MEAT & LIVESTOCK
AUSTRALIA

On farm

FINAL REPORT
NAP3.121

Improving nutritional management of grazing cattle: Improving reliability of faecal NIRS calibration equations

Project number NAP3.121

Report prepared for MLA by:

David Coates
CSIRO Sustainable Ecosystems

Meat & Livestock Australia Limited
Locked Bag 991
North Sydney NSW 2059

ISBN 1 74036 665 4

September 2004

CSIRO

Animal Production

TABLE OF CONTENTS

ABSTRACT

The aim of project NAP3.121 was to improve faecal NIRS calibration equations for predicting dietary crude protein, digestibility, faecal N, dietary non-grass proportions and growth rate in grazing cattle. Improvement was targeted at achieving levels of predictive reliability that would enable the technology to be used effectively by commercial producers across most, if not all, areas in the top half of Australia. The technology is primarily a decision support and educational tool designed to assist producers in the cost-effective nutritional management of their cattle and the efficient use of the pasture resource as well as being a powerful research tool. Project aims were largely achieved with significant improvements in the robustness of most calibration equations together with particularly encouraging results in relation to digestible dry matter intake predictions. The nature of NIRS is such that there is scope for significant and continued improvements to faecal NIRS technology and important areas requiring additional research were identified. Beneficial use of faecal NIRS by industry is dependent on other technologies and further research is needed in these areas to allow faecal NIRS to be exploited effectively.

EXECUTIVE SUMMARY

Project objectives and description

The primary objective of Project NAP3.121 was to improve faecal NIRS (Near Infrared Reflectance Spectroscopy) calibration equations for predicting the diet quality of cattle grazing pasture systems of northern Australia. With respect to industry application, the reason for the work was to provide the northern beef industry (producers, consultants and agribusiness) with an educational and decision support tool for improved nutritional management of grazing cattle. However, the work was also carried out to provide rangeland scientists with a new and powerful research tool to enhance the capture of knowledge from grazing experiments, to provide new insights and understanding in studies involving grazing cattle, to improve the cost effectiveness of research and to open up new avenues for research.

The project focused on a number of dietary and related attributes, viz. dietary crude protein (CP), the digestibility of the diet, grass and non-grass dietary proportions, faecal nitrogen (N) concentration and liveweight gain in growing cattle. The possibility of predicting faecal phosphorus (P) concentration was also investigated. Moreover, diet and faecal samples generated in the project will enable faecal NIRS calibration equations to be developed for predicting dietary neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the near future. Faecal NIRS calibration equations had already been developed for predicting dietary CP, digestibility, dietary non-grass proportions and liveweight gain but the equations needed further development to improve predictive reliability and accuracy when applied to faecal samples sourced from different locations and pasture communities right across northern Australia.

The calibration equation for each attribute or property is an independent entity derived from separate calibration sets though the same faecal spectra often occur in two or more of the calibration sets. Moreover, apart from dietary non-grass proportions, the reference values used to develop the calibration regression relationships between attribute and faecal spectra are direct measurements. Thus the reference values for dietary CP (diet CP = diet N x 6.25) are determined on samples of the actual diet; digestibility reference values are determined by *in vitro* analysis of diet samples or by *in vivo* digestibility trials; faecal N values are determined by chemical analysis of the faecal samples; and LWG values are determined by weighing cattle at regular, short intervals. Dietary non-grass proportions are calculated from faecal $\delta^{13}C$ values because this is the conventional method of determining non-grass proportions for cattle grazing tropical pastures. Thus, for this attribute, the primary faecal NIRS prediction was faecal $\delta^{13}C$. However, the reference values could just as easily have been dietary non-grass (%) calculated from the laboratory determination of faecal $\delta^{13}C$.

Project achievements

All aspects of the work were conducted according to plan and as set out in the contractual agreement except for the development of NDF and ADF equations. This was due to analytical problems and these two attributes were deleted from the initial agreement to be completed at a later date. Regarding the potential long term returns to the research funds invested it is considered that the achievements and outcomes are most satisfactory. While this “infant” technology needs more development to release its full potential, faecal NIRS at the completion of NAP3.121 represents a technology that can be used beneficially for both industry and research applications while providing a solid foundation for building future improvements.

The predictive reliability of calibration equations for dietary CP and digestibility was greatly improved by doubling the calibration set with respect to sample number and diet diversity. Of special significance were pen trials at Katherine Research Station and Brunchilly Station in the northern Territory, Swans Lagoon near Millaroo in north Queensland, and the Mt Cotton Research Farm near Brisbane where cattle were fed diets harvested from local pastures, as well as trials using oesophageal steers grazing a range of pasture species at Brian Pastures near Gayndah in south-east Queensland. Previously diets in the calibration set were largely restricted to hays and pastures from sites in north-east Queensland.

Calibration for predicting faecal N and dietary non-grass proportions were expanded with more than 400

new samples selected to broaden sample diversity with respect to pasture type and geographic source. The resultant equations are now considered to be robust and reliable for most situations.

One significant finding was that a single, universal calibration equation is inappropriate for predicting growth rate with sufficient accuracy to be of practical use. However, data from many monitor herds suggest that an alternative approach is to develop separate equations for either different pasture types or different regions. Sites within the eastern speargrass region together with sites from the Borthriochloa/Aristida regions of northern and southern Queensland appeared to be compatible such that the combined calibration set for these sites gave an equation with acceptable calibration statistics (Standard Error of Calibration (SEC) of 127 g/day and R^2 of 0.92).

A really encouraging outcome came to light with the results for predicting digestible dry matter intake (DDMI). An equation based on samples from 77 different forage diets gave surprisingly good calibration statistics with SEC of 0.6 g/kgLW.d and R^2 of 0.97.

Another notable achievement concerned the work conducted by Jim Gibbs in his PhD project where he demonstrated that faecal NIRS was effective in reliably predicting dietary CP and digestibility of diets consisting of hay with protein meal or cereal grain supplements. Calibration equations were developed for predicting these attributes for the forage component only (in mixed diets) or the total diet of forage plus supplement. This work will be described in detail in the PhD thesis and in subsequent journal papers.

Industry benefits and future research needs

There is a range of industry benefits arising from the work, both direct and indirect. The most obvious benefit is more efficient and cost effective beef production arising from improved nutritional management of grazing cattle. In this respect the role of faecal NIRS is as a decision support tool to help producers to decide when, what and how much supplement to feed cattle to meet production targets or goals in the most cost effective way. However, such decisions, if they are to be cost-effective, also require other technologies that can be designated, for want of more precise terminology, as “nutritional modeling” and “responses to supplements”. My assessment is that these technologies require substantial improvement if the full potential of faecal NIRS is to be captured. There are other possible benefits to industry but perhaps one of the most important is the unquantifiable benefit arising from faecal NIRS as an educational tool. Regardless of one’s expertise or knowledge, faecal NIRS can provide nutritional information to producers, extension personnel, consultants and scientists that cannot be acquired by alternative means.

A few industry members began using the technology in 1999 and the numbers have increased steadily year by year so that the total number of registered clients (by no means all current) now stands at over 650. I cannot comment on the benefits accruing to those who have submitted samples for analysis but feedback has generally been positive and many clients continue to submit samples on a continuing basis. There is also an increasing trend for stock feed (supplement) merchants to use the technology on behalf of their clients. I envisage that the benefits arising from faecal NIRS will be severely limited until the supporting technologies nominated above are improved. However, in the longer term everyone involved in the grazing industry will benefit from the technology – producers, extension staff, consultants, scientists and educators and hopefully consumers.

Faecal NIRS has now been accepted by most people as a legitimate technology for providing beneficial applications in both production and research. At the same time limitations to the technology must also be recognized, especially limitations with respect to accuracy. There is a need to balance faecal NIRS predictions against other critical observations and experience. NAP3.121 has successfully improved the predictive reliability of the suite of calibration equations as well as identifying problem areas, especially regarding predictions of growth rate but also in respect of diet quality predictions for specific pasture types. Future research is needed to address identified problem areas such as diets with high browse content, diets with a high proportion of non-leguminous forbs, and spinifex diets. Cattle breed effects on faecal NIRS predictions also need to be clarified and, if necessary, appropriate adjustments recommended. Finally, there is an urgent need for research to improve the supporting technologies and some specific areas of nutritional research (eg. browse and tannins) without which faecal NIRS cannot be properly exploited.

MAIN RESEARCH REPORT

Background and industry context

The importance of being able to quantify the diet quality of free grazing cattle is self-evident in the context of the nutritional management of grazing cattle. However, until relatively recently, the reliable estimation of diet quality proved to be either both difficult and costly at best or, in most situations, virtually impossible. The problems are due to the combination of pasture heterogeneity and selective grazing. In all but very intensive systems, trying to estimate diet quality from pasture measurements has been singularly unreliable. Fortunately a technological breakthrough in estimating the diet quality of free grazing cattle was made by a research team lead by Dr Jerry Stuth of Texas A&M University. Stuth and his team demonstrated that the crude protein (CP) and digestibility levels of the diets of grazing cattle could be reliably predicted using near infrared reflectance spectroscopy (NIRS) on faecal samples (Lyons and Stuth 1992).

In 1995 MLA provided funding support via Project CS.253 to develop faecal NIRS technology for application in the cattle industry of northern Australia and calibration equations were developed to predict dietary CP, digestibility and the proportion of non-grass in the diet as calculated from faecal $\delta^{13}C$. The calibration equation statistics (Final Report Project CS.253) clearly indicated the potential of the technology for beneficial commercial and research use but the 164 diets represented in the calibration sets for predicting dietary CP and digestibility were insufficient in both number and diversity to form the basis of a robust commercial technology.

Most of the diets sampled in CS.253 were from grazed pastures at sites in north-eastern Queensland (the CSIRO Lansdown Research Station south of Townsville, CSIRO research sites at Hillgrove Station north of Charters Towers and Cardigan Station south of Charters Towers, and the QDPI research site at Springmount west of Mareeba). In addition there were pen experiments where groups of cattle were fed a total of 54 different forage hays. Doubling the number of diets represented in the calibration sets for diet quality prediction, and increasing the diversity of diets with respect to pasture type and geographical origin, were deemed necessary for the development of robust calibration equations capable of providing reliable predictions on samples sourced across northern Australia.

Faecal samples in the calibration sets generated in CS.253 were intentionally restricted to those from unsupplemented cattle on roughage diets. When the resultant calibration equations were applied to faeces from cattle receiving protein and/or energy supplements, the effect on the predictions was variable depending on the type and intake of supplement. In general there was negligible effect of conventional urea based supplements fed as blocks or dry lick on the faecal NIRS predictions, i.e. the predictions remained the same as those from unsupplemented cattle consuming the same forage. Where cattle received significant amounts of true protein supplement such as cotton seed meal or fish meal, the effect on the dietary CP and digestibility predictions was still quite small, i.e. the predictions still related primarily to the forage component of the diet. Prior to NAP3.121 there was no information on the effect of energy supplements such as cereal grain, molasses and high lipid supplements on the faecal NIRS predictions. There was, therefore, an obvious need to further develop the faecal NIRS technology to cater for cattle receiving different types and amounts of supplement.

In addition to CS.253, MLA also provided some funding support to develop faecal NIRS calibration equations for predicting growth rate in cattle (NAP3.116). The outcome of NAP3.116 was similar to that of CS.253 in that the potential of the technology was established but additional work was needed to develop robust calibration equations that could be applied with sufficient confidence in the predictions to be of practical benefit to producers.

