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On Predicting Diet Digestibility and Crude Protein Content from the Faeces of Grazing Cattle

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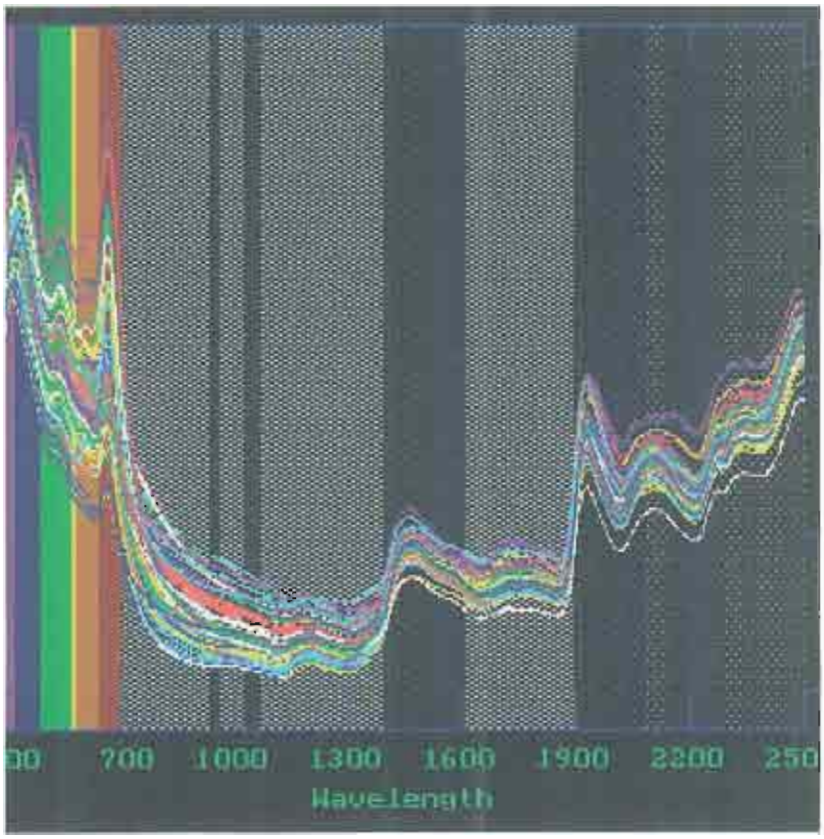
FINAL REPORT
PROJECT CS.253

Predicting diet digestibility and crude protein content from the faeces of grazing cattle



Meat Research Corporation

D.B. Coates
CSIRO Tropical Agriculture
Davies Laboratory
Townsville Q 4814

CSIRO

PART 1

ABSTRACT

CS.253
Predicting diet digestibility and crude protein content from the faeces of grazing cattle

A research projected conducted by CSIRO Tropical Agriculture

Principal Investigator:
D.B. Coates
Davies Laboratory
PMB. PO.
Aitkenvale Q 4814

ABSTRACT

Supplementation of grazing cattle is becoming increasingly important in the northern beef industry due to a range of production and marketing imperatives. Cost effective supplementation demands a knowledge of the diet quality from pasture alone because the quality of the base diet determines whether supplement is needed (when to feed), what to feed and how much to feed to meet a desired level of performance, as well as the biological and therefore economic response to feeding supplement. Unfortunately, no commercially applicable technology has been available for determining diet quality in grazing cattle. Recent developments in NIRS (near infra-red reflectance spectroscopy), however, have opened up new opportunities and overseas research has demonstrated the potential for predicting diet quality from faecal analysis using NIRS.

Project CS.253 was established to develop NIRS calibration equations for predicting diet digestibility and crude protein content from cattle faeces. The experimental methodology involved the collection and processing of faecal samples from both grazing and pen fed cattle and the acquisition and chemical analysis of forage samples representing the grazed and pen fed diets. Calibration equations were developed by determining the regression relationships between dietary reference values and faecal NIR spectra using the appropriate computer software. Additional work was performed to determine the potential of faecal NIRS for predicting forage intake and botanical composition and to resolve technical problems associated with the estimation of *in vivo* digestibility using *in vitro* techniques.

Project results demonstrated that faecal NIRS can provide accurate predictions of dietary N and dietary C_3/C_4 composition. The relationship between actual and predicted digestibility coefficients was less precise than for dietary N. Although faecal NIRS predictions of forage intake were poor, faecal NIRS showed good potential for predicting the intake of digestible dry matter. Additional data is needed to expand the size and diversity of calibration sample sets to improve the reliability of predictions before equations can be applied commercially with confidence and there is a need for on-going validation and refinement of calibration equations. Nevertheless, the sample sets and equations developed in CS.253 provide a valuable and sound foundation for future expansion and application. Overall, project results indicate that substantial benefits can be expected from faecal NIRS applications in industry and research.

Contrary to generally accepted dogma, *in vivo* digestibility was not a good indicator of forage quality and intake was not closely correlated with digestibility. Pepsin-cellulase *in vitro* digestibility was a better indicator of forage quality since forage intake was more closely related to *in vitro* than to *in vivo* digestibility. Faecal NIRS was a better predictor of the intake of digestible dry matter than any measure of digestibility. Accurate estimates of *in vivo* digestibility by pepsin-cellulase *in vitro* analysis were limited by the poor relationship between *in vivo* and *in vitro* digestibility. Project data showed that *in vivo* digestibility could be predicted more accurately by faecal NIRS than by *in vitro* analysis of dietary forage. The pepsin-cellulase *in vitro* digestibility of forage was lower than that of comparable extrusa. The difference was greater for grasses than legumes and the difference increased as digestibility decreased. These differences have important implications for estimating *in vivo* digestibility from *in vitro* analysis.

PART 2

EXECUTIVE SUMMARY

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EXECUTIVE SUMMARY

Background and industry context.

The diet quality of grazing cattle is difficult to measure or estimate but a knowledge of diet quality is needed to make informed decisions regarding strategies for cost effective supplementation. Since supplementation of grazing cattle is becoming increasingly important in the northern beef industry the need for a practical and effective technology for estimating the dietary nutritional status of grazing cattle is also a matter of some importance. Overseas work has demonstrated the potential of faecal analysis using near infra-red reflectance spectroscopy (NIRS) for the reliable prediction diet quality. As well having the potential to satisfy the requirements of reliability and accuracy, faecal NIRS can meet other requirements considered to be essential for commercial application, viz. ease of implementation in terms of sampling protocols, rapid turnaround and reasonable cost.

Project CS.253 was established for the purpose of developing calibration equations for the prediction of the digestibility and N content of the diet of free grazing cattle. The project was expanded to investigate and resolve technical problems associated with the estimation of *in vivo* digestibility from *in vitro* analysis of dietary forages. The expanded program also provided opportunities to increase the range of dietary attributes being predicted by faecal NIRS.

Objectives

1. Improve cost-effectiveness of supplementary feeding by developing technology for the reliable prediction of diet quality.

2. Enhance the efficiency and scope of grazing research by developing practical, low-cost technology for predicting diet quality and composition.

3. Measure the *in vivo* digestibility of a range of tropical forages and determine the potential of faecal NIRS for predicting diet quality attributes with calibration equations developed from reliably accurate reference values.

4. Determine the potential of faecal NIRS for predicting forage intake.

5. Compare the *in vitro* digestibilities of forage and extrusa samples and quantify any differences; develop regression relationships between *in vivo* and *in vitro* digestibility for feed and extrusa .

6. Determine the potential of faecal NIRS for predicting species composition of the diet.

Methodology

Calibration equations for faecal NIRS predictions of diet quality were developed by relating faecal NIR spectra to measured dietary attributes. Dietary attributes were determined by chemical analysis of dietary samples obtained from either grazed pastures or pen feeding experiments. Oesophageal fistulated steers were used to collect samples of selectively grazed forage from grazed pastures and dietary reference values for nitrogen concentration and *in vitro* digestibility were determined from the chemical analysis of extrusa. Appropriate adjustments for differences between the C_3/C_4 composition of extrusa and the diets selected by the grazing cattle were applied to improve the

accuracy of the dietary reference values. Dietary C_3/C_4 composition was determined from carbon isotope analysis of the faeces (faecal $\delta^{13}C$)[1] of the grazing cattle. Sample diversity was achieved by sampling pastures at different stages of growth from early wet to late dry season, by sampling at four locations with different soil types, by sampling over three years and by sampling a range of pasture species and mixtures. The grazed pasture sample set comprised 115 grazed diets represented by over 400 faecal samples and approximately 600 samples of extrusa.

Pen feeding experiments were conducted as conventional *in vivo* digestibility trials covering a range of forage qualities and forage species, both grasses and legumes. Eight pen trials comprising 37 different forage diets were conducted at Lansdown Research Station and data from an additional 17 forage diets were obtained from trials conducted at other locations. The pen trial sample set was represented by the 54 forage diets and over 200 faecal samples. Measurements of dietary N, *in vivo* dry matter digestibility, *in vitro* dry matter digestibility (IVDMD), forage intake and digestible dry matter intake (DDMI) were made for relating to faecal NIR spectra. *In vitro* analyses using the pepsin-cellulase technique were performed on both forage and extrusa samples.

Faecal spectra were obtained by scanning samples in a NIRSystems 6500 Spectroscope over wavelengths 400 to 2500 nm. Calibration equations were developed using ISI software - NIRS 3, version 3.10 (Infrasoft International) and modified partial least squares regression and a math treatment of 1,4,4,1 with SNV and DETREND.

[1] Dietary C_3/C_4 composition is linearly related dietary $\delta^{13}C$ and dietary $\delta^{13}C$ = faecal $\delta^{13}C$+ 1. Therefore dietary $C_3/C_4 \propto$ faecal $\delta^{13}C$.

Main results

Calibration equations and validation procedures showed that dietary N and faecal $\delta^{13}C$ could be reliably and accurately predicted using faecal NIRS (Standard Error of Performance -equivalent to RSD - of 0.2%N and 1.1 $\delta^{13}C$ units respectively). For widespread application the current calibration equations need to be expanded by increasing the diversity of samples within the calibration set and an on-going process of validation is required to ensure the reliability of predictions. Calibration statistics for pen trial data were better than those for grazed pastures, probably because of the greater accuracy of the reference values.

The predictive performance of calibration equations for dietary digestibility was judged to be inferior to that for dietary N and faecal $\delta^{13}C$. However, pen trial calibration equation statistics were excellent for both *in vivo* and *in vitro* digestibility and substantially better than those from grazed pasture. These differences were again considered to be due to the lack of precision and accuracy in the estimates of digestibility from grazed pastures.

Forage intake was not closely correlated with either forage or faecal NIR, indicating little scope for NIRS as a useful predictor of intake for tropical forages. However, faecal NIRS showed good potential as a predictor of DDMI. If confirmed in a larger calibration set that includes reliable DDMI estimates from pasture, this would be a major breakthrough since DDMI is closely correlated with animal performance. Calibration equation statistics are summarised in Table A.

In vivo dry matter digestibility was not well correlated with IVDMD using the pepsin-cellulase technique. These results indicate that estimates of *in vivo* digestibility derived from *in vitro* analysis of tropical forages may not be as accurate as the literature suggests. In contrast, faecal NIRS showed good prospects for the reliable prediction of *in vivo* digestibility so long as accurate reference values

are used in developing calibration equations. *In vivo* digestibility was not well correlated with forage intake for the range of forages offered and it was concluded that *in vivo* digestibility is not a good indicator of quality in tropical forages.

Extrusa samples differed from forage samples in pepsin-cellulase IVDMD. The *in vitro* digestibilities of extrusa were higher than those of forage. Differences were greater for grasses than legumes and differences increased as digestibility decreased. These differences have important implications when *in vivo* digestibility is estimated from *in vitro* analysis, implications relating to the botanical nature of the sample (grass or legume or mixture) and to the source of the sample (forage or extrusa).

