are to be adopted nationally.

We are under considerable pressure to come up with a statement of agreement in time for the inaugural annual conference of the AFIA, to be attended by the Federal Minister for Primary Industry, John Anderson, in mid-July.

RIRDC have advised that they can provide some additional funding for one meeting of lab personnel, in order to discuss and resolve all the issues outlined in this report. This meeting would need to be held before the end of June 1996.
RECOMMENDATION: That a national meeting of appropriate personnel from fodder testing laboratories be held before the end of June 1996 to obtain agreement on methodology, quality assurance and other issues raised in this report, and that this meeting include other specialists (e.g. Kaiser, Freer, Oddy) and any other member of the AFIA Steering Committee who is interested in the technical aspects of fodder analysis.

***COMMENTS ON THE ABOVE***

PF: Hence the meeting in Sydney on Tuesday!
METHOD 9.1  ESTIMATION OF DIGESTIBILITY USING THE PEPsin-CELLULASE TECHNIQUE

SCOPE

Applicable to plant material and feeds

OUTLINE OF METHOD

The sample is digested at 40°C with acidified pepsin, heated to 80°C, and then digested at 40°C with a buffered cellulase solution following pH adjustment to 4.6. Digestibility is measured as the disappearance of dry matter (or organic matter), adjusted using a linear regression based on similar samples of known in vivo digestibility.

APPARATUS

Analytical balance attached to computer
Pyrex test tubes, 25 x 150 mm (50 ml)
Plastic caps fitted with bunsen valves
Dispensers (3) to deliver 10, 15 and 0.8 ml of solutions
Magnetic stirrers and stirrer bars
Test tube racks
Covered water bath to accommodate at least 200 tubes
Immersion thermostat, to control water bath at 40°C
Immersion thermostat, to control water bath at 80°C
Sintered glass crucibles, porosity 1, 30ml
Adaptors for crucibles
Vacuum pump
Suction filtration manifold
Filtering flasks
Laboratory ovens
Muffle furnace

CHEMICALS

Hydrochloric acid, conc. A.R.
Pepsin A powder, 1:2000, BDH 39032 or equivalent
Sodium acetate, anhydrous A.R.
Sodium carbonate A.R.
Acetic acid, glacial A.R.
Cellulase, "Onozuka FA"

REAGENTS

1. Acidified pepsin solution (3% W/V in 0.125 M HCl)
Add 11.07 ml conc. HCl to 500 ml distilled water and dilute to 1 litre. Mix thoroughly and dissolve 3 g pepsin in the solution. Prepare this solution just before use. Adjust these quantities as required, depending on the number of samples in the batch (15 ml per tube).

2. **Sodium carbonate solution, 1 M**

Dissolve 106 g sodium carbonate in distilled water and dilute to 1 litre. Mix well.

3. **Buffered cellulase solution**

Dissolve 20.4 g sodium acetate and 8.7 ml acetic acid in distilled water and dilute to 1 litre. Dissolve 12.5 g cellulase in the solution and mix thoroughly. Prepare the solution just before use. Adjust these quantities as required, depending on the number of samples in the batch (10 ml per tube).

**PROCEDURE**

**Day 1**

1. Weigh out accurately about 0.25 g well-mixed sample onto a previously tared scoop and transfer carefully to a numbered 50 ml test tube containing a magnetic stirrer bar. Weigh out unknown samples in duplicate, *in vivo* standards in quadruplicate and include 3 blank tubes in each batch, spaced equally among the samples. Include at least 6 *in vivo* standards in each batch.

2. Concurrently, carry out a separate dry matter determination (Method 1.1) on each sample, and if organic matter digestibility is required carry out an ash determination as well (Method 2.1).

3. Add 15 ml acidified pepsin solution to each tube, mix using magnetic stirrer, and place cap in tube.

4. Place tubes (in their racks) in a water bath, previously heated to 40°C. Ensure that the bath is maintained at this temperature, with sufficient water for 24 hours.

**Day 2**

5. After 24 hours, remove tubes from water bath and place in another water bath, previously heated to 80°C, for 45 minutes.

6. After 45 minutes, remove tubes from 80°C water bath, and add 0.8 ml sodium carbonate solution to each tube. Mix using magnetic stirrer.

7. Add 10 ml buffered cellulase solution to each tube. Mix using magnetic stirrer.

8. Cap tubes and return them to the 40°C water bath for a further 24 hours.

9. Dry matter and ash determinations can conveniently be completed at this stage, if necessary.
10. Place clean sintered glass crucibles in 100°C oven overnight.

**Day 3**

11. Remove crucibles from 100°C oven, place in desiccator for 1 hour, and weigh.

12. Remove tubes from 40°C water bath after 24 hours and filter under vacuum into the sintered glass crucibles. Ensure all residue is transferred from the tubes using distilled water in a wash bottle. No other washing is required.

13. Dry crucibles overnight in an oven at 100°C.

**Day 4**

14. Remove crucibles from oven, place in desiccator for 1 hour, and weigh.

15. If organic matter digestibility is required, place crucibles in a COLD muffle furnace and heat to 550°C, maintaining this temperature for 2 hours.

16. Turn furnace off, allow to cool BELOW 200°C, place crucibles in desiccator for 1 hour, and weigh.

17. If organic matter digestibility is not required, still place crucibles in a COLD muffle furnace and heat to 550°C for 2 hours, to enable the crucibles to be easily washed. No weighing at this stage is necessary.