Initial, in house calibration equations for predicting faecal N concentration were developed by CSIRO without external funding support and the faecal N calibration set also needed to be expanded.

In terms of industry context, a range of on-farm applications of the technology has been described already (Coates 2000). Briefly these applications relate mainly to the nutritional management of grazing cattle (supplementation decisions, grazing management, husbandry such as timely weaning decisions), nutritionally governed marketing decisions, and resource monitoring and education (nutritional profiling and the influence of climate, season, soil type, stage of growth and botanical composition of pasture, fire and stocking rate on diet quality at paddock, property and regional scales). Research applications of the technology offer huge potential benefits to industry by providing scope for improved efficiency of research efforts and greatly increased scope for making progress in many areas of research.

With this background in mind, the primary focus of NAP3.121 was to improve the predictive reliability and utility of existing calibration equations by increasing the number and diversity of diets and the number and diversity of cattle represented in the calibration sets. The purpose of the project was to provide a powerful decision-support and educational technology for the northern beef cattle grazing industry as well as a powerful research tool for rangeland scientists.

Project objectives

Project objectives as stated in the contractual agreement between MLA and CSIRO were as follows:

"By December 2003, to improve the reliability of calibration equations so that dietary crude protein, dietary digestibility, dietary NDF (Neutral Detergent Fibre) and ADF (Acid Detergent Fibre), DOMI (Digestible Organic Matter Intake), dietary non-grass content, faecal N, faecal P and growth rate can be accurately assessed.

"The more general objective is to deliver a technology for the prediction of diet quality and animal performance as a decision support tool for the management of grazing cattle, but particularly as a key component of an integrated package for the nutritional management of grazing cattle."

It should be noted that:

(i) There were no existing calibration equations for predicting dietary NDF, dietary ADF and faecal P prior to NAP3.121.
(ii) Faecal P was added to the list of attributes at the request of MLA though earlier investigations had revealed that the development of a calibration equation for reliably predicting faecal P concentration was an unlikely outcome.
(iii) Results will be presented as DDMI (Digestible Dry Matter Intake) rather than DOMI because a calibration equation for DDMI was presented in the report for CS.253.

Methodology

Diet quality attributes (CP, digestibility, NDF, ADF and DDMI)

Basis of methodology

Faecal NIRS for predicting diet quality relies on the development of calibration equations incorporating data from experiments where diet quality can be measured so that appropriate reference values can be related to faecal NIR spectra. In the earlier project, CS.353, approximately two thirds of the diets were from pastures grazed by (i) small groups (3-5 head) of cattle from which the faecal samples were obtained, and (ii) oesophageal fistulated (OF) steers. The reference values for diet quality were determined by analysis of the extrusa samples collected from the OF steers but with appropriate adjustments to ensure, as far as possible, that the reference values were a valid estimate of the diet of the resident cattle (Coates 1999). The remaining one third were hay diets fed to cattle in pens. Results

from CS.253 clearly indicated that dietary reference values were determined more accurately in pen feeding experiments than from the grazed pasture trials and this was revealed in the calibration statistics where SEC and SECV (Standard Error of Calibration and Standard Error of Cross Validation) values were significantly lower for the calibration equations derived from the pen fed cattle.

Pen feeding experiments

Because of the problems of obtaining accurate dietary reference values from the grazed pasture trials, the emphasis in NAP3.121 was placed on pen feeding experiments. Moreover, in an effort to simulate the diets of grazing cattle, the emphasis was also placed on feeding pasture harvested direct from the paddock (fresh forage trials) rather than feeding hay. The basic requirements of the fresh forage trials were (i) to make every effort to maintain diet uniformity with respect to quality and botanical composition during the feeding period, and (ii) to maintain the feeding period long enough for the faeces to reliably represent the diet being fed.

With respect to requirement (i) it was necessary to select swards of uniform botanical composition and stage of growth. Obviously the weather conditions, especially rain, during trials could result in significant changes in diet quality with the risk that trials might have to be aborted before completion. Fortunately this occurred at only one site and on two occasions. Sub-sampling and analysis of all batches of harvested forage enabled any changes in diet quality from day to day to be monitored. Pasture with high moisture content was harvested and fed twice daily to maintain the integrity of the diet. In most of the pen experiments cattle were fed twice daily regardless of whether the feed was green or dry and the amount offered was in excess of voluntary intake (10-20% refusal rate).

With respect to requirement (ii), the minimum duration needed for faeces to equilibrate with the pen-fed diet was determined in a ten-day trial at Lansdown Research Station. Changes in faecal composition were monitored by daily sampling and NIRS analysis of faeces using existing equations. Faecal NIRS predictions levelled out after 3-4 days on the pen-fed diet. The feed in this trial was green and physiologically young (early wet season growth) so that rate of passage would have been quite fast relative to dry, mature tropical forage. Therefore, a minimum of 5 days pen feeding was deemed as the minimum duration for the pen feeding experiments. In practice, the diets were fed for more than 5 days in many of the trials. Regardless of the duration, faecal samples were collected daily to ensure that only spectra from samples collected after equilibration with the pen-fed diet were used in the calibration sets.

DM intake (DMI) measurements were made in pen feeding experiments conducted at Lansdown and Swans Lagoon. This involved weighing the amount of feed offered and the amount of feed not eaten (refusals); subsampling feed offered both morning and afternoon for dry matter determination (DM); subsampling refusals for DM determinations; and recording animal liveweights.

All short term pen feeding experiments provided reference values for dietary N (CP = 6.25N) and pepsin-cellulase *in vitro* DM digestibility of feed. The trials at Lansdown and Swans Lagoon also provided reference values for voluntary DMI expressed as gDM/kgLW.day.

During the course of the 3-year project pen feeding experiments were conducted at the following locations (number diets in brackets):

Lansdown Research Station near Townsville (46)

Swans Lagoon Research Station, Millaroo (22)

Janibee Station, Capella (2)

Penrose Station, Comet (1)

Brian Pastures Research Station, Gayndah (2)

Mt Cotton Research Farm, Brisbane (13)

Croxdale Station, Charleville (4)

Katherine Research Station, Katherine (23)

Brunchilly Station, Tennant Creek (13)

Camden Park, Bowral (2)

Total pen fed diets (128)

The diets fed are detailed in Appendix 1

Grazed pasture

Despite the perceived deficiencies of the OF sampling technique for obtaining dietary reference values, this technique is still useful and some of the targeted number of diets acquired during the course of NAP3.121 were obtained using this technique. OF sampling was confined to Brian Pastures where a team of OF steers was available. The majority of diets sampled were obtained from two pasture types, native speargrass (*Heteropogon contortus*) pasture and a bluegrass (*Bothriochloa insculpta*) pasture. The OF steers were alternated between the pasture types every fortnight and extrusa and faecal samples were collected at the end of each fortnight. Sampling continued for a full year. Other OF samplings were conducted as part of a PhD project investigating the efficiency of microbial protein synthesis in cattle grazing a range of tropical pastures (Maree Bowen, University of Queensland). In these samplings oesophageal extrusa samples were collected for estimating diet quality and faecal samples were obtained from rumen fistulated (RF) as well as the OF steers. In all, 32 different grazed pasture diets were sampled.

The OF trials provided reference values for dietary N and pepsin-cellulase *in vitro* DM digestibility of extrusa. The *in vitro* digestibility of extrusa differs from that of the feed due to the action of saliva (See Final Report CS.253).

In vivo digestibility experiments

As part of NAP3.121, funding was provided in support of a PhD project conducted by Mr Jim Gibbs at the Queensland University. A major part of the PhD project was to develop faecal NIRS calibration equations that could be applied to cattle consuming roughage diets supplemented with protein meal or cereal grain supplements for predicting the quality of both the entire diet (forage plus supplement) or the forage component only. The data were generated from hundreds of *in vivo* digestibility trials where cattle were fed 10 different grass hays alone and in combination with different amounts of either sorghum grain, or barley grain or cotton seed meal. The hays were also fed with a single rate of urea as a non-protein source of supplementary N. The project will be fully described in the PhD thesis but the results relating to straight forage diets also contribute to this report.

The *in vivo* digestibility trials provided reference values for dietary N, *in vivo* DM digestibility, pepsin-cellulase *in vitro* DM digestibility of the feed, DMI and DDMI.

Faecal attributes (faecal N, faecal P, and faecal $\delta^{13}C$)

Faecal N, faecal P, and faecal $\delta^{13}C$ are measured on the faeces themselves. Therefore, developing calibration equations is not dependent on specially designed experiments. Any faecal sample can be used in the calibration set, the only requirement being the scanning of the dried and milled sample to provide the NIRS spectrum and the laboratory analysis of the sample to determine the relevant reference value. However, for the resultant calibration equation to be sufficiently robust for widespread use the calibration set needs to be properly constructed and balanced with respect to the range of reference values and the diversity of samples. Samples for expanding existing calibration sets were therefore carefully selected from thousands of faecal samples so as to represent different pasture types, different diet qualities and diet compositions, different geographical locations, different seasons and different years. Reference faecal $\delta^{13}C$ values were determined by mass spectrometric analysis at Central Queensland University. Reference N concentrations were determined either by Kjeldahl digestion followed by colorimetric determination of N concentration at the CSIRO Long Pocket Laboratories or by the Dumas combustion method at Central Queensland University. Reference P concentrations were determined by Kjeldahl digestion followed by colorimetric determination of P concentration.

Growth rate or average daily gain (ADG)

Calibration equations for predicting growth rate require measured growth rate to be paired with faecal spectra. The difficulty is to accurately determine the growth rate that is valid for a specific point in time rather than the average growth rate over an interval between successive weighings. In fact, the change in liveweight between successive weighings may often be a very poor measure of what we mean by growth rate where growth rate implies tissue accretion (positive growth rate) or loss (negative growth rate). We know that short term changes in gut fill and/or body water content can override changes in tissue accretion and so result in erroneous measures of growth rate that are based simply on weight changes.

Monitor herds of growing cattle, either steers or heifers, were established at a range of sites and on different pasture types (Table 1). Stocking rates were conservative at all sites except for intentional, heavily stocked treatments at Swans Lagoon, so that growth rate would not be limited by low amounts of pasture DM except in severe drought. Sites were chosen so that specific mineral deficiencies such as P, S or Na, would not depress growth rate. Possible mineral deficiencies were remedied by supplementation (eg. S at Fletcherview). Apart from routine vaccination against clostridial diseases, no specific measures were taken to control diseases and parasites. However, regular inspection and handling of the cattle indicated that diseases and/or parasites were unlikely to have depressed growth rate to a significant extent.

At some sites more than one age group of cattle were present at the one time. This made it possible to obtain data on the influence of age/liveweight on growth performance. Herds at most sites were maintained for a minimum of 12 months and, if possible, data sets were obtained in annual increments. However, drought caused data acquisition to be terminated prematurely in some of the herds during 2002 and 2003. The preferred experimental protocol was to muster, weigh and faecal sample the cattle at 4-week or monthly intervals and to collect faecal samples (bulked within herd) from the paddock mid-way between successive weighings but there was some variation between sites with respect to weighing and faecal sampling intervals. Some (eg. the oldest) or all of the cattle within a paddock were replaced annually with young animals. Cumulative liveweight change curves (herd average) were plotted for each herd and growth rates for pairing with faecal samples were estimated from these curves. In some instances samples and data could not be accepted for adding to the calibration set because valid estimates of growth rate could not be calculated due to liveweight being confounded by changes in gut fill.

Table 1. Location and pasture type of monitor herds established for developing faecal NIRS calibration equations to predict growth rate in cattle.