Table A. Summary of calibration equation statistics for faecal NIRS predictions

Source	Attribute	No. of samples	Range	SEC	R^2
Grazed pasture	Dietary Nitrogen (%)	427	0.48 - 3.15	0.133	0.94
	Dietary IVDMD Coeff.	437	0.44 - 0.83	0.033	0.80
	Faecal $\delta^{13}C$	347	13.1 - 28.6	0.79	0.96
Pen trials	Dietary Nitrogen (%)	196	0.31 - 4.06	0.087	0.99
	Dietary IVDMD Coeff.	215	0.28 - 0.83	0.022	0.97
	In vivo DMD	187	0.37 - 0.73	0.025	0.89
	Intake (g/kgLW)	189	7.3 - 29.5	1.80	0.79
	DDMI (g/kgLW)	183	3.7 - 20.1	1.03	0.89

Table B. Summary of linear regression relationships derived from pen experiments

Regression equation	*n*	R^2	RSD
In vivo DMD = 0.474 (*in vitro* DMD feed) + 0.308	54	0.64	0.045
In vivo DMD = 0.714 (*in vitro* DMD extrusa) + 0.155	34	0.73	0.040
In vivo DMD grass = 0.508 (*in vitro* DMD grass) + 0.302	39	0.65	0.044
In vivo DMD legume = 0.567 (*in vitro* DMD leg) + 0.218	10	0.87	0.026
Intake (g/kg/LW) = 38.965 (*in vivo* DMD) - 3.077	47	0.47	2.75
Intake (g/kgLW) = 24.792 (IVDMD feed) + 4.678	47	0.68	2.12
Intake (g/kgLW) = 29.703 (IVDMD extrusa) + 0.670	27	0.57	2.18
DDMI (g/kgLW) = 38.907 (*in vivo* DMD) - 11.731	47	0.75	1.55
DDMI (g/kgLW) = 0.784 (Intake) - 3.902	47	0.90	1.00
DDMI (g/kgLW) = 22.222 (IVDMD feed) - 1.553	47	0.80	1.39
DDMI (g/kgLW) = 28.434 (IVDMD extrusa) - 6.082	27	0.80	1.19
IVDMD legume extrusa = 0.958 (IVDMD forage) + 0.033	5	0.98	0.01
IVDMD grass extrusa = 0.791 (IVDMD feed) + 0.163	31	0.97	0.02

Conclusions

The outcomes of CS.253 herald a major breakthrough in the practical prediction of diet quality in free grazing herbivores which can be applied equally well in both commercial and research situations.

Reliable predictions of dietary N (or crude protein) and the C_3/C_4 composition of the diet (from faecal δ13C) can be made with current calibration equations for cattle though there is a need for further validation. Predictions of dietary digestibility are less reliable but may be improved with a more diverse calibration set of samples and more accurate reference values. Of major significance are the good prospects for the prediction of digestible dry matter intake by means of faecal NIRS. The encouraging outcomes of the completed project indicate scope for increasing the range of attributes predicted by faecal NIRS and for improvements in the reliability of prediction of all attributes as calibration equations are expanded and refined. Faecal NIRS prediction of diet quality and composition in free-ranging cattle will provide opportunities for advancements in production efficiencies and resource management in the commercial field and for innovative and cost-efficient research.

Animal performance is driven by the intake of digestible energy, the two determinants of which are *in vivo* digestibility and intake. The relatively poor relationship between *in vivo* digestibility with both voluntary intake and the intake of digestible energy observed in CS.253, throws a question mark over the usefulness of *in vivo* digestibility as a reliable indicator of quality in tropical forages. Other findings concerning relationships between the pepsin-cellulase *in vitro* digestibility of forage, the *in vitro* digestibility of extrusa, and forage *in vivo* digestibility also question the integrity of current procedures. A review of the relative importance attached to digestibility as a measure of forage quality and of current practices in estimating *in vivo* digestibility would seem to be warranted. Similarly, there is a need to identify the most appropriate attributes as input data for driving nutritional models for predicting the performance of cattle grazing tropical pastures.

Recommendations for follow-up activities

A. ***Further development of faecal NIRS technology.*** There is a need to build on the foundation established in CS.253 by expanding the calibration sets of samples and reference values and to monitor the reliability of predictions by an on-going program of validation. In particular the expansion of the calibration set for predicting digestible dry matter intake is recommended. This expansion should be based on the acquisition of data and samples from grazing cattle and would necessarily involve the use of alkane technology to derive reference values. In addition, the range of attributes predicted should be increased by conducting additional chemical analysis on samples acquired during the course of CS.253. These analyses should include acid detergent fibre, neutral detergent fibre, acid detergent lignin of diet samples and faecal N and P concentrations.

B. ***Development of associated technology.*** The benefits of being able to predict diet quality can only be fully realised if dietary predictions can be interpreted in terms of potential animal performance. This requires an appropriate nutritional model and currently none exists for tropical/subtropical areas. Thus there is an urgent need for such a model, not only to use in conjunction with faecal NIRS technology, but for general use. Faecal NIRS technology itself would facilitate the development of such a model because it provides a cost-efficient means of relating observed animal performance to quantitative estimates of diet quality for developing the mathematical

relationships within the model. Data for animal performance - diet quality relationships can be easily accumulated from monitor herds grazing a range of pasture types at various locations. There is also a need for additional R&D to quantify responses to supplementation in relation to class of supplement, amount of supplement and diet quality afforded by the pasture.

C. ***Commercial exploitation.*** Commercial exploitation of faecal NIRS technology for cost-effective supplementation would be enhanced by further development of the technology as recommended in (A) above. Full commercial exploitation would also be dependent on coupling the faecal NIRS technology with a nutritional model as recommended in (B) above. Finally, a service laboratory for conducting the faecal analyses at reasonable cost, with rapid turn-around and streamlined procedures for the delivery of samples to the laboratory would be needed. CSIRO Tropical Agriculture is currently investigating various aspects relevant to establishing a commercial service.

PART 3

FINAL REPORT

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CONTENTS

BACKGROUND

Provided disease is controlled, diet quality is the over-riding determinant of animal productivity and, as such, is central to all animal production enterprises, both commercial and research. However, diet quality is usually difficult or impossible to measure accurately in grazing cattle due to the spatial heterogeneity of pasture quality coupled with selective grazing. The difficulty in measuring diet quality is accentuated in the extensive pastoral areas of Australia where large paddocks may encompass an enormous variation in soil type, plant species diversity and spatial differences in quality due to variation in environmental influences of rain, temperature etc. across the grazing unit.

The digestibility and crude protein (CP) or nitrogen (N) status of the diet have been identified as two of the main determinants of forage intake and productivity of grazing animals. An ability to predict these dietary attributes would have substantial benefits for both industry and research. Past attempts at developing technologies for this purpose have usually had little success until the reliable prediction of dietary CP and digestibility using Near Infrared Reflectance Spectroscopic (NIRS) analysis of cattle faeces was demonstrated by researchers in Texas, USA (Lyons and Stuth 1992). The advent of faecal NIRS technology in the USA offered the prospect of developing similar technology applicable to tropical pastures in northern Australia. Project CS.253 was initiated for this purpose with the primary objective of developing calibration equations for the prediction of the digestibility and N content of the diet of free grazing cattle.

In the industry context, being able to predict diet quality was seen as a critical component of cost-effective supplementation of grazing cattle. Informed decisions, with predictable economic consequences, in relation to what, when and how much supplement to feed, can only be made in the knowledge of the current and short-term future nutritional status of unsupplemented animals. This is because the dietary nutritional status of the unsupplemented animal determines whether a response to a supplement will occur or not, the class of supplement needed to give the best response, the response relationship in terms of increased production relative to intake of supplement, and the amount of supplement which needs to be consumed to meet a desired level of performance. Rapid turn-around, ease of implementation, and low cost would be have to characterise any technology for adoption by industry for predicting diet quality. Faecal NIRS has the inherent characteristics to satisfy these requirements. The ability to monitor the diet quality and composition of grazing animals was seen to have additional benefits to industry in the areas of resource monitoring, resource management and property planning, and sustainable production.

After the project had commenced it became apparent that there were difficulties associated with deriving accurate estimates of dietary *in vivo* digestibility using accepted sampling and analytical procedures. The project was expanded to incorporate additional work aimed at resolving the difficulties that had been identified. The additional work focussed on a series of conventional *in vivo* digestibility trials with cattle to provide accurate *in vivo* digestibilities for a range of forage diets and to re-investigate relationships between *in vivo* and *in vitro* estimates of digestibility. The digestibility trials also provided the opportunity to investigate the potential of faecal NIRS for predicting forage intake.

OBJECTIVES

The specific objective of the original project was to develop reliable predictive equations for determining the digestibility and crude protein content of the diet of free grazing cattle on tropical rangeland pastures using Near Infrared Reflectance Spectroscopy (NIRS) on cattle faeces.

The broader practical objectives were to:

1. Develop technology to allow producers to increase the cost-effectiveness of supplementary feeding by providing critical information to assist in decision making with regard to the timing of supplementary feeding and with regard to the formulation of supplements necessary to achieve the desired response.

2. Develop technology to provide a research tool that will enhance the efficiency and scope of research dealing with productivity and sustainability issues in rangeland science.

The project was then enlarged to incorporate additional objectives, viz.:

3. Determine the *in vivo* digestibility of a range of tropical pasture species and/or mixtures of varying quality for the purpose of developing calibration equations based on reliable data for estimating dietary digestibility from faecal NIR.

4. Determine whether a useful relationship can be determined which will allow DM intake to be estimated from either feed or faecal NIRS.

5. Determine whether the *in vitro* DM disappearance during pepsin-cellulase digestion is different in samples of extrusa obtained from fistulated steers than in the forage from which the extrusa was derived; quantify any difference between *in vitro* digestibility of extrusa and feed samples; and develop regression relationships between *in vitro* digestibility and *in vivo* digestibility appropriate for samples of feed and samples of extrusa respectively.

6. Determine the potential of faecal NIRS for predicting species composition of the diet.

METHODOLOGY

The development of calibration equations for predicting attributes by means of NIRS always requires the matching of NIR spectra with laboratory reference values for a large set of samples (the calibration set) so that statistical correlations between spectra and attribute value can be determined. For the calibration equation to be of useful predictive value the calibration set of samples must cover the spectral diversity likely to be encountered in the wider "unknown" population. Therefore calibration sets need to be diverse with respect to quality (attribute value), sample source, location, season and year. In most NIRS applications, such as forage analysis, the laboratory reference values and the NIR spectra are obtained from the same set of samples. For the application being developed in CS.253 the laboratory reference values necessarily had to be derived from dietary samples while the NIR spectra were obtained from the faecal samples of cattle consuming those diets. The difficulty, therefore, was clearly related to obtaining representative samples of the diet to match with faecal samples.

Sampling methodology

Calibration sample sets were obtained from grazed pastures and pen feeding experiments.

Grazed pastures

Sample collection. Faecal samples were collected from cattle (steers, heifers or cows) that were resident in the paddock for at least a week prior to sampling. The number of cattle varied from 1-6 but was generally 3 - 5. Samples of extrusa, obtained from a group of 6 oesophageal fistulated (OF) steers, were analysed to obtain the dietary reference values for matching with faecal NIR spectra. Diversity of samples in the calibration set was achieved by:-

(i) sampling at 4 locations (Lansdown Pasture Research Station, Springmount west of Mareeba, Cardigan Station south of Charters Towers and Hillgrove Station north of Charters Towers). Apart from the geographic spread, the sites represented different soil types, vegetation classes and climatic characteristics.

(ii) sampling different pasture types at each location representing a wide range of pasture species including grasses and legumes, native and introduced species.

(iii) sampling across seasons to cover the range of young, leafy, green, high quality diets through to mature, stemmy, dry, low quality diets.

(iv) sampling over a 3-year period to encounter non-specific differences that occur between years.

Overall, 115 separate "grazed diets" were sampled (site, paddock within site, occasion within paddock). These diets were represented by about 600 extrusa samples and 450 faecal samples.