**CALCULATIONS**

\[
\text{DDM\%DM} = \left(\frac{\text{sample DM} - (\text{residue DM} - \text{blank DM})}{\text{sample DM}}\right) \times 100
\]

\[
\text{DOM\%OM} = \left(\frac{\text{sample OM} - (\text{residue OM} - \text{blank OM})}{\text{sample OM}}\right) \times 100
\]

06/95
Appendix 8.5. AFIA fodder sampling protocol

HOW TO TAKE FODDER SAMPLES FOR ANALYSIS

The accuracy of fodder analysis depends on the sample you send to the laboratory. It is critical that the sample represents the average composition of the "lot" of fodder sampled, otherwise the laboratory tests will not be useful.

A "lot" is defined as hay or silage taken from the same cutting, at the same stage of maturity, the same species (pure or mixed) and variety, the same paddock, and harvested within 48 hours. Other factors influencing the definition of a "lot" include rain damage, weed content, soil type, treatment after cutting and storage effects. A "lot" of baled hay or cubes should not exceed 200 tonnes.

Sampling hay

Representative hay samples can only be obtained with a probe or core sampling device. Do not rely on a couple of handfuls or a "flake" from one bale. Corers are commercially available in Australia from HAYCORE, P.O. Box 31, Dunkeld, Victoria 3294 (phone 03 5577 2216), and there are several types also marketed in the USA. Alternatively, they can be home-made using 32 mm steel tubing, and should be at least 450 mm long with a slightly scalloped and sharp cutting edge. Corers are driven either using a hand brace or an electric drill (where practicable). Some cordless drills may not be suitable if they lack power or turn too fast. A portable generator is useful and can be justified if many samples are taken.

Small square bales

Sample between 10 and 20 small square bales, selected at random from the “lot”. Take one core from each bale selected, probing near the centre of the "butt" end, at right angles to the surface. Ensure that the corer does not get hot. Combine all cores into a single sample in a bucket, and mix thoroughly. The whole sample should be kept intact and not subdivided.

Large round or square bales

Sample between 5 and 10 large bales, again selected at random. Take one core from each side of all bales selected, probing at right angles to the surface at different heights. Combine all cores into a single sample in a bucket, and mix thoroughly. The whole sample should be kept intact and not subdivided.

Cubes or pellets

Select a handful of cubes or pellets from at least 6 locations or bags which make up the complete lot. Combine the sub-samples in a bucket and mix thoroughly to obtain a final sample not exceeding 500 grams.

Sampling silage
Silage is best sampled at least 3 weeks after it has been ensiled, and as close to the time of feeding as practicable. In theory, there should be minimal losses in quality if ensiled correctly, but in practice this is not always the case, depending on time of wilting, rain or heat damage, mould and the presence of air.

**Pit or bunker silage**

Before opening the pit or bunker, core samples for analysis can be obtained using a long coring device that extends deeply into the pit or bunker. Alternatively, random handfulls can be taken from at least 10 locations across a freshly cut face of the stack, although this will not provide such a good representative sample. Combine all the material into a single sample in a bucket and mix thoroughly to obtain a final sample not exceeding 500 grams, reducing the sample by the quartering process if necessary.

**Wrapped baled silage**

Sample between 5 and 10 large bales at random, using a coring device in the same manner as for large hay bales. However, this procedure is acceptable only if great care is taken to reseal the holes made in the plastic by the corer. Combine all cores into a single sample in a bucket, and mix thoroughly. The whole sample should be kept intact and not subdivided.

**Sample handling**

Immediately after sampling and mixing, the final fodder sample must be placed in a robust (preferably "press-seal") plastic bag and tightly sealed to exclude air. This is to ensure that the laboratory report of dry matter will approximate the dry matter content of the lot when it was sampled.

Samples must be delivered to the laboratory as quickly as possible after being taken. In particular, silage samples must be frozen immediately after being taken, unless they can reach the laboratory on the same day they were collected. This is especially important during hot weather. Avoid mail delays over the weekend by posting samples early in the week.

Ensure that you closely follow the laboratory's instructions for labelling samples and filling out all the required details on the sample submission sheet.

If you have any further queries or problems regarding sampling or sample handling, contact the appropriate AFIA-recommended laboratory for further information.
Appendix 8.6. AFIA quality grading system for hay and silage

(1) **AFIA grades for legume and pasture hay and silage (NACMA ref. CSF-1)**

<table>
<thead>
<tr>
<th>DMD%</th>
<th>ME (MJ/kg DM)</th>
<th>Crude protein (% of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&gt; 19</td>
</tr>
<tr>
<td>&gt; 66</td>
<td>&gt; 9.5</td>
<td>A1</td>
</tr>
<tr>
<td>60-66</td>
<td>8.6-9.5</td>
<td>B1</td>
</tr>
<tr>
<td>53-59.9</td>
<td>7.5-8.6</td>
<td>C1</td>
</tr>
<tr>
<td>&lt; 53</td>
<td>&lt; 7.5</td>
<td>D1</td>
</tr>
</tbody>
</table>

DMD = dry matter digestibility  
ME = metabolisable energy  
DM = dry matter

(2) **AFIA grades for cereal hay and silage (NACMA ref. CSF-2)**

<table>
<thead>
<tr>
<th>DMD%</th>
<th>ME (MJ/kg DM)</th>
<th>Crude protein (% of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&gt; 10</td>
</tr>
<tr>
<td>&gt; 66</td>
<td>&gt; 9.5</td>
<td>A1</td>
</tr>
<tr>
<td>60-66</td>
<td>8.6-9.5</td>
<td>B1</td>
</tr>
<tr>
<td>53-59.9</td>
<td>7.5-8.6</td>
<td>C1</td>
</tr>
<tr>
<td>&lt; 53</td>
<td>&lt; 7.5</td>
<td>D1</td>
</tr>
</tbody>
</table>

DMD = dry matter digestibility  
ME = metabolisable energy  
DM = dry matter
9. References