Site	Pasture type
Lansdown, Townsville	Urochloa/stylo and buffel/stylo
Swans Lagoon, Millaroo	Predominantly native pasture
Forest Home, Mingela	Indian couch
Fletcherview, Charters Towers	Buffel grass
Wambiana, Charters Towers	Native pasture
Berrigurra, Comet	Buffel grass
Research Station, Alice Springs	Predominantly buffel grass
Rosebank, Longreach	Mitchell grass downs
Toorak, Julia Creek	Mitchell grass downs
Morungle, Richmond	Predominantly Mitchell grass

Calibration procedures

Calibration was carried out with ISI software (Infrasoft International) and was based on modified partial least squares regression (MPLS) (Shenk and Westerhaus 1991), wavelengths 700-2500 nm, standard normal variates (SNV) with detrend (Naes et al. 2002) for noise and scatter correction, and both first and second derivatives of the log 1/R reflectance measurements (1,4,4,1 and 2,4,4,1).

Results and discussion

Dietary attributes

Dietary N

In all, 578 faecal spectra suitable for inclusion in the calibration set for predicting dietary N were obtained from 160 different diets sampled in the course of NAP3.121. Reference values for dietary N ranged from 0.24 – 4.39% (1.5 – 27.4%CP). However, diet N was in the low range of 0 – 1% for over 70% of the samples and there were few diets with high N concentrations (Table 2). Forage harvesting pasture for pen feeding was the main cause of the very high proportion of low N diets. It was clear that the quality of forage-harvested material was much lower than that of diets selected by grazing cattle. This was due to a higher proportion of stem and lower proportion of non-grass fractions in harvested forage compared with diets selected by grazing animals. The N content of forage-harvested pasture rarely exceeded 1%. However, in some of the pen feeding experiments diet quality was increased by adding hand harvested material such as Leucaena (*Leucaena leucocephala*) leaf or high quality hay such as lucerne. Differences between mechanically harvested forage and the diets selected by cattle grazing the same pasture are well illustrated by data from Brunchilly Station on the Barkly (Table 3)

In the earlier project, CS.253, where most of the diet-faecal pairs were derived from grazed pasture, diet quality was noticeably higher than for the mechanically harvested diets fed in NAP3.121 (Table 2). When diet faecal pairs from CS.253 were combined with those from NAP3.121, the distribution of samples with

respect to dietary N was still weighted towards diets of low N concentration but not as severely as the sample set from NAP3.121 alone.

Table 2 Distribution of reference diet N concentrations for samples (spectra) in the calibration set.

	Number of samples			Percentage of samples		
Diet N%	CS.253	NAP3.121	Total	CS.253	NAP3.121	Total
0 – 0.49	30	85	115	5	15	9
0.50 – 0.99	190	313	503	30	54	41
1.00 – 1.49	219	106	325	34	18	27
1.50 – 1.99	95	25	120	15	4	10
2.00 – 2.49	81	17	98	13	3	8
2.50 – 2.99	14	6	20	2	1	2
>3.00	14	26	40	2	5	3

Table 3. Crude protein concentration in diets selected by grazing cattle and in forage harvested feed from the same pastures at Brunchilly Station. The protein in selected diets and non-grass proportions of both selected diets and forage harvested material are faecal NIRS predictions. The protein in forage harvested feed was determined by chemical analysis.

Trial and date	Pasture type	Grazed diets		Forage harvested diets	
		CP%	Non-grass%	CP%	Non-grass%
Trial 1 Aug 2000	Flinders grass	10.0	60	4.6	<10
Trial 2 Oct 2000	Mitchell grass	9.4	50	3.3	<10
	Flinders grass	11.1	66	3.4	14
Trial 3 Dec 2000	Flinders grass	10.0	34	4.7	11
Trial 4 Apr 2001	Mitchell grass	8.9	54	5.7	25
	Flinders grass	9.3	42	4.4	8
Trial 5 Jul 2001	Mitchell grass	11.5	84	4.1	18
	Flinders grass	9.0	53	4.1	14
Trial 6 Mar 2002	Weeping Mitchell	11.9	44	6.3	3
	Bull Mitchell	13.1	59	6.8	13
	Barley Mitchell	10.5	49	6.8	8

New calibration equations were developed on the combined calibration set of samples representing over 300 different forage diets. Calibration equation statistics are presented in Table 4.

Table 4. Faecal NIRS calibration equation[1] statistics for predicting dietary nitrogen concentration.

Equation	Math treat	No. samples	Range in N%	SEC	SECV	R^2
DNIT1441.EQA	1,4,4,1	1202	0.24 – 4.39	0.165	0.172	0.949
DNIT2441.EQA	2,4,4,1	1203	0.24 – 4.39	0.157	0.163	0.954

[1] MPLS, SNV & detrend, wavelengths 700 – 2500

From the results in Table 4 it can be seen that there was a small advantage in using the second derivative over the first derivative with slightly lower SEC and SECV and slightly higher R^2. However, it is doubtful whether there would be any practical advantage of one equation over the other when predicting diet N from samples outside the calibration set.

When residuals (Reference diet N – predicted diet N) were plotted against reference diet N there was evidence that predictions tended to be over-estimated on average at low diet N (preponderance of negative residuals) and under-estimated on average at high diet N (preponderance of positive residuals) (Fig.1). In fact, mean residual was indeed negative when diet N was ≤ 1%, mean residual was close to zero when diet N was between 1-2%, and mean residual became increasingly more positive as diet N rose above 2%.

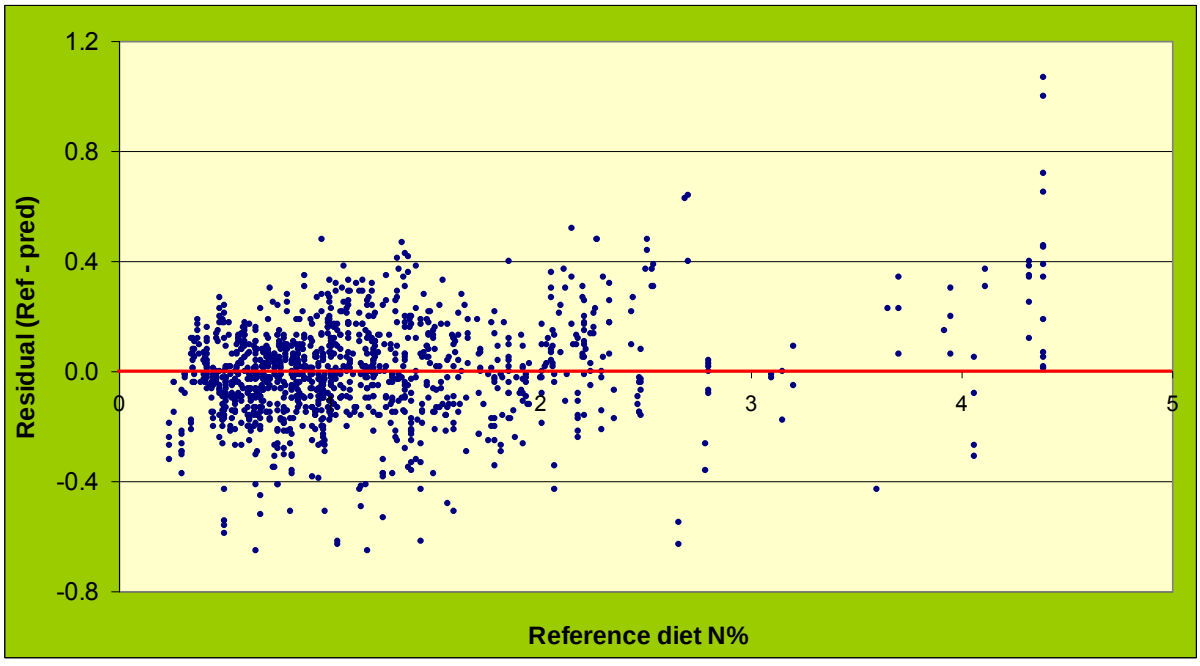

Figure 1. Residuals (Reference diet N – Predicted diet N) plotted against reference diet N for samples in the calibration set for predicting diet N; predictions were made with equation DNIT1441.EQA.

To overcome bias at low and high diet N concentrations the calibration set was segmented into 3 subsets based on reference values: a subset with diet N up to 2% (low N); a subset with diet N between 1.5 and 2.5% (medium N); and a subset with diet N of 1.5% and over (medium-high N). Separate calibration

equations were developed for each subset. Statistics for the equations developed on first derivative spectra are presented in Table 5.

Table 5. Faecal NIRS calibration equation[1] statistics of partial equations developed on subsets of the full calibration set of samples.

Equation	No. samples	Range in N%	SEC	SECV
LNIT1441.EQA	1050	0.24 – 2.00	0.134	0.136
MNIT1441.EQA	218	1.49 – 2.51	0.139	0.152
M-HN1441.EQA	273	1.49 – 4.39	0.161	0.182

[1] MPLS, SNV & detrend, wavelengths 700 – 2500

SEC values were improved for samples with low and medium diet N and bias was largely eliminated for both low and high N samples, thus indicating an advantage in favour of the partial equations compared with the universal equation.

From a practical standpoint, predictive accuracy can also be gauged by the distribution of samples in relation to the difference between predicted and reference N. Within the calibration set there was a clear improvement using the partial equations compared with the single, universal equation. This is clearly illustrated in Table 6 which shows the proportion of samples where the difference between predicted and reference value was > 0.16%N (i.e. >1%CP). The differences between the partial and universal equations in predictive accuracy demonstrated on samples in the calibration set would presumably be of equivalent magnitude in the open population. Therefore, it may be concluded that the partial equations offer a significant advantage over the universal equation. When faecal NIRS is used as a decision support tool, predictive accuracy is of particular importance when dietary protein levels are low. In the dietary range of 0 – 6.2% CP (0 – 1%N), it was pleasing to note that predicted diet CP% was within 1%CP of reference CP for 86% of samples.

Table 6. The effect of partial equations versus universal equation on the proportion of samples where the difference between predicted and reference N exceeded 0.16%N.

		Proportion (%) of samples where difference between predicted and reference N > 0.16	
Reference N%	No. samples	Universal equation	Partial equations
0.00 – 1.00	630	23	14[a]
1.01 – 1.75	380	32	27[a]
1.76 – 2.25	120	32	17[b]
>2.25	90	58	34[c]

[a] predicted with LNIT1441.EQA; [b] predicted with MNIT1441.EQA; [c] predicted with M-HN1441.EQA

In theory, reference values are meant to be error free. This does not occur in practice and it has been shown that when significant random error occurs in reference values, NIRS predictions can, in fact, be more accurate than the reference method (Coates 2002a). Making accurate determinations of dietary N concentration for cattle fed roughage diets is very difficult and errors can be quite large. It is highly probable, therefore, that the measures of predictive performance presented in the various tables above, under-estimate the true predictive performance of the calibration equations.

Nevertheless, for a proportion of samples, the difference between predicted and reference diet N was

unacceptably large and could not be attributed simply to errors in the reference value. For example, the difference between predicted and reference value was greater than 0.24%N (1.5% CP) in 5% of samples with diet N up to 1% and the proportion was 10% of samples with diet N in the range of 1.01 – 1.75%N. The proportion increased as diet N concentration increased. The sample set was therefore closely inspected in an attempt to determine whether specific types of diet may have been associated with large prediction errors.