Sample processing. Fresh faecal samples were cooled by placing on ice and later frozen for storage. Frozen samples were then dried in a forced draft oven at 60°C for 48 hours before grinding in a Tecator Cyclotec Laboratory Mill to pass a 1mm screen. Ground samples were packaged and stored in a deep freeze prior to obtaining NIR spectra. A sub-sample of milled faeces was processed in a Tema mill for later $\delta^{13}C$ analysis.

Extrusa samples were cooled by placing on ice and later frozen for storage. Frozen samples were dried in a forced draft oven at 60°C for 48 hours before grinding through a Christy & Norris Laboratory Mill to pass a 1mm screen. Ground extrusa samples were packaged and stored in cool conditions until later analysis. A sub-sample of milled extrusa was processed in a Tema mill for later $\delta^{13}C$ analysis.

Sample analysis. Faecal spectra were obtained by scanning in a NIRSystems 6500 spectroscope fitted with a spinning sample cup holder. Samples were scanned through the bandwidth of 400-2500nm which includes part of the visible band as well as the near infrared band. Prior to scanning the faecal samples were re-dried overnight at 60°C and maintained in a dry state until scanning was complete.

The nitrogen concentration of all extrusa samples was determined by standard laboratory Kjeldahl digestion and analysis. *In vitro* dry matter digestibility (IVDMD) was determined by the 2-stage pepsin-cellulase dry matter disappearance method (McLeod and Minson 1978).

$\delta^{13}C$ determinations were made on all faecal and extrusa samples (Le Feuvre and Jones 1992).

Determination of laboratory reference values. Samples of extrusa collected from OF steers do not necessarily match closely the integrated diet of the resident cattle. Differences can be due to differences in the botanical composition between OF extrusa and resident diets or to differences in the plant parts selectively grazed. In grass/legume pastures, which formed the majority of grazed diets in CS.253, differences in legume content between OF extrusa and resident diets were considered the main determinant of differences in chemical composition, particularly N concentration. Simple linear regressions were determined to describe the relationship between N concentration and $\delta^{13}C$ of extrusa samples, either within a paddock or within a group of paddocks. Dietary $\delta^{13}C$ values of resident cattle (faecal $\delta^{13}C$ + 1) were then used to calculate dietary N concentration from the regression equation. Where appropriate, the same process was used to calculate the dietary digestibility values for resident cattle. In the majority of cases, however, the relationship between digestibility and $\delta^{13}C$ of extrusa was not significant and the digestibility of forage consumed by resident cattle was taken as the average of the extrusa samples.

Pen feeding experiments

Pen feeding experiments were conducted as conventional *in vivo* digestibility trials. Each pen trial consisted of a 9-day adaptation period followed by an 8-day total collection period. Diets were fed to satisfy voluntary consumption (feed offered at approximately 10% more than previous day's intake). In all, 8 separate pen trials comprising 37 different diets were conducted at Lansdown Pasture Research Station. Data and samples from *in vivo* digestibility trials conducted at other locations and comprising another 17 forage diets were also obtained. The diets fed covered a range of qualities and included grasses (predominantly C_4 grasses), legumes (predominantly tropical legumes) and mixed diets (Table 9 and Figure 9).

At Lansdown, faecal samples for NIRS analysis were obtained by collecting fresh, uncontaminated samples from each of the last 3 days of the collection period and bulking within animals.

Representative samples of the forages fed and of the uneaten residues were dried, ground and analysed for N concentration and *in vitro* digestibility. In addition, some of the forages were fed to oesophageal fistulated steers to obtain samples of extrusa which were then processed and analysed in the usual way to determine the *in vitro* digestibility of extrusa for comparison with that of the feed offered.

NIRS analysis

Faecal samples were scanned in the first instance at the Victoria Agriculture FeedTest Centre at Hamilton, Victoria, under the direction of Dr Peter Flinn. CSIRO, Tropical Agriculture in conjunction with James Cook University, acquired its own instrument (NIRSystems 6500) towards the end of 1996. All samples were then scanned on site at the Davies Laboratory in Townsville.

Developing calibration equations

ISI (Infrasoft International) software (NIRS 3, Version 3.10) was used for all spectral analyses, data manipulation and spectra calibrations. The calibration statistics presented in this report were all derived from Modified Partial Least Squares regression using the full bandwidth of wavelengths (400 - 2500 nm) and a math treatment of 1,4,4,1 with SNV and Detrend.

RESULTS

Calibration equations for predicting dietary attributes from faecal NIRS

Grazed pastures

There was a good relationship between faecal NIR predicted dietary N and the laboratory reference values of the 427-sample calibration set. The coefficient of determination (R^2) was 0.94 and the standard error of calibration (SEC) was 0.133 (Table 1, Figure 1). These values compare favourably with reported estimates for cattle (Lyons and Stuth 1992) and goats (Leite and Stuth 1995) in the United States.

Likewise, the calibration equation statistics for faecal $\delta^{13}C$ indicated a good relationship between laboratory reference values and NIR predicted values with SEC of 0.79 and R^2 of 0.96 (Table 1, Figure 2). There are no published data with which to compare these statistics.

The relationship between NIR-predicted and laboratory reference values for *in vitro* DM digestibility (IVDMD) (SEC = 0.033; R^2 = 0.80) was poorer than that for either dietary N or faecal $\delta^{13}C$ (Table 1, Figure 3). Nevertheless, when allowances are made for scaling differences between *in vitro* and *in vivo* estimates of digestibility (1 unit of *in vivo* is equivalent to approximately 1.5 units of pepsin-cellulase *in vitro*), the SEC was similar to values reported by Lyons and Stuth (1992) and Leite and Stuth (1995).

Problems associated with determining appropriate laboratory reference values may have contributed to the poorer correlation between laboratory estimated and NIR predicted values for IVDMD compared with those for dietary N. There was often a substantial range in the IVDMD of extrusa samples collected from individual paddocks suggesting a less than desirable level of precision in the determination of laboratory reference values. Pepsin-cellulase *in vitro* digestion was used for determining IVDMD in project CS.253 whereas Lyons and Stuth (1992) and Leite and Stuth (1995) estimated *in vivo* OMD from the *in vitro* digestion of extrusa samples with rumen liquor followed by the NDF procedure. The latter technique may be more amenable to prediction by faecal NIRS than the pepsin-cellulase method.

Table 1. Calibration equation statistics for the grazed pastures sample set

	No. of samples	Range	SEC	R^2
Nitrogen (%)	427	0.48 - 3.15	0.133	0.94
IVDMD Coeff.	437	0.44 - 0.83	0.033	0.80
Faecal $\delta^{13}C$	347	13.10 - 28.59	0.79	0.96

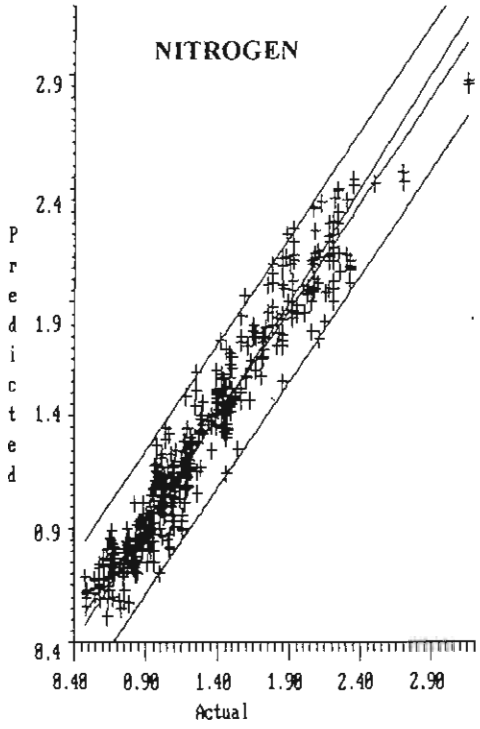

Figure 1. Relationship between predicted (faecal NIRS) and actual (laboratory reference values) values of dietary nitrogen (%) in the grazed pastures calibration set.

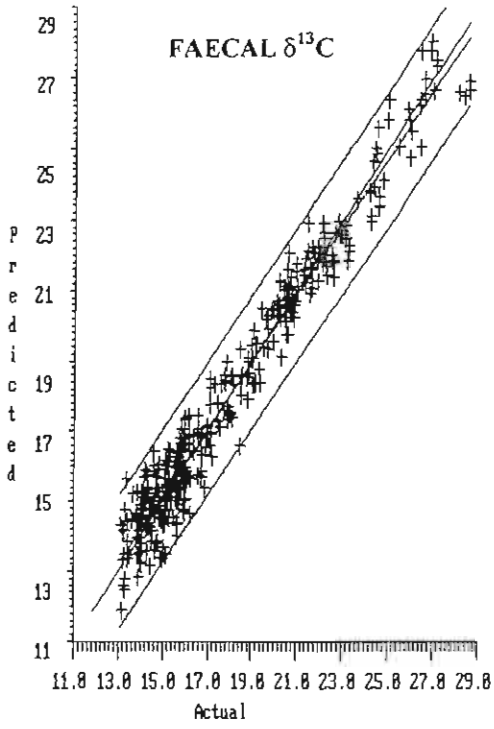

Figure 2. Relationship between predicted (faecal NIRS) and actual (laboratory reference values) values of faecal $\delta^{13}C$ in the grazed pastures calibration set.

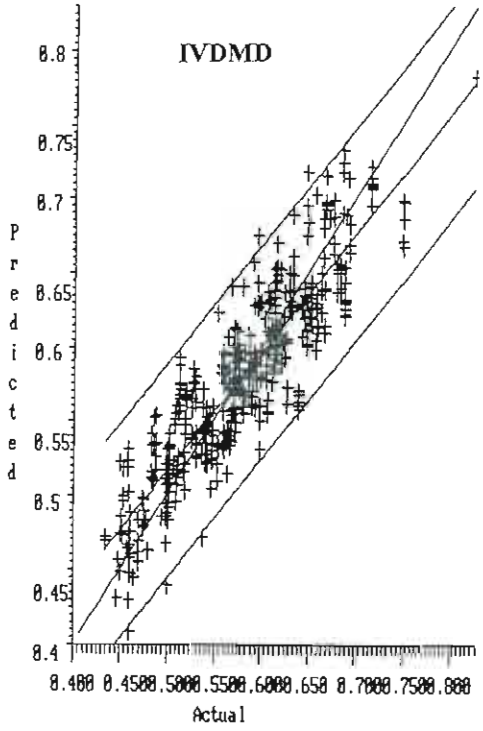

Figure 3. Relationship between predicted (faecal NIRS) and actual (laboratory reference values) values of dietary IVDMD in the grazed pastures calibration set.

Pen feeding experiments

N concentrations in the 54 forages ranged from 0.31 to 4.06% N. Calibration statistics for the 196 faecal sample set were excellent with a SEC of 0.087 and an R^2 of 0.99 (Table 2, Figure 4). The relationship between NIR predicted dietary N and the laboratory reference values was appreciably better than for grazed pasture. This was no doubt due, at least in part, to the greater accuracy of the laboratory reference values for pen fed diets.

The calibration statistics for faecal NIRS prediction of *in vitro* digestibility were again noticeably superior to those for grazed pastures with a SEC of 0.022 and an R^2 of 0.97 (Table 2, Figure 5), and once again the difference in calibration statistics between the grazed pasture and pen feeding data sets would have been due primarily to the relative accuracy of laboratory reference values. The *in vitro* digestibility coefficients of pen fed diets were determined on representative samples of the feed and not on samples of extrusa.

The *in vivo* digestibility of a feed is a function not only of the feed but also of the animal. It is not surprising, therefore, that the calibration statistics for *in vivo* digestibility were inferior to those for *in vitro* digestibility which is purely a function of the feed (Table 2, Figure 6). Nevertheless, the SEC of 0.025 and the R^2 of 0.89 were highly acceptable.