Large prediction errors, either under- or over-prediction, did not appear to be consistently associated with specific forage types except perhaps for forage sorghum diets where dietary N was substantially over-predicted. However, there were only 2 forage sorghum diets represented by 8 spectra in the entire calibration set so no firm conclusion could be made. What did become apparent, however, was that the probability of severe under-prediction (reference N – predicted N > 0.16%N) on faecal samples from low N diets (0 – 1%N) was very low at 3% whereas the probability of severe over-prediction was much higher at 10%. Conversely, the probability of severe over-prediction on samples from high N diets (> 2%N) was very low at 6% whereas the probability of severe under-prediction was quite high at 20%. An understanding of the probabilities of severe under- or over-prediction of dietary N from faecal NIRS is important with respect to using the technology as a decision support tool such as making decisions on the need to commence N supplementation. The above probabilities indicate a greater risk of over-estimating rather than under-estimating dietary N in protein deficient diets and therefore a greater risk of deferring supplementation rather than supplementing prematurely.

Another cause of prediction error was revealed in diets composed of fractions with contrasting quality attributes. Various composite diets consisting of poor quality grass hay mixed with some high quality plant material (eg. lucerne or green oaten hay) were fed in some of the pen trials and faecal NIRS often under-predicted dietary N in these composite diets (Table 7).

While under-prediction may not be initially obvious from the data in Table 7 for the lucerne containing diets in Trial C, this was due to diet N being seriously over-predicted for the native pasture control diet. Regarding all the mixed diets represented in Table 7, under-prediction was observed in those containing high quality lucerne hay, green oaten hay, and flowers of *Albizia lebbek* but not in the diets containing leaves of *Carissa lanceolata* or Cavalcade hay. Under-prediction of diet N in the mixed diets occurred where there were large differences in digestibility between the dietary components. The digestibilitities of the lucerne hay, green oaten hay and flowers of *Albizia lebbek* were known to be much higher than the digestibility of the basal grass component (at least 25 percentage units higher) whereas the difference was much less for Calvalcade and minimal for the *Carissa* leaves. Thus, faecal proportions of the highly digestible components would have been significantly less than the dietary proportions and this probably resulted in the observed under-prediction of diet N.

The question poses itself whether similar mixed diets consisting of fractions of widely different N concentration and digestibility may occur naturally. Two examples readily come to mind: (i) where the diet consists of old season dry grass mixed with new, green leaf such as often occurs at the break of the growing season, and (ii) where the diet consists of mature C_4 grass together with high quality “herbage” such as in common across the Mitchell grass black soil downs. In both cases one needs to be aware that faecal NIRS is likely to under-estimate diet N.

Table 7. Faecal NIRS predictions and reference diet N for diets consisting of poor quality tropical grass mixed with high quality plant material.

Diet	Ref. N% basal forage	Ref. N% total diet	Predicted diet N%
Trial A			
Native pasture (NP) hay	0.40	0.40	0.40
NP + 20% green oaten hay	0.40	1.09	0.81
NP + 20% Albizia flowers[1]	0.40	0.85	0.63
NP + 20% Carissa leaf[2]	0.40	0.55	0.58
Trial B			
NP hay	0.36	0.36	0.39
NP + lucerne hay	0.36	1.33	0.91
NP + green oaten hay	0.36	1.31	1.06
NP + Cavalcade hay[3]	0.36	0.86	0.88
Trial C			
NP fresh forage	0.30	0.30	0.59
NP + 6% lucerne	0.30	0.50	0.68
NP + 12% lucerne	0.30	0.69	0.76
NP + 19% lucerne	0.30	0.95	0.94
NP + 34% lucerne	0.30	1.42	1.33
NP + 56% lucerne	0.30	2.16	1.94

[1] flowers of *Albizia lebbek*

[2] *Carissa lanceolata*

[3] *Centrisema pascuorum* cv. Cavalcade

Digestibility of the diet

In vivo digestibility

In CS.253, reference data and faecal samples were collected form *in vivo* digestibility trials involving 51 different hay diets. Reference data and faecal samples were obtained from an additional 27 hay diets in the course of NAP3.121 and the samples from CS.253 and NAP3.121 were combined into an expanded calibration set for recalibration. There was no discernable advantage in using math treatment 2,4,4,1 compared with 1,4,4,1. Calibration statistics for the original CS.253 equation, together with those for the expanded set are shown in Table 8

Table 8. Faecal NIRS calibration equation[1] statistics for predicting *in vivo* dry matter digestibility. Statistics for the equation developed in project CS.253 are shown together with the expanded calibration set derived by combining samples from 27 new diets with those from CS.253. Reference *in vivo* digestibility values were those determined for individual animals.

Source	No. diets	No. samples	Range (%)	SEC	SECV	R^2
CS.253	51	187	37 - 73	2.5	2.9	0.89
CS.253 & NAP3.121	78	313	31 - 85	3.9	4.1	0.80

[1] MPLS, SNV & detrend, 1-4-4-1, wavelengths 700 – 2500

The results in Table 8 show a significant deterioration in SEC, SECV and R^2 as a consequence of expanding the calibration set. An inspection of the measured (reference) and predicted values for individual samples, each representing an individual animal, revealed the probable cause of the relatively poor calibration statistics of the new equation. In most of the trials four animals were fed the same diet but the number varied between 2 and 10. For a significant proportion of the diets, between animal variation of measured *in vivo* DMD was surprisingly large, as high as 14 percentage units and more than 10 percentage units in 11 of the 77 diets. On the other hand, between animal variation in predicted digestibility was quite small and exceeded 4 percentage units in only 11 of the 77 diets (maximum variation was 7 units) and was under 3 percentage units for two thirds of the diets. Moreover, predicted digestibility was not correlated with measured digestibility within individual diets. It was clear, therefore, that the faecal NIRS equation could not predict animal differences in the *in vivo* digestibility and that predictions were related to the feed characteristics as “seen” in the undigested residues.

While the results showed that faecal NIRS was unable to predict *in vivo* digestibility differences between animals consuming the same diet, the small between-animal variation in predicted digestibility indicated that faecal NIRS was better suited to predicting mean *in vivo* digestibility of roughage diets. Therefore, mean rather than individual *in vivo* digestibilities would be more appropriate to use as reference values. Accordingly, the reference values were amended and, two atypical diets, a very stemmy Setaria hay and a low quality native pasture hay with 20% dry *Albizia lebbek* leaves, were removed from the calibration set on the basis that their inclusion would harm rather than improve predictive reliability. Recalibration was performed with much improved calibration statistics (Table 9).

Table 9. Faecal NIRS calibration equation[1] statistics for predicting *in vivo* dry matter digestibility. Reference *in vivo* digestibility values were the means for the different diets.

Source	No. diets	No. samples	Range (%)	SEC	SECV	R^2
CS.253 &NAP3.121	75	295	42 - 72	1.72	2.17	0.95

[1] MPLS, SNV & detrend, 2-4-4-1, wavelengths 700 – 2500

The use of mean rather than individual animal measures of *in vivo* digestibility has important consequences regarding the determination of reference values. If digestibility determinations for individual animals were appropriate reference values, then the number of animals fed a specific diet would be of no concern; determinations from say two or even one animal would be acceptable as valid reference values. However, if reference values are to represent the mean digestibility of a diet, the accuracy will clearly depend on the number of animals contributing to the mean. Because of the large between-animal variation in the *in vivo* digestibilities of many roughage diets, a minimum of four animals per diet, and preferably more, would seem to be necessary for deriving valid reference values. Reference values for approximately one third of the diets represented in the above calibration set were the means of less than 4 determinations. Therefore, the calibration statistics presented in Table 9 were probably adversely affected by inaccurate reference values. Nevertheless, the results of the calibration are very acceptable.

In vitro digestibility of the feed

Reference values for the pepsin-cellulase *in vitro* digestibility (measured simply as dry matter disappearance) were derived for all pen feeding experiments representing 187 different forage diets. The calibration statistics were most acceptable (Table 10).

Table 10. Faecal NIRS calibration equation[1] statistics for predicting *in vitro* dry matter disappearance of dietary forage.

Source	No. diets	No. samples	Range (%)	SEC	SECV	R^2
CS.253 &NAP3.121	187	633	16 - 86	2.8	3.2	0.95

[1] MPLS, SNV & detrend, 2-4-4-1, wavelengths 700 – 2500

The lower SEC and SECV values for predicting *in vivo* digestibility (Table 9) were quite unexpected. However, the larger calibration set for the *in vitro* equation, twice the size with respect to both sample numbers and the diversity of diets, probably contributed to the higher SEC and SECV values for the *in vitro* calibration. Therefore, a separate calibration was developed on the same set of samples used for the *in vivo* calibration so that a direct comparison of calibration statistics could be made (Table 11).

Table 11. Faecal NIRS calibration equation[1] statistics for predicting *in vitro* dry matter disappearance of dietary forage using the same set of samples used for the *in vivo* digestibility calibration (see Table 9).

Source	No. diets	No. samples	Range (%)	SEC	SECV	R^2
CS.253 & NAP3.121	75	293	27 - 83	2.3	3.0	0.97

[1] MPLS, SNV & detrend, 2-4-4-1, wavelengths 700 – 2500

Although SEC and SECV were still higher than for the *in vivo* calibration, the range of *in vitro* digestibilities was larger than for the *in vivo* digestibilites. The ratio of the range in values to the SEC, or SECV, provides a legitimate comparison and this revealed that the *in vitro* calibration was indeed superior to the *in vivo* calibration.

In vitro digestibility of the extrusa

Reference values for digestibility of the 32 grazed pasture diets sampled during the course of NAP3.121 were necessarily derived from the *in vitro* analysis of extrusa samples. The 125 faecal samples from these 32 diets were added to the calibration set generated in CS.253 and calibration was performed on the expanded calibration set (Table 12).

Table 12. Faecal NIRS calibration equation[1] statistics for predicting *in vitro* dry matter disappearance of dietary extrusa.

Source	Math treat.	No. diets	No. samples	Range (%)	SEC	SECV	R^2
CS.253	1,4,4,1	155	597	38 - 83	3.3	3.4	0.84
CS.253 & NAP3.121	1,4,4,1	187	717	38 - 83	3.5	3.6	0.85
CS.253 & NAP3.121	2,4,4,1	187	720	38 - 83	3.2	3.5	0.88

[1] MPLS, SNV & detrend, wavelengths 700 – 2500

There was little change in calibration equation statistics with the addition of the extra samples but there was some advantage in using the second derivative of faecal spectra compared with the first derivative. The ratio of the range of digestibility to the SEC or SECV indicated much better calibration statistics for *in vitro* digestibility of feed than for *in vitro* digestibility of extrusa (Table 13). The difference was probably due mainly to extrusa samples being less representative of the true diet than forage samples obtained from pen feeding experiments (Coates *et al*. 1987). This would result in larger errors in reference values for the dietary extrusa equation than for the dietary forage equation.

Table 13. The ratio of analyte range to the SEC and SECV for faecal NIRS calibrations[1] of (i) *in vitro* digestibility of forage eaten and (ii) *in vitro* digestibility of dietary extrusa.

Equation	No. samples	Range	SEC	SECV	Range/SEC	Range/SECV
Forage	633	70	2.8	3.2	25	22
Extrusa	720	45	3.2	3.5	14	13

[1] MPLS, SNV & detrend, 2-4-4-1, wavelengths 700 – 2500

Dry matter intake and digestible dry matter intake

Intake was measured for 73 different forage diets in NAP3.121 and the faecal samples with matching dry matter intake (DMI) reference values from these trials were added to the calibration set generated in CS.253. Conventional *in vivo* digestibility trials were carried out for 27 of the 73 forage diets and DDMI reference data from the *in vivo* pen trials were also combined with the CS.253 calibration set. Recalibration produced the results shown in Table 14.