Voluntary intake ranged from 7.3 to 29.5 g/kgLW. The relationship between NIR predicted intake and actual intake (SEC = 1.80; R^2 = 0.79) was weaker than for dietary N and digestibility (Table 2, Figure 7).

DDMI (g/kgLW) integrates digestibility and intake to give a measure of digestible energy which is closely correlated with animal performance. The good relationship (SEC = 1.03; R^2 = 0.89; Table 2, Figure 8) between predicted and actual DDMI in the pen trial data set was, therefore, an outcome of particular significance. Useful predictions of DDMI or DOMI in grazing cattle would be of immense benefit with regard to the prediction of animal performance.

Table 2. Calibration equation statistics for pen feeding sample set

	No. of samples	Range	SEC	R^2
Nitrogen (%)	196	0.31 - 4.06	0.087	0.99
IVDMD Coeff.	215	0.28 - 0.83	0.022	0.97
In vivo DMD	187	0.37 - 0.73	0.025	0.89
Intake (g/kgLW)	189	7.3 - 29.5	1.80	0.79
DDMI (g/kgLW)	183	3.7 - 20.1	1.03	0.89

Grazed pasture and pen feeding experiments combined

When the field and pen data sets were combined, there was still a good relationship between predicted and laboratory reference values for dietary N (SEC = 0.129, R^2 = 0.96). The SEC for the combined calibration equation was marginally lower than for the grazed pasture alone but appreciably higher than for the pen experiments alone.

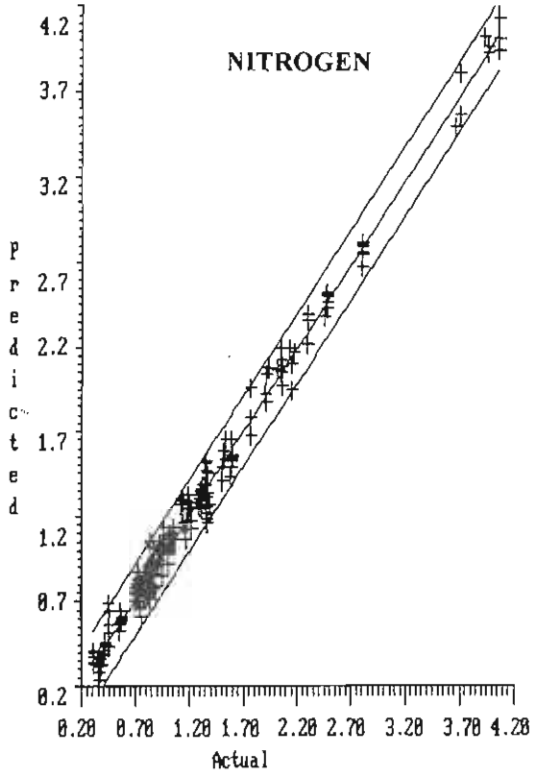

Figure 4. Relationship between predicted (faecal NIRS) and actual (laboratory reference values) values of dietary nitrogen (%) in the pen trial calibration set.

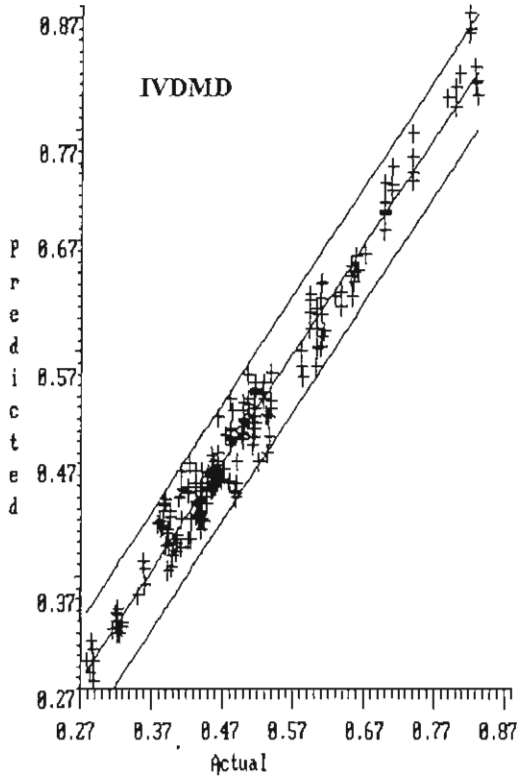

Figure 5. Relationship between predicted (faecal NIRS) and actual (laboratory reference values) values of dietary IVDMD in the pen trial calibration set.

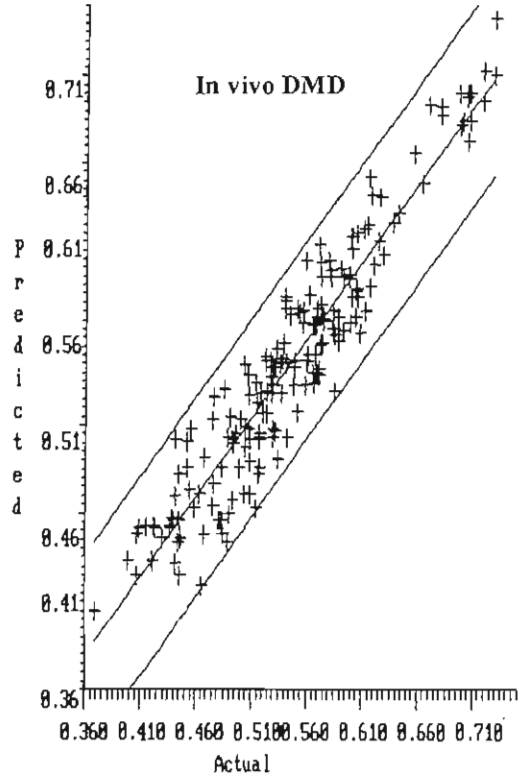

Figure 6. Relationship between predicted (faecal NIRS) and actual measures of dietary *in vivo* DMD in the pen trial calibration set.

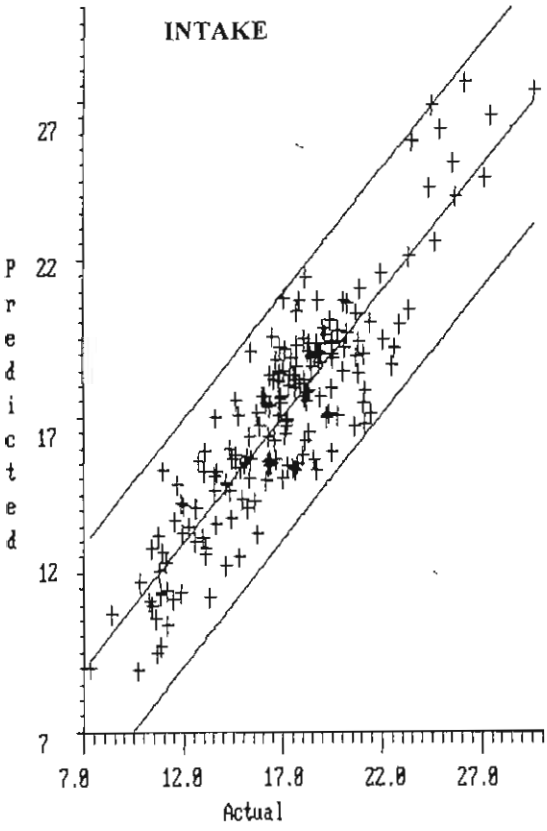

Figure 7. Relationship between predicted (faecal NIRS) and actual measures of forage intake (g/kgLW) in the pen trial calibration set.

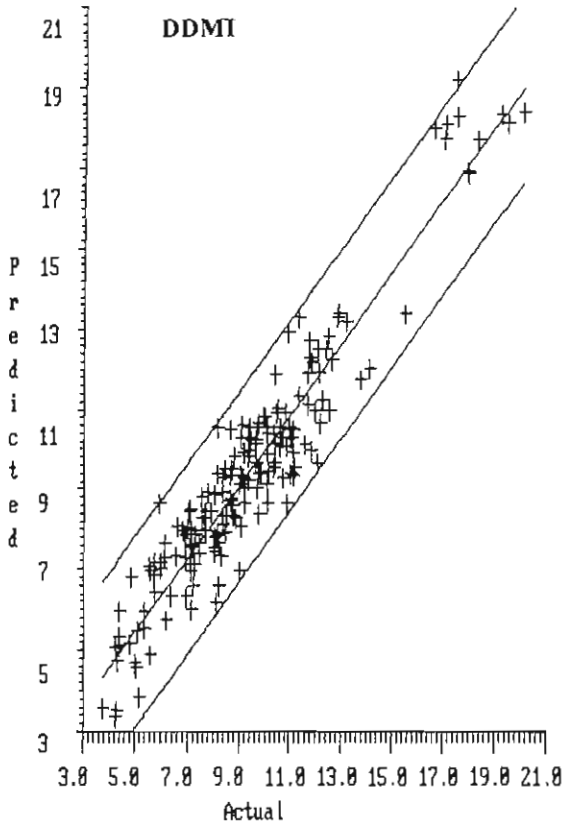

Figure 8. Relationship between predicted (faecal NIRS) and actual measures of digestible dry matter intake (DDMI, g/kgLW) in the pen trial calibration set.

Validation

Grazed pasture

(i) Validation by every second sample

A calibration equation was developed from half the total number of samples in the calibration set by selecting the odd numbered samples by position number. This equation was then used to make predictions on the remaining samples (even numbers). The prediction statistics were quite acceptable in relation to the calibration statistics. Good correlations between NIR predictions and laboratory reference values were achieved for dietary N and faecal $\delta^{13}C$ with SEPs (standard error of performance) of 0.20 ($R^2 = 0.90$) and 1.10 ($R^2 = 0.92$) respectively (Table 3). Predictions of IVDMD were less satisfactory but consistent with the calibration equation statistics.

Table 3. Validation statistics where a calibration equation, developed from half the samples in the calibration set, was used to predict constituent values for the remaining samples.

	Nitrogen (%)	IVDMD Coeff	Faecal $\delta^{13}C$
SEP	0.20	0.04	1.10
Bias	0.04	0	0.09
R^2	0.90	0.75	0.92

(ii) Validation by taking out 25% of diets

A validation set of 102 faecal samples (representing 28 of the grazed pasture diets) was removed from the full calibration set of samples. A calibration equation was derived from the remaining 75% and this equation was used to predict dietary attributes on the validation set.

Predictions of dietary N and faecal $\delta^{13}C$ were acceptable while predictions of IVDMD were less than satisfactory with a SEP of 0.04 and an R^2 of 0.61. The poorer statistics for these predictions compared with the statistics for the calibration samples (for the full or partial sets) was probably due to a reduction in the spectral diversity in the calibration set when samples from 25% of the diets were removed.

Table 4. Validation statistics where a calibration equation, developed from 75% of the diets in the calibration set, was used to predict constituent values of the samples in the remaining diets.

	Nitrogen (%)	IVDMD Coeff	Faecal $\delta^{13}C$
SEP	0.22	0.04	1.09
Bias	-0.01	0.01	0.01
R^2	0.82	0.61	0.92

(iii) Validation using a data set from the Brigalow Research Station

Faecal samples were obtained from steers grazing buffel grass pastures at Brigalow Research Station in southern Queensland. Dietary N concentrations had been determined by analysis of extrusa samples collected from fistulated steers. Faecal NIRS predictions of dietary N (mean of 6 steers) were compared with mean extrusa N concentrations. The agreement between NIRS predicted dietary N and extrusa N was considered to be excellent (Table 5.)

Table 5. Comparison of faecal NIRS dietary N estimates with the N concentration of extrusa samples collected from oesophageal fistulated steers.