Table 14. Faecal NIRS calibration equation[1] statistics for predicting dry matter intake (DMI) and digestible dry matter intake (DDMI) in cattle fed roughage diets.

Source	Math treat.	No. diets	No. samples	Range (%)	SEC	SECV	R^2
			DMI (g/kgLW.d)				
CS.253	1,4,4,1	44	189	7.3 – 29.5	1.80	2.00	0.79
CS.253 &NAP3.121	1,4,4,1	117	472	3.6 – 30.4	2.40	2.51	0.74
CS.253 & NAP3.121	2,4,4,1	117	472	3.3 – 30.4	2.17	2.42	0.79
			DDMI (g/kgLW.d)				
CS.253	1,4,4,1	43	183	3.7 – 20.1	1.03	1.16	0.89
CS.253 & NAP3.121	1,4,4,1	70	276	1.4 – 20.1	1.26	1.39	0.88
CS.253 & NAP3.121	2,4,4,1	70	276	1.4 – 20.1	1.27	1.42	0.88

[1] MPLS, SNV & detrend, wavelengths 700 – 2500

Expansion of the calibration sets resulted in an increase in SEC and SECV values for both DMI and DDMI. This was probably due partly to the increased diversity of diets and, in the case of DMI, to larger errors in the reference values for fresh forage trials where the duration of feeding was much shorter than in conventional *in vivo* digestibility trials.

In Table 14, SEC, SECV, R^2, Range/SEC and Range/SECV (values for Range/SEC and Range/SECV not shown) all indicate that calibration statistics were superior for the DDMI equations than for the DMI equations. That faecal NIRS is able to predict DDMI more accurately than DMI suggests that DDMI is more closely related to faecal spectral characteristics than is DMI. This may be because of the peculiar relationship between digestibility and DMI in tropical forages where forages of similar digestibility can have vastly different intakes and, conversely, feeds of similar intake can have vastly different digestibilities. This in turn is probably associated with the interaction between of mean residence time of digesta in the rumen with particle size reduction and rate of passage. Thus, while low DDMI in fibrous feeds is usually associated with low digestibility, it is sometimes associated with moderately high digestibility but very low intake due to slow rate of digestion, slow particle size reduction, and slow rate of passage. Whatever the underlying mechanism, it is fortuitous that faecal NIRS has the apparent ability to predict DDMI more accurately than DMI because DDMI integrates the measures of both digestibility and intake and provides the critical estimate of the intake of metabolisable energy.

It has already been pointed out that the evidence from this project indicates that faecal NIRS does not have the ability to predict *in vivo* digestibility differences between cattle consuming the same diet and that calibration statistics were markedly improved when the mean digestibilitiy values instead of individual

animal digestibilities were used as reference values (cf. Tables 8 and 9). An inspection of the relationship between reference and predicted values also revealed that faecal NIRS was unable to correctly identify between-animal differences within diets for DMI or DDMI. Therefore, calibration equations were developed using the diet means for DMI and DDMI as reference values (Table 15). The result was a dramatic improvement to calibration statistics. Those for the DDMI equations are extraordinarily good for a functional property. The plot of predicted DDMI (equation with math treatment 2,4,4,1) against diet mean reference values is shown in Fig. 2.

Table 15. Faecal NIRS calibration equation[1] statistics for predicting dry matter intake (DMI) and digestible dry matter intake (DDMI) in cattle fed roughage diets. Reference values were the means for the different diets.

Source	Math treat.	No. diets	No. samples	Range (%)	SEC	SECV	R^2
			DMI (g/kgLW.d)				
CS.253 &NAP3.121	1,4,4,1	117	471	4.2 – 28.6	1.92	1.98	0.81
CS.253 & NAP3.121	2,4,4,1	117	472	4.2 – 28.6	1.69	1.85	0.85
			DDMI (g/kgLW.d)				
CS.253 & NAP3.121	1,4,4,1	70	276	1.9 – 18.5	0.83	0.90	0.95
CS.253 & NAP3.121	2,4,4,1	70	276	1.9 – 18.5	0.59	0.75	0.97

[1] MPLS, SNV & detrend, wavelengths 700 – 2500

Although the statistics in Table 15 demonstrate the potential for faecal NIRS to accurately predict DDMI in cattle consuming roughage diets, the current equations are probably based on a calibration set that is far too small in terms of sample number and diet diversity to be considered robust for practical use. Nevertheless, the results are extremely encouraging and indicate that expansion of the calibration set is an objective well worth pursuing.

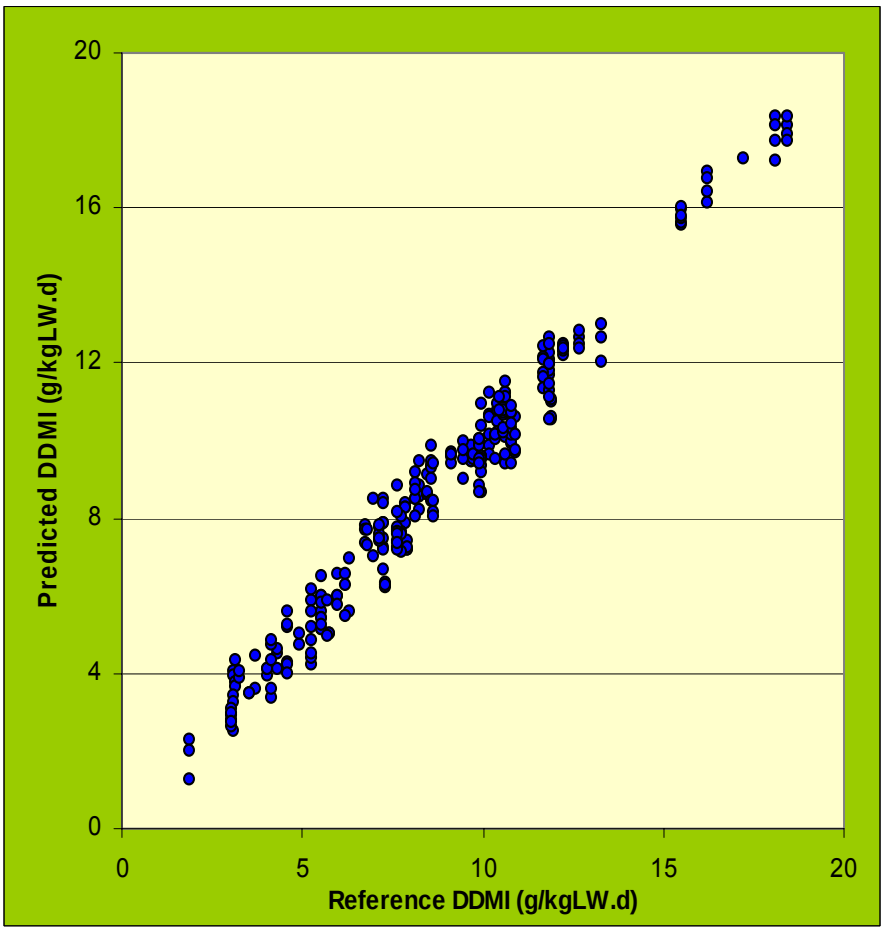

Figure 2. Plot of predicted digestible dry matter intake (DDMI) against reference DDMI (mean for each diet) for samples in the calibration set.

Faecal N concentration

The calibration set of samples for predicting faecal N concentration immediately prior to starting NAP3.121 contained approximately 550 faecal spectra with laboratory determined constituent values. Over 400 samples were added to the calibration set in stages during the course of the project and recalibrations were conducted on the expanded sets. Calibration equation statistics for pre-project and post-project equations are presented in Table 16.

Table 16. Calibration equation[1] statistics for the prediction of faecal N concentration

	No. of samples	Range	SEC	SECV	R^2
Pre-project	564	0.70 – 2.58	0.071	0.078	0.96
Post-project	987	0.70 – 3.16	0.078	0.080	0.96

[1] MPLS, SNV & detrend, 1-4-4-1, wavelengths 700 – 2500

There were only small increases in SEC and SECV values for the post-project equation compared with the pre-project equation despite the wider range in faecal N and the increased diversity of samples. Validation exercises were carried out prior to each of the four expansions of the calibration set. Validation SEP values were 0.07, 0.08, 0.13 and 0.14. The higher values were associated with sample types previously not represented in the calibration set such as high faecal N samples from cattle grazing improved, temperate grass/clover pastures. The post-project calibration equation for predicting faecal N is the most robust of all the faecal NIRS equations. Predictive accuracy would be almost comparable to standard chemical analysis.

There is a highly significant correlation between faecal N and dietary N concentrations. This is illustrated in Fig. 3 where diet N is plotted against faecal N using the samples contained in the calibration set for predicting diet N. The diet N values are laboratory determined reference values while the faecal N values are predictions from the post-project faecal N equation.

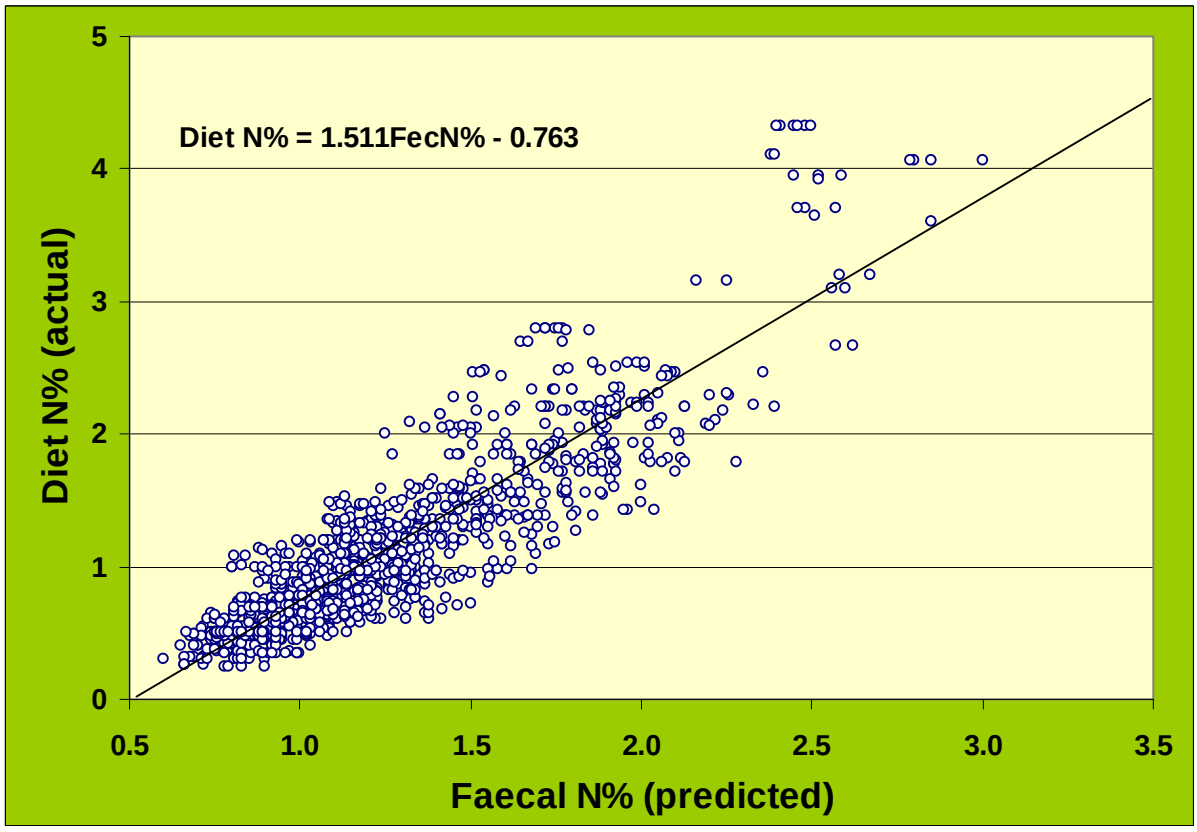

Figure 3. Relationship between diet N% and faecal N% using samples where cattle consumed diets of known N concentration (n = 1208)

Although the correlation is highly significant ($P < 0.0001$), the relationship is of limited use for predictive purposes. When diet N was regressed on faecal N using the data presented in Fig. 3, the SE of the regression was 0.31(%N). This is twice the SEC of the faecal NIRS equation for predicting diet N. Nevertheless, the regression relationship between diet N and faecal N predictions can be used as a guide to the validity of faecal NIRS predictions for diet N. Thus, the 95% probability range of diet N levels for any given faecal N prediction can be calculated from the linear regression equation and the SE of the regression (Table 17). If predicted diet N% lay outside the range then the reliability of the prediction would be questionable.