Sampling date	Extrusa N (%)	NIRS N (%)
22 Dec 94	1.90	1.78
20 Feb 95	1.41	1.21
31 mar 95	1.34	1.33
19 Jun 95	1.33	1.25
23 Nov 95	1.49	1.91
1 Feb 96	1.34	1.55
2 Apr 96	0.75	0.76
9 May 96	1.81	1.77
11 Jul 96	0.96	0.90
29 Aug 96	0.74	0.97

Pen feeding experiments

(i) Validation by every second sample

When every second sample was dropped from the calibration set, predictions of dietary N for samples in the validation set remained satisfactory; predictions of IVDMD and *in vivo* digestibility were reasonable, DDMI predictions were less than desirable and predictions of intake were poor (Table 6). The prediction statistics were considered to be quite reasonable in relation to the restricted calibration set of samples once every second sample was removed. Even the full calibration set of faecal samples from the pen experiments is considered to be too small for the development of a robust calibration equation.

Table 6. Validation statistics where a calibration equation, developed from half the samples in the calibration set, was used to predict constituent values for the remaining samples.

	Nitrogen (%)	IVDMD Coeff.	In vivo Coeff	Intake (g/kgLW)	DDMI (g/kgLW)
SEP	0.18	0.04	0.03	2.62	1.65
Bias	-0.02	0	-0.01	0.02	-0.42
R^2	0.95	0.88	0.86	0.48	0.73

(ii) Validation by removing 25% of the diets.

When samples from 25% of the pen trial diets were removed from the calibration set, the NIR predictions on the samples removed were less satisfactory, as judged by SEP, than when every second sample was dropped (Table 7). Coefficients of determination actually improved but bias increased. This was probably due to the greater impact of removing whole diets from the calibration set, than from removing samples across all diets, on the spectral diversity of the calibration set. Prediction of dietary N remained acceptable but prediction of the other attributes, uncorrected for bias, was unsatisfactory. As the calibration set increases in diversity, validation statistics or predictive performance will generally approach the calibration statistics.

Table 7. Validation statistics where a calibration equation, developed from 75% of the diets in the calibration set, was used to predict constituent values of the samples in the remaining diets.

	Nitrogen (%)	IVDMD Coeff.	In vivo Coeff	Intake (g/kgLW)	DDMI (g/kgLW)
SEP	0.20	0.06	0.04	2.71	1.87
Bias	0.1	-0.02	-0.01	-0.99	-1.04
R^2	0.98	0.89	0.85	0.73	0.87

Forage NIR calibrations

Milled samples of the diets offered in the pen experiments were analysed in the NIRS instrument and relationships of the forage spectra with N concentration, IVDMD, *in vivo* DMD, intake and DDMI were determined (Table 8). Calibration equation statistics were excellent for N and IVDMD. Results from this limited data set indicated that *in vivo* digestibility could not be predicted accurately from NIR analysis of the forage. In fact, the calibration statistics for the prediction of *in vivo* DMD by faecal NIR were substantially better than those by forage NIR. There appeared to be little scope for forage NIR as a predictor of voluntary intake. In contrast, forage NIR showed considerable potential as a useful predictor of DDMI. Interestingly, the evidence to date suggests that DDMI can be predicted equally well from both forage and faeces (cf. Tables 2 and 8).

Table 8. Calibration equation statistics for forages fed in pen experiments.

	No. of samples	Range	SEC	R2
Nitrogen (%)	75	0.31 - 4.08	0.088	0.99
IVDMD coeff	75	0.29 - 0.82	0.021	0.97
In vivo DMD	47	0.42 - 0.69	0.034	0.70
Intake (g/kgLW)	47	9.54 - 26.85	2.635	0.42
DDMI (g/kgLW)	47	4.36 - 18.45	1.073	0.85

In vivo digestibility trials

In vivo dry matter digestibility, intake, DDMI and faecal output were determined for 37 different forages at Lansdown. Data relating to an additional 17 forages were obtained from trials conducted at other locations but the intake, DDMI and faecal output (% body weight) data from 7 of the additional forages were considered to be spurious and therefore rejected. Digestibility data from these 7 diets were retained. Data relating to the 54 forages are presented in Table 9 and Figure 9.

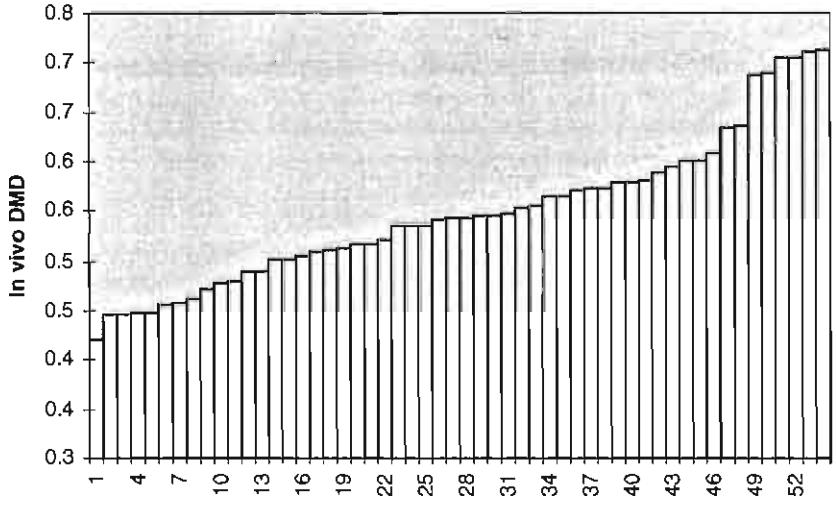

Figure 9. *In vivo* dry matter digestibility (DMD) of the 54 forages fed in pen experiments

Table 9. In vivo dry matter digestibility, digestible dry matter intake (DDMI), faecal output, in vitro dry matter digestibility (IVDMD) measured in in vivo digestibilitytrials at Lansdown (LDN) and other locations.

FEED	SITE	NO. HEAD	LWT kg	IN VIVO DMD	IVDMD FEED	INTAKE g/kgLW	DDMI g/kgLW	FAECAL OUTPUT % Body wt
BUFFEL 3	LDN	3	398	0.420	0.388	13.95	5.86	0.81
SECA/BUFFEL	LDN	6	242	0.447	0.413	15.94	7.12	0.88
GLYCINE	LDN	3	262	0.448	0.499	18.17	8.14	1.00
NATIVE(95)	LDN	3	251	0.449	0.292	10.76	4.83	0.59
URO 3 (Low)	LDN	4	270	0.455	0.407	17.38	7.91	0.95
NATIVE(96)	LDN	3	253	0.457	0.327	9.54	4.36	0.52
HUMIDICOLA	LDN	3	418	0.461	0.372	12.49	5.76	0.67
PERTUSA	LDN	2	383	0.472	0.358	14.17	6.68	0.61
NATIVE(95)	LDN	3	342	0.480	0.292	9.09	4.36	0.48
SECA	LDN	4	382	0.489	0.470	12.53	6.13	0.64
LDN VERANO	LDN	4	431	0.501	0.462	15.15	7.60	0.74
URO 2 (Med-low)	LDN	3	411	0.502	0.424	13.56	6.81	0.78
URO 1 (Med)	LDN	3	260	0.510	0.463	19.07	9.73	0.93
MITCHELL	LDN	4	419	0.512	0.430	18.57	9.51	0.91
GLYCINE	LDN	3	357	0.513	0.499	15.85	8.13	0.77
VERANO/BUFF	LDN	6	212	0.518	0.554	16.63	8.61	0.80
CLITORIA	LDN	4	430	0.534	0.620	20.28	10.83	0.94
BUFF 2 (High)	LDN	4	407	0.534	0.534	19.41	10.37	1.04
BLUE COUCH	LDN	4	354	0.535	0.423	14.77	7.91	0.69
BUFF 1(Med)	LDN	4	432	0.540	0.489	15.28	8.25	0.81
PEANUT/FCR(70:30)	LDN	3	418	0.544	0.555	17.81	9.68	0.81
URO 1 (Med)	LDN	4	404	0.547	0.464	17.79	9.73	0.92
RHODES	LDN	4	384	0.554	0.507	18.81	10.42	0.84
HARTS VERANO	LDN	4	390	0.565	0.579	21.06	11.89	0.92
PURPLE PIGEON	LDN	4	398	0.565	0.478	18.40	10.39	0.78
FINE CUT	LDN	3	363	0.571	0.455	19.11	10.91	0.82
PANIC	LDN	4	391	0.573	0.397	12.58	7.21	0.54
FINE CUT	LDN	3	266	0.573	0.455	17.81	10.21	0.76
CAVALCADE	LDN	4	393	0.577	0.608	17.31	9.99	0.73
IND COU	LDN	4	393	0.579	0.528	19.04	11.03	0.81
FCR/VERANO	LDN	4	386	0.594	0.549	17.12	10.18	0.69
MILLET	LDN	3	395	0.600	0.604	22.17	13.30	0.89
BRACHIARIA	LDN	4	351	0.600	0.394	14.07	8.44	0.56
LUCERNE	LDN	4	233	0.687	0.813	26.86	18.45	0.84
WHEAT	LDN	4	216	0.688	0.648	18.48	12.72	0.58
OATS/FCR	LDN	4	246	0.713	0.791	22.73	16.21	0.66
LUCERNE/OATS	LDN	4	472	0.710	0.816	25.44	18.06	0.74
RHODES RUN 9	McLEN	2	235	0.477	0.413	12.29	5.86	0.64
RHODES RUN 8	McLEN	2	234	0.521	0.413	19.19	9.99	0.92
BUFFEL 2	POPPI	4	306	0.542	0.450	13.23	7.18	0.60
PARAMATTA GR	GRAFTON	3	255	0.545	0.332	15.80	8.62	0.71
BUFFEL 1	POPPI	4	310	0.546	0.537	18.77	10.24	0.81
RHODES 1Control	McLEN	5	345	0.553	0.433	19.61	10.84	0.87
RHODES/LUCERNE	ROCKY	6	290	0.579	0.516	17.67	10.23	0.74
SPEARGRASS	POPPI	4	319	0.588	0.485	15.58	9.16	0.65
LUCERNE (Restricted	ROCKY	18	316	0.607	0.698	21.92	13.31	0.86
RYEGRASS	POPPI	4	328	0.633	0.678	18.78	11.89	0.69
BUFFEL BP	BP	4	393	0.517	0.516			
SPEARGRASS BP	BP	4	393	0.490	0.501			
BLUEGRASS BP	BP	4	401	0.446	0.447			
SORGHUM BP	BP	4	401	0.506	0.593			
YOUNG OATS BP	BP	4	284	0.704	0.712			
MATURE OATS BP	BP	4	283	0.636	0.604			
RYEGRASS BP	BP	4	279	0.705	0.735			

In vivo - in vitro digestibility relationships

In vitro digestibilities are usually determined in the laboratory for the purpose of estimating *in vivo* digestibilities. Standards of known *in vivo* digestibility are included in the analyses so that adjustments can be made according to the relationship between *in vitro* dry matter disappearance and *in vivo* digestibility (McLeod and Minson 1978). Obviously the accuracy with which *in vivo* digestibility can be estimated from *in vitro* analysis will depend on the correlation between *in vitro* dry matter disappearance and *in vivo* digestibility as well as on the precision of the *in vitro* analysis technique.