Table 17. Average and 95% confidence limits of diet N% for different faecal N% predictions.

	Diet N%	
Faecal N%	Average	Range
0.75	0.37	0.20 – 0.99
1.00	0.75	0.20 – 1.37
1.25	1.13	0.51 – 1.75
1.50	1.50	0.88 – 2.12
1.75	1.88	1.26 – 2.50
2.00	2.26	1.64 – 2.88
2.25	2.64	2.02 – 3.26
2.50	3.01	2.39 – 3.63

Faecal δ^{13}C and dietary non-grass proportions

The δ^{13}C of forages and faeces are negative values but this simply indicates that the proportion of δ^{13}C in plant material is less than that in the limestone deposit which forms the reference against which other substances are compared when determining δ^{13}C values. Therefore, for the sake of simplicity the negative sign has been ignored in the results and discussion which follow.

The calibration set of samples for predicting faecal δ^{13}C immediately prior to starting NAP3.121 contained just over 800 faecal spectra with laboratory determined constituent values. Almost 700 samples were added to the calibration set in stages during the course of the project and recalibrations were conducted on the enlarged sets. Calibration equation statistics for pre-project and staged expansions are presented in Table 18.

Table 18. Calibration equation[1] statistics for the prediction of faecal δ^{13}C

	No. of samples	Range	SEC	SECV	R^2
Pre-project	825	13.04 – 28.59	0.735	0.762	0.95
Stage 1	997	12.95 – 28.59	0.688	0.729	0.95
Stage 2	1058	12.86 – 28.59	0.689	0.726	0.95
Stage 3	1123	12.55 – 28.59	0.700	0.731	0.95
Stage 4	1314	12.43 – 28.35	0.760	0.795	0.94
Stage 5	1447	12.37 – 27.65	0.747	0.781	0.94
Post-project	1501	12.27 – 27.65	0.759	0.781	0.94

[1] MPLS, SNV & detrend, 1-4-4-1, wavelengths 700 – 2500

There were increases in SEC and SECV values for the post-project equation compared with the pre-project equation associated with the increased diversity of samples but the calibration statistics remained satisfactory. With the increased size and diversity of the calibration set the post-project calibration equation should be substantially more robust than the pre-project equation. Validation exercises were carried out prior to the staged expansions and validation statistics are presented in Table 19. The validation statistics are comparatively poor because the samples used were deliberately selected as being spectrally different from samples in the current calibration set at that time. For example, the 187 samples for the Stage 4 validation (Table 19) were samples from a Leucaena grazing experiment. The calibration set at that time had few samples from cattle grazing Leucaena and the results in Table 19 and Fig. 4a clearly show the poor accuracy of prediction using the then equation to predict faecal $\delta^{13}C$ on samples from cattle with Leucaena in their diets. The poor predictions were characterized by a large bias resulting in most predictions being overestimated and a poor coefficient of determination (R^2). When recalibration was done after adding these samples to the calibration set the new calibration equation was able to make reliable predictions on samples sourced from Leucaena diets (Fig 4b).

Table 19. Validation statistics for the prediction of faecal $\delta^{13}C$

	No. of samples	Range	SEP	Bias	Slope	R^2
Stage 1	182	13.29 – 25.94	2.15	-0.06	0.71	0.50
Stage 2	72	13.43 – 25.83	1.07	0.49	1.01	0.90
Stage 3	63	12.55 – 20.52	0.98	-0.77	0.87	0.89
Stage 4	187	12.51 – 23.71	1.84	-1.41	1.16	0.85
Stage 5	141	12.37 – 27.65	1.14	-0.55	0.92	0.91
Stage 6	22	12.27 – 19.81	0.83		0.86	0.82

The apparently poor R^2 (0.82) and slope (0.86) statistics for the Stage 6 validation were due to the limited range of faecal $\delta^{13}C$ reference values and the small number of samples respectively. The SEP, however, was pleasingly low at 0.83.

Samples in the calibration set were intentionally sourced from cattle consuming diets where all the grasses were C_4 species and all the non-grasses, except for sedges, were C_3 species. In terms of the relationship between faecal spectra and reference $\delta^{13}C$, the reference faecal $\delta^{13}C$ values were therefore simply measures or indices of either C_4 grass or C_3 non-grass concentrations in the faeces rather than measures of ^{12}C:^{13}C *per se*. This became apparent when the calibration equation was applied to the faeces of cattle eating temperate or C_3 grass (eg. ryegrass or oats). Predicted faecal $\delta^{13}C$ was around 16 units indicating that the relevant spectral characteristics were equivalent to faeces derived from a diet of about 80% C_4 grass and 20% C_3 non-grass. One might surmise that an equivalent situation would exist when cattle consumed a C_4 forb diet: predicted faecal $\delta^{13}C$ would probably be around 25 – 26 units, i.e. similar to that from a diet of about 20% C_4 grass and 80% C_3 forbs. However, this latter scenario has not been tested.

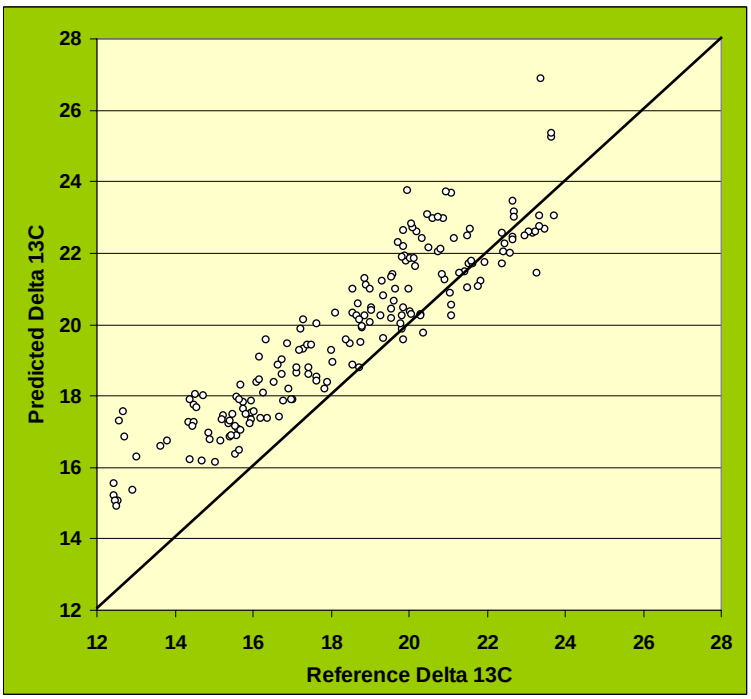

Figure 4a. Validation plot showing the relationship between predicted and actual faecal $\delta^{13}C$ values of samples from cattle grazing Leucaena/grass pastures.

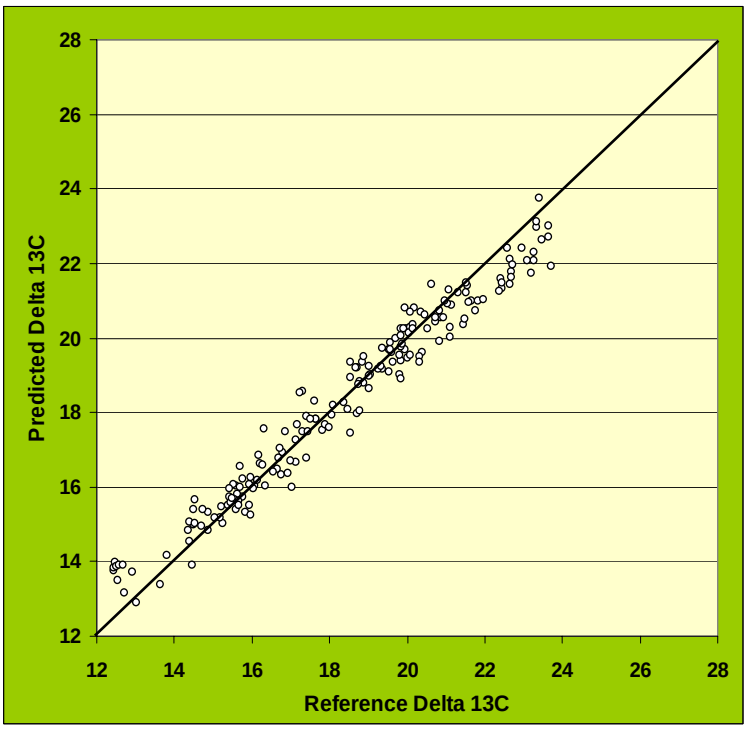

Figure 4b. Plot showing the relationship between predicted and actual faecal $\delta^{13}C$ values of samples from cattle grazing Leucaena/grass pastures after incorporation into the calibration set.

The formula for calculating the proportion of non-grass in the faeces assumes a diet where grasses are C_4, and non-grasses are C_3, such that:

Non-grass in faeces(%) = (faecal $\delta^{13}C$ – 13.5)*7

This formula assumes:

the average $\delta^{13}C$ value of C_4 grasses is 12.5 units

the average $\delta^{13}C$ value of C_3 non-grass species is 26.5 units

faecal $\delta^{13}C$ is one unit more than the corresponding $\delta^{13}C$ of the diet.

If the calibration equation is applied to faeces from cattle grazing pastures that contain C_4 grasses with both C_3 and C_4 forbs such as often occurs on Mitchell grass downs, faecal $\delta^{13}C$ will be over-estimated but the proportion of non-grass is likely to be under-estimated. Conversely, if the calibration equation is applied to faeces from cattle grazing pastures that contain C_3 non-grass with C_3 or both C_3 and C_4 grasses, such as in southern Australia, faecal $\delta^{13}C$ will be under-estimated but the proportion of non-grass is likely to be over-estimated.

If the digestibility of the grass and non-grass dietary components are not the same, then the proportion of non-grass in the faeces will not equate with the proportion of non-grass in the diet. However, because it is not possible to determine the differential digestibilities of the grass and non-grass dietary components, the formula applied for calculating dietary non-grass proportions is actually the formula for calculating the proportion of non-grass in the faeces. Therefore, if the digestibility of dietary non-grass is higher than that of the grass fraction (eg. when cattle graze pasture containing winter herbage of legume or other forbs) and mature grass, the faecal NIRS prediction of non-grass is likely to be under-estimated. Conversely, when cattle eat a mixture of grass and low digestibility browse (eg. mulga or wattle), the non-grass prediction is likely to be an over-estimate.