The results of the 54 forages fed in the pen trials indicated that *in vivo* dry matter digestibility could not be accurately predicted from the *in vitro* dry matter digestibility of the feed (Fig. 10):
In vivo DMD = 0.474 (*in vitro* DMD feed) + 0.308 (n = 54; R^2 = 0.64; RSD = 0.045)

Note that the comparable statistics for the faecal NIR estimates of *in vivo* digestibility were an R^2 of 0.89 and an RSD of 0.025 (Table 2)

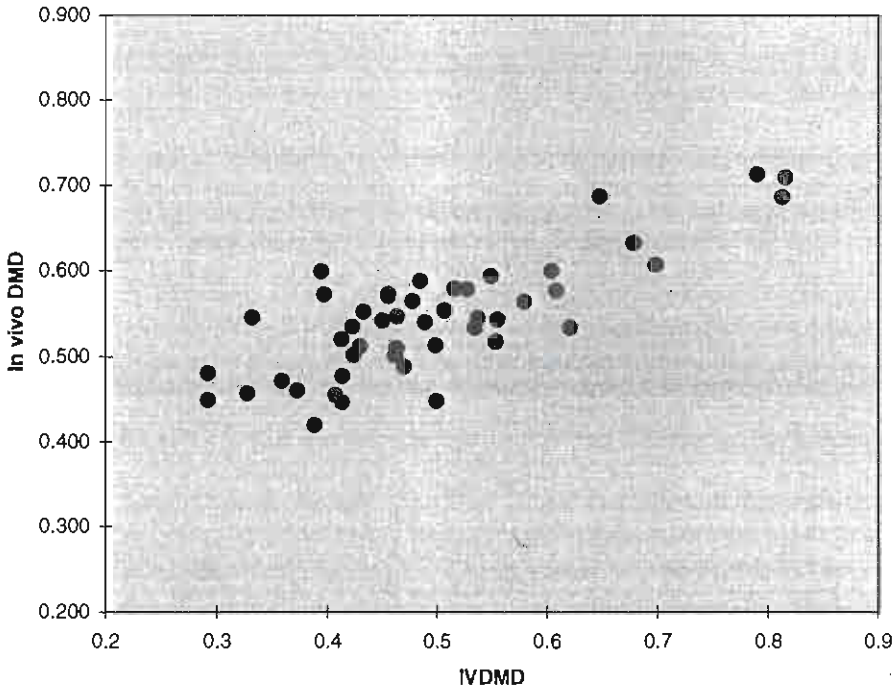

Figure 10. Relationship between *in vivo* dry matter digestibility (DMD) and *in vitro* dry matter digestibility (IVDMD) of the 54 forages fed in pen experiments.

The relationship between *in vivo* DMD and the *in vitro* dry matter digestibility of extrusa samples was marginally better:
In vivo DMD = 0.714 (*in vitro* DMD extrusa) + 0.155 (n = 34, R^2 = 0.733; RSD = 0.040)

When grasses and legumes were treated separately, there was no improvement in the *in vivo - in vitro* relationship for the grasses:

In vivo DMD grass = 0.508 (*in vitro* DMD grass) + 0.302 (n = 39; R^2 = 0.65; RSD = 0.044)

There was, however, a good relationship for the legume forages (Fig. 11) with a much reduced RSD though there were only 10 legume hays:
In vivo DMD leg = 0.567 (*in vitro* DMD leg) + 0.218 (n = 10; R^2 = 0.87; RSD = 0.026)

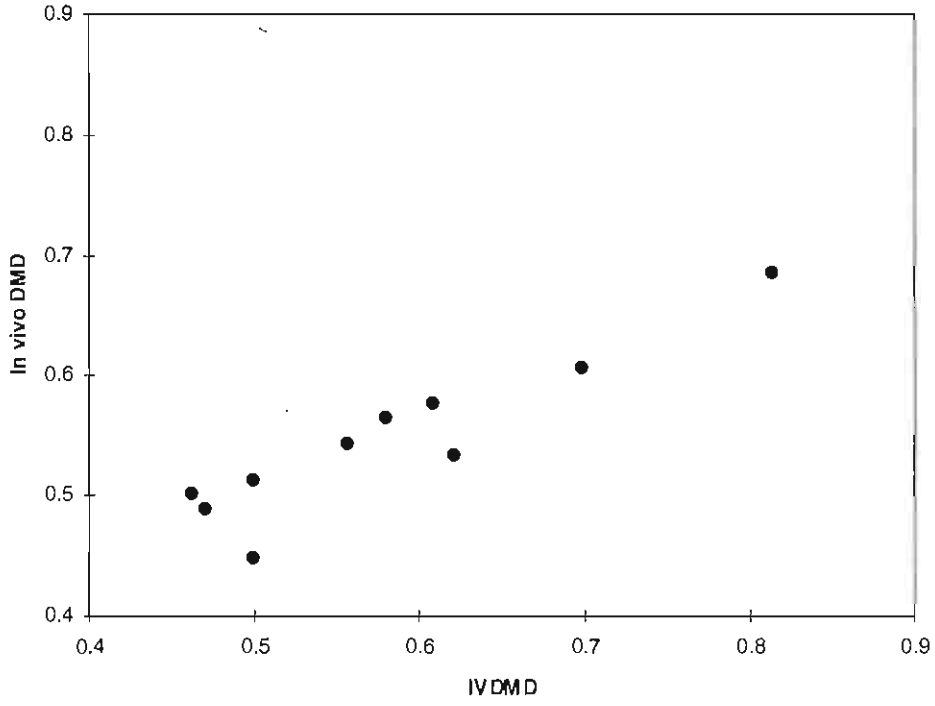

Figure 11. Relationship between *in vivo* dry matter digestibility (DMD) and *in vitro* dry matter digestibility (IVDMD) of feed for 10 legume forages.

The results from these digestibility trials indicated that the prediction of *in vivo* DMD from *in vitro* analysis requires different regression relationships for grasses and legumes. Grass/legume mixtures would be intermediate.
The results also indicate that faecal NIRS has the potential to predict *in vivo* digestibility more accurately than the pepsin-cellulase *in vitro* analysis of the forage.

Digestibility and intake relationships

In vivo DMD was a poor predictor of relative intake (g/kgLW) and accounted for less than half of the variation in intake (Fig. 12).
INTAKE (g/kg/LW) = 38.965 (*in vivo* DMD) - 3.077 (n = 47; R^2 = 0.47; RSD = 2.75)

DDMI was more closely related to intake than it was to *in vivo* DMD (Figs. 13 and 14):
DDMI (g/kgLW) = 38.907 (*in vivo* DMD) - 11.731 (n = 47; R^2 = 0.754; RSD = 1.55)
DDMI (g/kgLW) = 0.784 (INTAKE) - 3.902 (n = 47; R^2 = 0.897; RSD = 1.00)

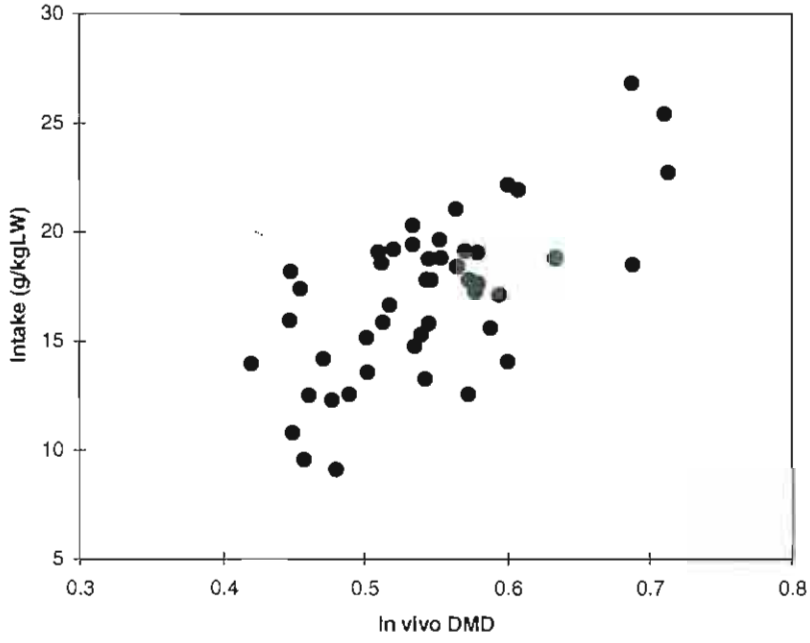

Figure 12. Relationship between Relative Intake (g/kgLW) and *in vivo* dry matter digestibility (DMD) for 47 forages fed in pen experiments.

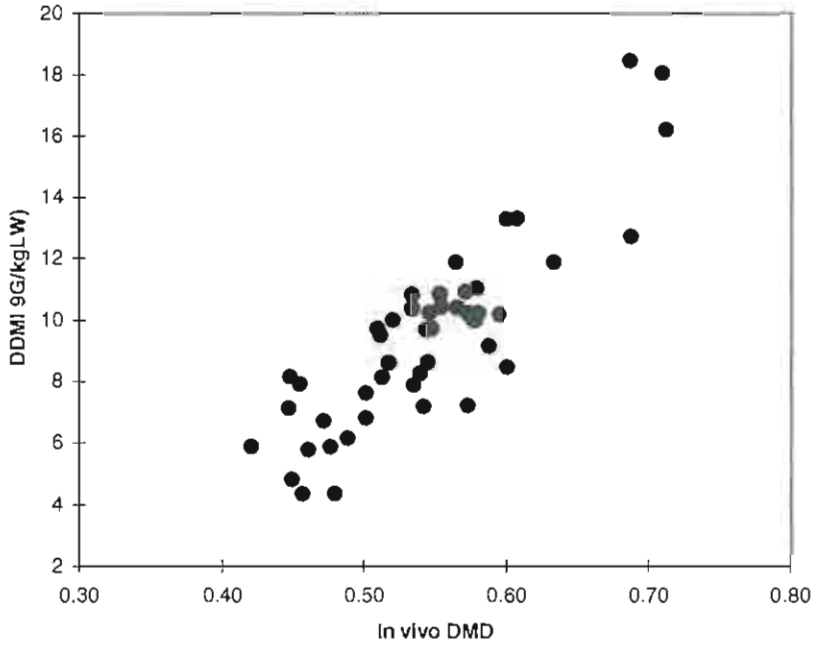

Figure 13. Relationship between digestible dry matter intake (DDMI) and *in vivo* dry matter digestibility (DMD) for 47 forages fed in pen experiments.

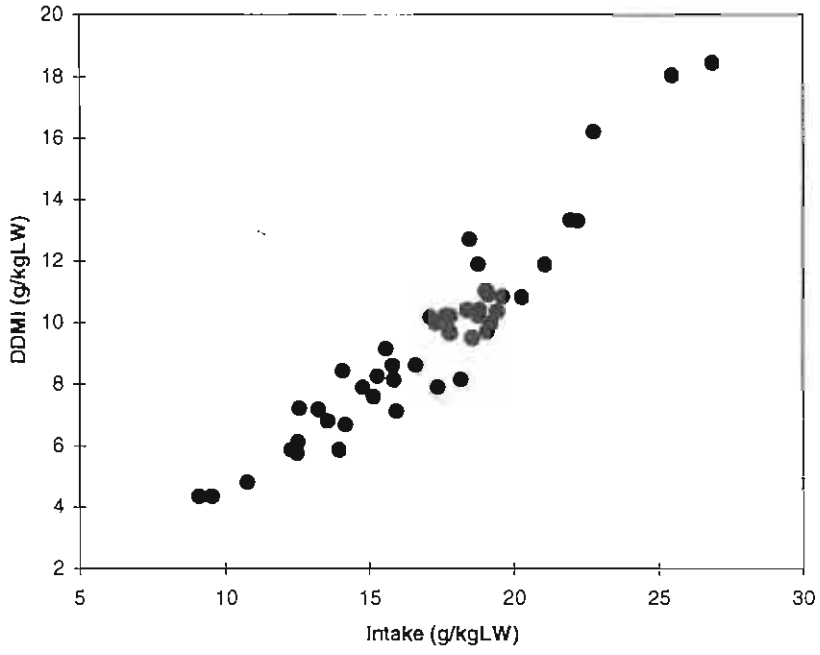

Figure 14. Relationship between digestible dry matter intake (DDMI) and Intake for 47 forages fed in pen experiments.

Thus, it appears that the *in vivo* digestibility of tropical forages is not a good indicator of forage quality. For example, the Gatton panic (*Panicum maximum*) and *Brachiaria decumbens* hays had high *in vivo* digestibility coefficients at 0.573 and 0.600 respectively, but the intakes were low at 12.58 and 14.07 g/kgLW respectively. In contrast, cattle fed Mitchell grass (*Astrebla* spp) hay and one of the Urochloa (*Urochloa mosambicensis*) hays had relatively high intakes (18.57 and 19.07 g/kgLW respectively) despite having appreciably lower *in vivo* digestibilities (0.51). Similarly, one of the Urochloa hays and a native pasture hay both had *in vivo* digestibility coefficients of 0.45, but the intake of the Urochloa hay was almost double that of the native pasture (17.38 vs 9.54 g/kgLW).