The above considerations demonstrate the importance of understanding the basis of the predictions and the sources of error contributing to faecal NIRS predictions of dietary non-grass. It is clear that current equations should not be applied to cattle grazing southern, cool season pastures that contain C_3 grasses. Similarly, information on the type of non-grass species available to the grazing animal can be very helpful in determining the likelihood of faecal NIRS predictions of dietary non-grass being over- or under-estimates. For example, the presence of C_4 forbs and the generally higher digestibility of the non-grass fraction compared with the grass fraction means that there is a much greater probability of dietary non-grass being under-estimated than over-estimated in cattle grazing Mitchell grass pastures.

Faecal phosphorus

Faecal P concentrations were determined on 250 faecal samples selected to ensure a wide range in P concentrations and adequate diversity in terms of sample origin (geographic location, season and year, pasture type, class of cattle etc). The calibration statistics (Table 20) indicated that prediction errors were too large for faecal NIRS to be of practical use in predicting faecal P concentration.

Table 20. Faecal NIRS calibration equation 1 statistics for predicting faecal phosphorus concentration.

No. samples	Range in P%	SEC	SECV	R^2
246	0.12 – 0.71	0.07	0.08	0.67

Dietary NDF and ADF

Difficulties were encountered with chemical analysis procedures for determining NDF and ADF dietary concentrations at the required level of accuracy and precision for use as reference values. As a consequence, no calibration equations have yet been developed. However, the chemical analysis procedures have been resolved and faecal NIRS for predicting dietary NDF and ADF will be dealt with in a separate report.

Growth rate

Faecal spectra and matching growth rate reference values for more than 35 monitor-herd-years were accumulated during NAP3.121 from the sites listed in Table 1. These data were added to the calibration set developed in the earlier project NAP3.116 and calibration was carried out on the expanded set (Table 21).

Table 21. Faecal NIRS calibration equation [1] statistics for predicting growth rate (g/hd.day) in cattle.

Source	Math treat.	No. samples	Range (g/day)	SEC	SECV	R^2
NAP3.116	1,4,4,1	629	-430 – 1880	138	146	0.86
NAP3.116 & NAP3.121	1,4,4,1	1153	-790 – 1880	157	162	0.88
NAP3.116 & NAP3.121	2,4,4,1	1157	-790 – 1880	155	164	0.88

[1] MPLS, SNV & detrend, wavelengths 700 – 2500

Although the calibration equation statistics for the expanded set, and especially the R^2, appeared satisfactory compared with the earlier equation, there was an increase in the SEC and SECV values indicating some loss of predictive accuracy. The improvement in R^2 was merely a consequence of the increased range in the reference values. In addition to the higher SEC and SECV values, a significant number of samples were rejected as outliers during the calibration process (the ISI software package automatically eliminates outliers based on the T statistic of residuals). An inspection of the outliers revealed that more than half (17 of 31) were from Brigalow Research Station representing approximately 12% of all samples from that site. Moreover, five of the 14 samples from Alice Springs were outliers representing a very high rejection rate from that site. The remainder were from Berrigurra (2), Toorak (2), Galloway Plains (2), Rosebank (1), Forest Home (1) and Wambiana (1). The distribution of outliers across sites/pasture types suggested that it would be a useful exercise to plot predicted against reference values for each site and these are presented in Fig. 5.

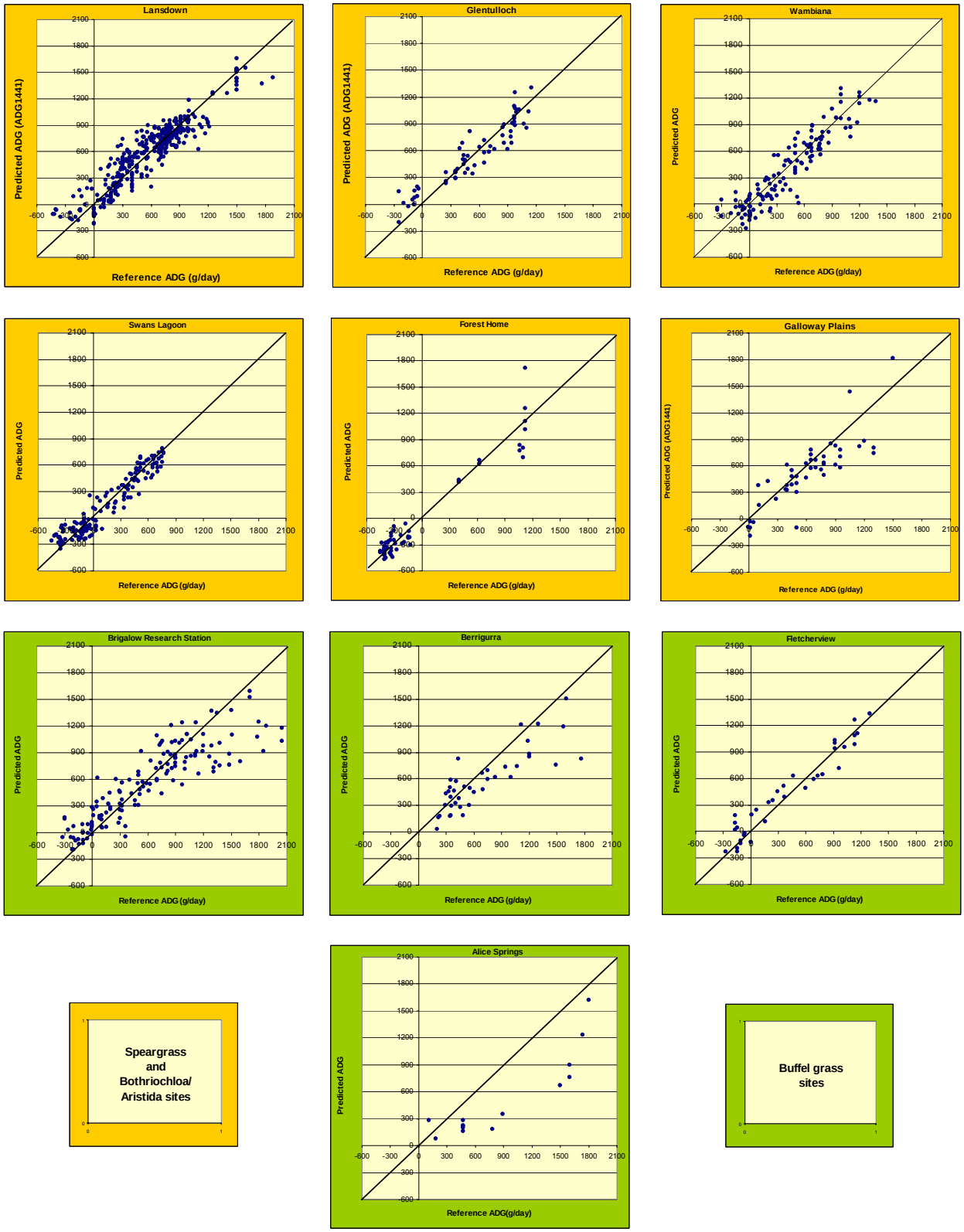

Figure 5 (part 1). Predicted average daily gain (ADG in grams) plotted against reference ADG for individual sites. Predictions were made using the math treatment 1,4,4,1 equation and outliers are included. Speargrass and Bothriochloa/Aristida sites and buffel grass sites.

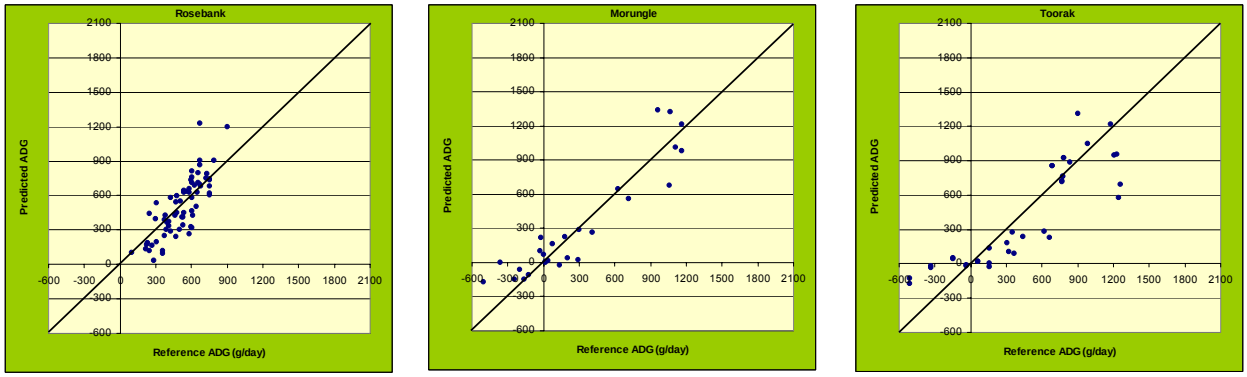

Figure 5 (part 2). Predicted average daily gain (ADG in grams) plotted against reference ADG for individual sites. Predictions were made using the math treatment 1,4,4,1 equation and outliers are included. Mitchell grass sites.

It is clear from the plots in Fig. 5 that the sites with the poorest correlation between predicted and reference ADG were Brigalow Research Station, Berrigurra, Alice Springs and Toorak. Since three of these sites had buffel grass pastures it was concluded that the relationship between predicted and reference ADG may have been influenced by pasture type and that separate calibration equations may be required to represent different pasture types. Therefore, the calibration set was partitioned into three subsets: one subset representing the black speargrass region (Lansdown, Swans Lagoon, Forest Home and Galloway Plains) together with the Bothriochloa/Aristida native pastures (Glentulloch and Wambiana); one subset representing buffel grass pastures (Brigalow Research Station, Berrigurra, Fletcherview and Alice Springs); and one subset representing Mitchell grass pastures (Rosebank, Toorak and Morungle). Recalibration was performed on each subset (Table 22).

Table 22. Faecal NIRS calibration equation [1] statistics for predicting growth rate (g/hd.day) in cattle grazing different pasture communities.

Pasture type	No. samples	Range (g/day)	SEC	SECV	R^2
Speargrass and Bothriochloa/Aristida	808	-450 – 1760	127	133	0.92
Buffel grass	229	-320 – 1880	202	217	0.86
Mitchell grass	128	-514 – 1258	109	139	0.91

[1] MPLS, 1,4,4,1, SNV & detrend, wavelengths 700 – 2500

Clearly, calibration equation statistics were much improved for the speargrass and Bothriochloa/Aristida subset and for the Mitchell grass subset while there was an apparent deterioration for the buffel grass subset. However, comparing calibration statistics of the subset equations with the universal equation (all sites equation) can be misleading because of the different sizes and structures of the calibration sets. For example, whereas 24 samples from buffel grass sites were rejected as outliers during calibration of the universal set, only 5 samples were rejected during calibration of the buffel grass subset. Therefore a comparison of the actual predictions from subset equations with those from the universal equation provides a better indication of the improvement resulting from partitioning the calibration set (Fig. 6).