The *in vitro* digestibility of the feed or the extrusa was a better predictor of intake than *in vivo* digestibility.

INTAKE (g/kgLW) = 24.792 (IVDMD feed) + 4.678 (n = 47; R^2 = 0.68; RSD = 2.119)
INTAKE (g/kgLW) = 29.703 (IVDMD extrusa) + 0.670 (n = 27; R^2 = 0.57; RSD = 2.181)

Similarly, *in vitro* digestibility was a better predictor of DDMI than *in vivo* digestibility.

DDMI (g/kgLW) = 22.222 (IVDMD feed) - 1.553 (n = 47; R^2 = 0.80; RSD = 1.385)
DDMI (g/kgLW) = 28.434 (IVDMD extrusa) - 6.082 (n = 27; R^2 = 0.80; RSD = 1.191)

In vitro digestibility of feed and extrusa

When comparisons were made between the pepsin-cellulase *in vitro* digestibility of samples of forage and samples of extrusa it was found that the digestibility of the extrusa was usually higher

than that of the unmasticated forage. Grasses behaved differently from legumes, there being little difference between feed and extrusa for the legumes.
IVDMD legume extrusa = 0.958 (IVDMD forage) + 0.033 (R^2 = 0.98; RSD = 0.009)

For the grasses, the digestibilities of forage and extrusa were similar for forages of high digestibility but there were substantial differences at low levels of digestibility (Figure 15).
IVDMD extrusa = 0.791 (IVDMD feed) + 0.163 (R^2 = 0.97; RSD = 0.017)

The regression relationship above indicates that the IVDMD of feed and extrusa would be the same at a feed IVDMD of 80%, but the IVDMD of extrusa would be 10 units higher when feed IVDMD is 30%.

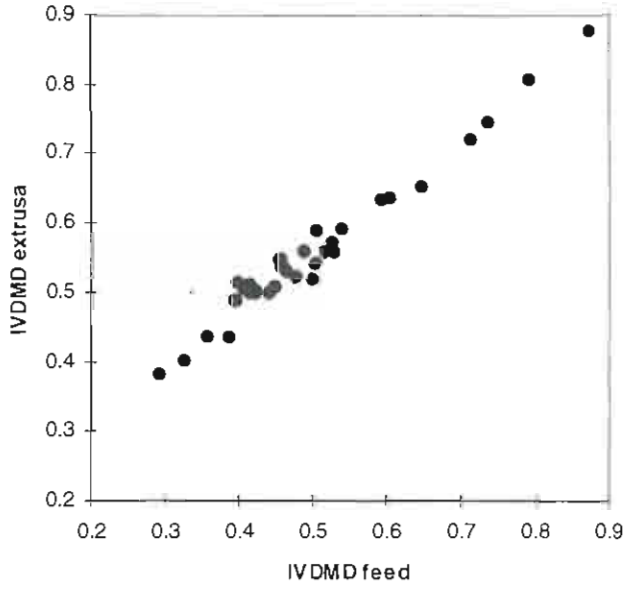

Figure 15. Relationship between *in vitro* dry matter digestibility (IVDMD) of extrusa and IVDMD of the feed for grass diets.

The higher *in vitro* digestibility of extrusa compared with the forage offered was common to each of the 6 OF steers and further investigations revealed that the increase was associated in some way with salivary contamination. Comparable increases occurred when saliva was mixed with ground samples of forage. The dry matter in saliva (1% DM) causes a slight increase in the apparent digestibility of the sample since the salivary DM is totally digestible. However, salivary DM is unlikely to account for more than a 2 percentage units increase in dry matter digestibility.

The difference between the *in vitro* digestibility of forage and extrusa is of major significance. Where *in vitro* dry matter disappearance is used to predict or estimate a correlated parameter (such as *in vivo* digestibility, or intake, or DDMI), the predictive relationships for forage and extrusa will be different. In the past, regression relationships between the known *in vivo* and *in vitro* digestibilities of forages have been used to estimate the *in vivo* digestibility of unknown samples from *in vitro* analysis, regardless of whether the unknown samples are forage or extrusa. Results from this project clearly indicate that separate relationships need to be developed for different applications: forage vs extrusa, and grass vs legumes vs mixtures.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The following conclusions can be drawn with respect to faecal NIRS technology.

- Faecal NIRS can be used to obtain accurate predictions of the dietary N of free grazing cattle.
- Faecal NIRS prediction of dietary N is far superior to any alternative technology in terms of scope, accuracy. practical application and cost.
- Faecal NIRS can be used to obtain accurate predictions of the dietary $\delta^{13}C$ of free grazing cattle.
- Faecal NIRS can be used to obtain predictions of the dietary digestibility of free grazing cattle with only moderate accuracy.
- Faecal NIRS cannot provide accurate predictions of forage intake in cattle consuming tropical forages.
- Faecal NIRS shows promise for the prediction of DDMI in free grazing cattle.
- Lack of accuracy in laboratory reference data used for developing calibration equations is a major limitation to the accuracy of faecal NIRS predictions of diet quality. Such a limitation is related primarily to data obtained from grazed pastures where samples of extrusa from oesophageal fistulated cattle are used for determining laboratory reference values.
- Reliability/accuracy of current predictions of all dietary attributes is limited to a certain extent by the size and diversity of the calibration set. This is a greater limitation with respect to attributes associated with the energy status of the diet (digestibility and digestible energy intake) than to N and dietary C_3/C_4 composition. Therefore, additional data is required to expand the size and diversity of calibration sample sets to improve the reliability of faecal NIRS predictions of diet quality and the geographic spread of the potential area of application. Likewise. there is a need to demonstrate the reliability of dietary predictions by an on-going process of validation.
- Faecal NIRS has the great advantage that predictions of all attributes for which calibrations equations have been developed can be made from a single NIR analysis. Moreover, if spectral information is stored, predictions can be made retrospectively or modified at any future time if new calibration equations are developed or if existing calibration equations are expanded or modified.
- There is considerable scope for an increase in the range of attributes that may be predicted using faecal NIRS.

- The usefullness and applications of faecal NIRS technology can be substantially increased by building on the data and relationships developed in CS.253. Faecal NIRS technology has the potential to make really significant and worthwhile contributions to both industry and research in the areas of pasture-animal relations, nutritional management of grazing cattle and the management of resources for sustainable production.

Additional conclusions listed below relate to the measurement or estimation of digestibility in cattle consuming tropical forages.

- First and foremost, results from the pen feeding experiments demonstrated that *in vivo* DMD cannot be considered as a good indicator of diet quality in the north Australian context because *in vivo* DMD is not closely correlated with the intake of digestible energy. This statement is made on the basis of diet quality being a measure of the productive capacity of the diet, the productive capacity being determined by the intake of digestible energy.

- *In vivo* digestibility is not a good indicator of forage quality because forage intake is not well correlated with *in vivo* digestibility in tropical grasses.

- The pepsin-cellulase *in vitro* DMD of tropical forages is a better indicator of forage quality than *in vivo* digestibility, the former being more closely correlated with both forage intake and the intake of digestible energy.

- Faecal NIRS offers better opportunities for predicting the intake of digestible energy than any measure of digestibility.

- Accurate estimates of *in vivo* digestibility in cattle by the pepsin-cellulase *in vitro* analysis of forage or dietary samples is limited by the relatively poor relationship between *in vivo* and *in vitro* digestibility of tropical grass diets. The *in vivo* - *in vitro* relationship is different for grasses compared with legumes.

- Faecal NIRS has the potential to predict *in vivo* digestibility more accurately than the pepsin-cellulase *in vitro* analysis of the forage.

- *In vitro* digestibility measurements made on forage differ from those made on extrusa. In general, IVDMD of extrusa is higher than that of the uncontaminated forage. The difference in grasses is much more marked than in legume forages. The differences are greater at low digestibility, diminishing linearly as the digestibility increases.
- The differences in IVDMD between forage and extrusa, and the differences between grasses and legumes in the forage-extrusa relationship, have important implications in estimating *in vivo* digestibility from *in vitro* analysis, implications that previously have not been recognised nor accounted for.

Recommendations

General recommendations

Results from project CS.253 have demonstrated that faecal NIRS offers substantial benefits to industry and research but the potential benefits can only be reaped with additional development of the technology to improve the predictive accuracy of existing calibration equations and to add to the range of attributes being predicted. In addition, there is an urgent need to develop related technologies so that faecal NIRS dietary predictions can be effectively used in decision making or for the optimal interpretation of experimental results. The following recommendations are made with these goals in mind.

1. Proceed with a planned schedule of low-level sampling (diets and faeces) to validate and expand calibration equations for predicting dietary N and dietary IVDMD by faecal NIRS.

2. Expand and validate calibration equations for predicting dietary C_3/C_4 composition, especially with respect to diets with a browse component.

3. Initiate a sampling program for generating additional data and calibration samples for predicting dietary DDMI. The most pressing need is for accurate data relating to animals on pasture, especially green feeds. Alkane techniques provide the technology by which accurate reference values can be determined for matching with faecal (and forage) NIR spectra. Since such a sampling program could be accomplished without using fistulated cattle, it could be best accomplished by a multi-site, multi-institutional approach. Chemical analyses would need to be conducted in either a single, certified laboratory or alternatively in a number of laboratories with cross laboratory quality control measures.

4. Maintain a *search-and-acquire* program for obtaining data and samples from pen trials conducted with cattle consuming roughage diets anywhere in Australia. Such samples would be of great benefit for validating and expanding calibration equations.

5. Increase the range of attributes predicted from faecal NIRS. It is expected that accurate to useful predictions of dietary fibre fractions (ADF, NDF and ADL) and of faecal N concentration could be made once relationships are developed. The process would be relatively simple and cost effective since large sample sets accumulated from CS.253 are already available. Additional samples will become available in the course of carrying out recommendation 1. The only requirement is to conduct the appropriate chemical analyses on the stored diet (forages from pen trials and extrusa from pasture samplings) and faecal samples (faecal N) as appropriate.

6. Develop a nutritional model for reliably predicting animal performance from a knowledge of current nutritional status (dietary N and dietary energy status) that is applicable to pastures and environments in northern Australia. Existing models like GRAZFEED and CAMBEEF and current nutritional standards (eg. NRC, SCA) do not perform well when applied to tropical pastures. There is, therefore, a need to re-define the feeding standards for cattle grazing tropical/subtropical pastures and to develop new or modified nutritional models that can be applied in northern Australia.

7. Additional research is required to adequately predict the biological response of cattle to supplementation in relation to the type of supplement, the amount of supplement and the nutritional status of the animal from pasture alone.

Recommendations for commercial exploitation

CS.253 has developed calibration equations for predicting diet quality attributes from faecal NIRS in grazing cattle. Additional work, as detailed in the general recommendations, is required to improve the reliability of existing equations and to broaden the range of attributes being predicted. Assuming that these outcomes can be achieved, there are additional requirements for the effective commercial exploitation of the technology with respect to the nutritional management of grazing cattle. The prediction of diet quality needs to be linked to an appropriate nutritional model so that the diet quality information can be assessed in terms of animal performance. There is an additional need to be able to predict responses to feeding various classes of supplement in a range of situations. Models for predicting responses to supplements must, by necessity, relate back to the base diet provided by the pasture.

There are, therefore, three essential components to the nutritional management of grazing cattle in the field of cost effective supplementation:

(i) technology for predicting diet quality in free grazing cattle

(ii) technology (nutritional model) for predicting animal performance from diet quality

(iii) technology for predicting responses to supplements

None of the components can stand in isolation, each being dependent on the others. All three components are necessary to make informed decisions on when, what and how much supplement to feed in order to meet a specified production target.

The three components of the nutritional management of grazing cattle were the focus of a technical workshop held at the CSIRO, Tropical Agriculture's Cunningham Laboratory in March, 1998. The workshop was convened as a necessary step towards the commercial exploitation of the faecal NIRS technology developed in CS.253. A report on the workshop, including outcomes and recommendations will be available in due course. In addition, a market assessment of the faecal NIRS and related technologies is being conducted by CSIRO, Tropical Agriculture to determine the likely demand from the various branches of the production arm of the beef industry (producers, analytical service providers, feed/supplement merchants, agri-consultants).

SUCCESS IN MEETING OBJECTIVES

The project was successfully conducted and completed in line with the stated methodologies, scheduled research program and agreed milestones. In fact, the sampling schedule far surpassed the agreed work program in that the number of pasture diets sampled was almost double the agreed minimum of 60 different pasture diets. The success in meeting the specific objectives is detailed below.

Main objective: To develop reliable predictive equations for determining the digestibility and crude protein content of the diet of free grazing cattle using faecal NIRS.

This objective was largely achieved with respect to the prediction of dietary crude protein concentration (estimate 90% achieved). Calibration equations were developed with excellent calibration statistics (ie. SEC values). Validation procedures indicate that the equations are able to predict dietary nitrogen with Standard Errors of Performance (SEP) of approximately ± 0.2%N. However, there is scope for developing more robust calibration equations by expanding the calibration set and continued validation of the current and any future equation/s is required.

The project clearly demonstrated a range of problems associated with the attempted prediction of dietary digestibility. One of the major problems encountered was the difficulty in obtaining accurate reference values for relating to faecal NIR spectra. It became clear that accurate reference values could not be obtained by the *in vitro* analysis of dietary samples obtained by sampling pastures with fistulated steers. Where reliable reference values were obtained from pen feeding experiments, the project results indicated that faecal NIRS is likely to be a better predictor of dietary *in vivo* digestibility than the *in vitro* analysis of dietary forage by the pepsin-cellulase method. However, the current calibration set of faecal samples with accurate matching data for *in vivo* digestibility is considered too small and too restricted for the reliable prediction of dietary digestibility in free grazing cattle, especially as all such samples in the calibration set were derived from hays.

Equations were developed for predicting the *in vitro* digestibility of the diet with reasonable accuracy. Once again the major problem with the development of calibration equations was the difficulty in obtaining accurate reference values when pastures were sampled with fistulated steers. Calibration equation statistics were excellent when accurate reference values were obtained from the pen feeding trials. Overall, the current equations are considered to be useful and reasonably robust but, as for N, there is a need for expansion of the calibration set and for the continued validation of current and future equations.

The main objective was seen as providing the means by which related objectives with industry and research applications, respectively, could be achieved.

1. Develop technology to allow producers to increase the cost-effectiveness of supplementary feeding.

The ability to predict diet quality from faecal analysis was always seen as just one component of a broader technology package focusing on cost-effective supplementation of grazing cattle, the other components being the ability to predict animal performance from a knowledge of current diet quality and the ability to predict responses to the provision of supplementary nutrients. Biological (and therefore economic) responses to supplementary feeding depend on the amount

and formulation of the supplement consumed relative to the current and on-going nutritional status of the animals not receiving supplement. Given the existence or development of adequate nutritional models for the latter two components, the "what", "when" and "how much" supplement to feed (the critical decisions for cost effective supplementation) will then depend on a knowledge of current and on-going diet quality in the absence of supplement. Project CS.253 has gone a long way towards providing the technology for predicting the diet quality of grazing animals. The reliable prediction of dietary N concentration seems assured (90% achieved) but further work is needed for the reliable prediction of dietary energy status (70% achieved). The project clearly demonstrated the limitations of digestibility as an index of dietary energy status. This itself is a significant achievement. Fortunately, the project also demonstrated the exciting prospect of being able to predict the intake of digestible energy (DDMI). The fulfilment of such a prospect would represent a really significant advance in pasture-animal relationships. Current predictions of DDMI based on the calibration equation developed from pen feeding data are considered to be useful but lacking in known reliability. There is a need to expand the calibration set of faecal samples and diets, particularly with respect to grazed rather than pen-fed diets. One of the encouraging aspects of the prediction of diet quality from faecal NIRS is that the technology is likely to become more and more useful and applicable as the foundation developed in CS.253 is built on and expanded.

2. Develop technology to provide a research tool that will enhance the efficiency and scope of research dealing with productivity and sustainability issues in rangeland science.

This objective has been achieved to the extent that useful applications for enhancing the efficiency and scope of research can already be made. However, the objective has not been fully achieved due to (i) the current deficiencies in the faecal NIRS technology as described above and (ii) the almost limitless scope for faecal NIRS and associated technologies to expand and improve with time.

Current predictions of dietary N are considered to be reliable with a SEP of ± 0.2% N. Similarly, dietary C_3/C_4 composition can be predicted with a high level of confidence. While estimates of dietary digestibility and DDMI are less reliable, treatment rankings and relativities with respect to these dietary attributes are likely to be valid even if predicted values are not accurate. Once samples have been subjected to NIRS, spectra can be stored and the predictions can be revised as calibration equations are expanded and improved. Following the success achieved in project CS.253, the suite of dietary attributes predicted will be expanded in due course.

Currently, frequent, regular monitoring of diet quality using faecal NIRS is being conducted on herds at Lansdown, Swans Lagoon, Rosebank (Longreach), Brigalow Research Station, Brian Pastures Research Station and Glentulloch (Injune).

3. Determine the *in vivo* digestibility of a range of tropical pasture species and/or mixtures of varying quality for the purpose of developing calibration equations based on reliable data for estimating dietary digestibility from faecal NIR.

This objective was successfully achieved. Eight pen experiments, covering 37 different diets, were conducted at Lansdown Research Station. In addition, samples and data were obtained from a further 17 diets fed in pens at other locations. Diets included 25 different introduced pasture species fed separately or in mixtures as well as mixtures of native pasture grasses. *In vivo* dry

matter digestibilities ranged from 42% to 71%. The calibration equations developed and the relevant calibration statistics confirmed the improved reliability of pen trial data compared with that from field sampling with fistulated steers. The calibration statistics indicate the potential for the accurate prediction of diet quality using faecal NIRS when calibration equations are developed with accurate laboratory reference data. The reliability of dietary predictions will improve with an expanded calibration set but the sample set accumulated in CS.253 provides an extremely valuable foundation for future expansion as more pen experiment data come to hand.

4. Determine whether a useful relationship can be determined which will allow DM intake to be estimated from either feed or faecal NIRS.

Data from pen trials indicated that accurate predictions of forage intake in cattle consuming tropical forages could not be made using either feed or faecal NIRS. It was also demonstrated that forage intake is not closely related to forage digestibility. Tropical forages of similar digestibility may have markedly different intakes or conversely, tropical forages with similar intakes may differ widely in digestibility.

A highlight of the project, however, was the demonstration of a good relationship between either faecal or forage NIRS and DDMI. The prediction of DDMI is considered to be far more useful than the prediction of intake.

5. Determine whether the *in vitro* DM disappearance during pepsin-cellulase digestion is different in samples of extrusa obtained from fistulated steers than in the forage from which the extrusa was derived; quantify any difference between *in vitro* digestibility of extrusa and feed samples; and develop regression relationships between *in vitro* digestibility and *in vivo* digestibility appropriate for samples of feed and samples of extrusa respectively.

This objective was fully achieved. Project results indicated clearly that the dry matter disappearance during pepsin-cellulase *in vitro* digestion differed between samples of forage and samples of extrusa derived from the forage. Overall, IVDMD of extrusa was higher than that of the forage from which it was derived. C_4 grasses behaved differently from legumes with differences being larger for the grasses. Within the grasses, differences between extrusa and forage increased as digestibility decreased. Regression relationships were developed for estimating *in vivo* digestibility of grasses and legumes from *in vitro* analysis of either feed or extrusa.

6. Determine the potential of faecal NIRS for predicting species composition of the diet.

This objective was achieved to the extent that good relationships were developed between faecal NIRS and faecal $\delta^{13}C$, the latter being an index of the dietary C_3/C_4 composition. In tropical pastures the C_3/C_4 composition usually equates with the non-grass/grass composition, the non-grass proportion comprising native or introduced legumes, forbs and browse. Stylo species were the dominant C_3 species in the pastures sampled but other legumes, both native and introduced, a wide range of forbs and some browse were also present. The current calibration equation is based on a set of approximately 350 faecal samples derived from over 100 diets. (Further

differentiation of species composition of the diet would require the development of "local" calibration equations for application in specified situations with defined limitations.)

INTELLECTUAL PROPERTY

The Intellectual Property arising out of CS.253 is represented in (i) the calibration equations for predicting dietary attributes from faecal NIRS, (ii) the faecal NIR spectra files and matching laboratory reference values for the relevant dietary attributes and (iii) the calibration sets of faecal and dietary samples being held in cold storage. The calibration equations are not static and will be subject to continued expansion and refinement. Equations can only be transferred to other users if the NIRS instruments are cross-standardised. However, other users could generate their own calibration equations if they had access to the faecal samples for scanning on their own instruments and the relevant laboratory reference data for relating to the spectra. In other words, the Intellectual Property can only be shared, transferred or sold by agreement between relevant parties and by following a protocol of agreed procedures.

FUNDING

The Meat Research Corporation provided funding support to CS.253 over the 3-year period, 1995/96 - 1997/98. The total contribution by the Corporation amounted to $100,585. The CSIRO contribution to the project to June 1998 was estimated to be in the order of $400,000.

IMPACT

The impact of the project and its results within Australia as at the date of the Final Report was predominantly in the area of awareness and expectancy. The project received publicity by way of radio talks and interviews in Queensland and the Northern Territory, together with articles in newsletters, newspapers and magazines. Producer interest in the technology has been widespread with enquiries coming from Western Australia and New South Wales as well as from many areas within Queensland. Enquiries have come from sheep as well as cattle producers and from intensive as well as extensive operations. Widespread interest from within the scientific community has also been apparent. While the technology has its sceptics, there seems to be a general desire that the technology will be effective in achieving the desired outcomes since the ability to predict diet quality is recognised as being of enormous potential benefit to research capability as well as having worthwhile commercial applications.

Since mid-1997, faecal samples have been received from a member of the Feed Smarter group of producers at Roma on a regular basis for faecal profiling (ie. using faecal NIRS to monitor diet quality at regular intervals to establish a profile of the nutritional status of animals grazing a specified pasture). The lack of a nutritional model to estimate animal performance from diet quality seriously limits the usefulness of the NIR predictions but the producer involvement has been helpful in progressing the technology. Faecal samples have also been obtained from a commercial herd of cattle grazing sown, tropical grass/legume pasture on the Atherton Tablelands. The technology is being used to monitor diet quality on grazing trials in the Northern Territory (Kidman Springs and Newcastle Waters), and Queensland (Rosebank near Longreach, Glentulloch near Injune, and Swans Lagoon near Ayr) and in a series of monitor herds at Lansdown, Swans Lagoon, Brigalow Research Station and Brian Pastures. The aim of the monitor herds is to provide information for building or modifying a nutritional model for

predicting animal performance from diet quality. At present CSIRO Tropical Agriculture is bearing the processing and analytical costs of all external samples.

The impact of the technology over the 5-year period 1998/99 - 2003/04 will depend on the level of external funding support since additional work is still required to meet the technical requirements for effective commercialisation of the technology. Adoption rates subsequent to the commercial availability of the technology are difficult to estimate as are the financial consequences since benefits can come by way of not feeding supplements unnecessarily, feeding less for the same response or feeding more for a greater response, feeding in time to meet a market within a given time frame, improved resource management with long term benefits, improved decision making in relation to marketing strategies or simply through a much improved understanding of the nutritional potential and deficiencies of a grazing enterprise.

FUTURE COMMITMENT

CSIRO Tropical Agriculture has a commitment to continue with the development and commercialisation of the technology but the commitment is necessarily contingent upon adequate external funding support as a result of the Commonwealth Government policy relating to appropriation to external funding ratios.

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