Simple linear regressions were computed for predicted ADG on reference ADG and this demonstrated that correlations between predicted and reference ADG were improved by using subset equations compared with the universal equation for all sites except Berrigurra, Galloway Plains and Swans Lagoon. In particular there were significant improvements for the Mitchell grass pastures at Rosebank and Toorak and for buffel grass pasture at Brigalow Research Station. The evidence from these comparisons therefore suggests that there is some benefit to be gained by developing separate calibration equations

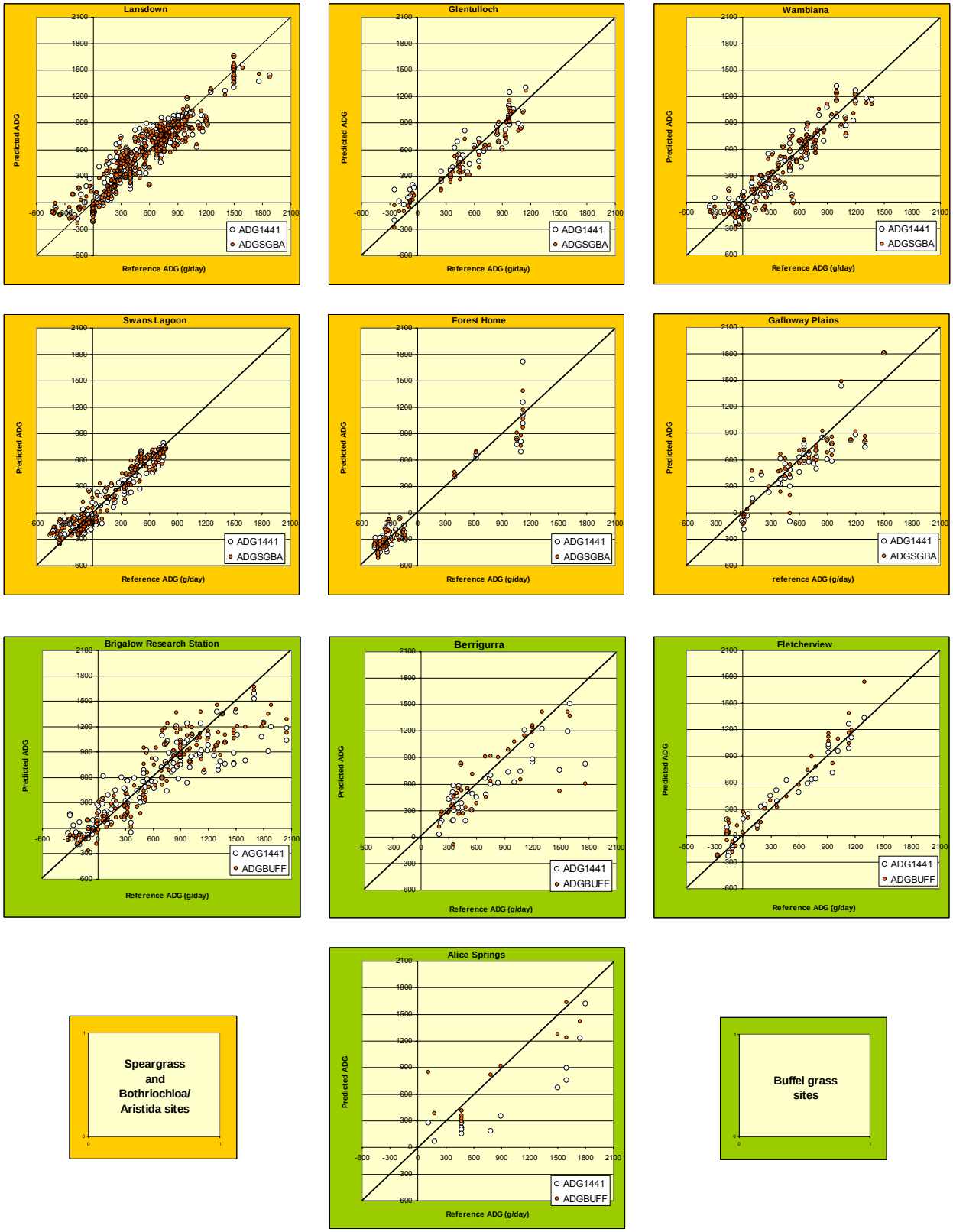

Figure 6 (part 1). Predicted average daily gain (ADG in grams) using the universal equation (ADG1441) and subset equations plotted against reference ADG. ADGSGBA is the subset equation for speargrass and Bothriochloa/Aristida sites and ADGBUFF is the subset equation for buffel grass sites.

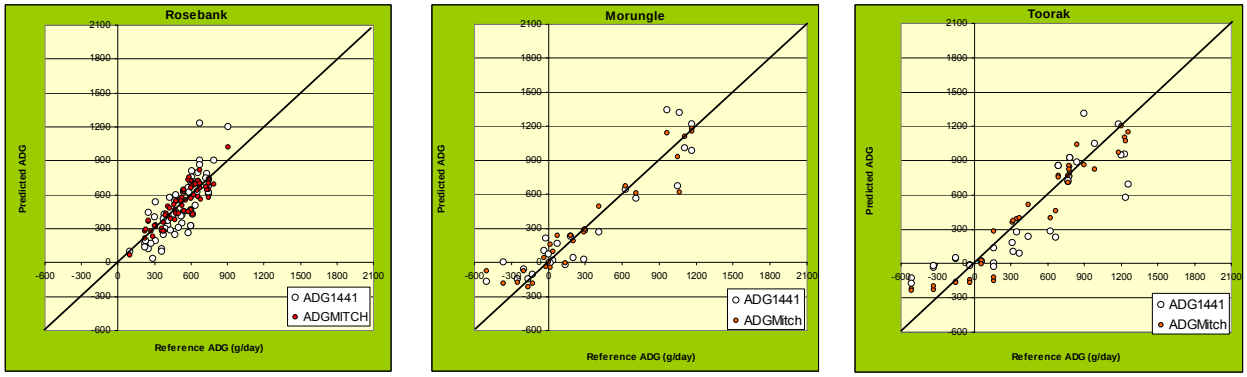

Figure 6 (part 1). Predicted average daily gain (ADG in grams) using the universal equation (ADG1441) and subset equations plotted against reference ADG. ADGMITCH is the subset equation for Mitchell grass sites.

for different pasture types and/or regions. It is noteworthy that the calibration statistics for the calibration set from the combined Lansdown and Swans Lagoon sites are very acceptable (Table 23). This calibration set represents 24 monitor-herd years from two sites where the pastures were quite different with respect to species composition but where the climate was similar and where the cattle were genetically similar (Brahman crossbreds at Swans Lagoon and Droughtmasters at Lansdown).

Table 23. Faecal NIRS calibration equation [1] statistics for predicting growth rate (g/hd.day) in cattle using data from the Lansdown and Swans Lagoon sites.

Region	No. samples	Range (g/day)	SEC	SECV	R^2
North-east coastal speargrass [2]	517	-450 – 1760	102	114	0.94

[1] MPLS, 1,4,4,1, SNV & detrend, wavelengths 700 – 2500

[2] Pastures at Lansdown were introduced pastures, predominantly Urochloa/stylo while those at Swans Lagoon were predominantly native grass.

The problem of inaccurate reference values is a serious one in setting up calibration sets for predicting growth rate due to the confounding effect of changes in gut fill and body water on liveweight measurements and the calculation of growth rates from serial liveweights. Calculating valid estimates of ADG early in the growing season is of particular concern. Gains are usually highest at that time, compensatory gains are often significant and changes in body water content and gut fill may be disproportionately large between successive weighings due to rapidly changing weather and pasture conditions. Difficulties in accurately calculating reference ADG and the subsequent errors in reference values probably account for part of the large differences between reference and predicted ADG that were very prominent at the Brigalow Research Station, Berrigurra, and Galloway Plains sites at high reference ADG values (Figs. 5 and 6).

Mixed diets of forage plus supplements of cereal grain or protein meal

The work conducted on roughage diets that included cereal grain and cotton seed meal supplements will be presented in full in Jim Gibbs' PhD thesis. Results from the hay-only diets have been included in this report. With respect to the forage-supplement diets, suffice it to say that calibration equations were developed that can reliably predict crude protein and digestibility for the total diet or for the forage component alone. This represents a significant and important extension to the capability of faecal NIRS technology.

Success in achieving objectives

The planned research work as set out in the agreement between CSIRO and MLA was successfully completed. The objective of improving the predictive accuracy and usefulness of calibration equations varied among the attributes being predicted. These are discussed below.

Dietary crude protein

The number of diets represented in the calibration set was more than doubled and the diversity of diets with respect to pasture type, location, season and year, as well as the diversity of cattle consuming the diets, was markedly increased. There is no doubt that current equations are much more robust than those developed prior to NAP3.121. Therefore, the post-project equations should provide more accurate predictions of dietary CP than was previously possible for most pasture types and regions across northern Australia. There are, however, some regions and forage communities where dietary CP predictions remain unreliable or suspect. In saying this it must be understood that predictions of dietary CP cannot be validated unless the actual diet can be obtained for chemical analysis and this cannot be done with grazing cattle. Forage communities where circumstantial evidence indicates that faecal NIRS predictions of dietary CP are suspect or unreliable are discussed below.

(i) Diets with a substantial amount of native browse. Currently there are only two diets that contained browse species represented in the calibration set. These diets contained prickly pine (*Bursaria spinosa*) shoots and the leaves of currant bush (*Carissa lanceolata*) at dietary proportions of 11 and 20% respectively. Although the agreement between reference CP and predicted CP was satisfactory for these 2 diets there is still a question-mark over the reliability of the current equations when applied to samples derived from high browse diets simply because of the lack of representation of such diets in the calibration set.

(ii) Diets with a high proportion of non-leguminous forbs. There is a good representation of diets with high dietary proportions of pasture legumes in the calibration set. These were derived from pen fed diets containing leguminous hay and from grazed grass/stylo pastures sampled with OF steers. However, diets containing substantial amounts of non-leguminous forbs are not represented. Such diets are particularly important throughout the Mitchell grass country. Although there is no evidence to suggest that the predictive accuracy of current equations applied to samples from high forb diets is any different from that in respect of diets well represented in the calibration set, the situation is similar to that of diets containing browse; i.e. the possibility of poor predictive accuracy simply because the diets are not represented in the calibration set.

(iii) Spinifex diets. Predictive accuracy on samples from cattle grazing spinifex pastures is suspect on the same basis as the two instances described above in that spinifex diets are currently not represented in the calibration set. Evidence from many samples coming from the Pilbara in Western Australia suggests that predictions of dietary N for cattle grazing spinifex pastures are over-estimated. No doubt there are other grasses not represented in the calibration set but spinifex by its very nature is more likely to be a problem than most other grasses. Moreover, spinifex is important because of its wide distribution.

Digestibility

As with dietary CP there is no doubt that faecal NIRS calibration equations for predicting digestibility have been improved. Results for predicting *in vivo* digestibility (Table 9) were particularly encouraging but the number of diets represented in the calibration set is still too small for the equation to be considered robust.

New faecal NIRS equations for predicting the *in vitro* digestibility of dietary extrusa were probably little changed from the previous equations because grazed pastures sampled with OF steers, as distinct from pen feeding experiments, accounted for only 20% of the diets tested in NAP3.121. Conversely, the

calibration set for predicting the *in vitro* digestibility of dietary forage was almost trebled by the addition of more than 400 samples representing about 130 different diets. Faecal NIRS calibration statistics developed on the expanded set are very good and comparable to those for forage NIRS equations. This is an exceptionally good outcome because DDMI is closely correlated with the pepsin-cellulase *in vitro* digestibility of tropical forages. In fact, the relationship between DDMI and *in vitro* DMD of forages is far superior than the relationship between *in vivo* DMD and *in vitro* DMD (Fig. 7).

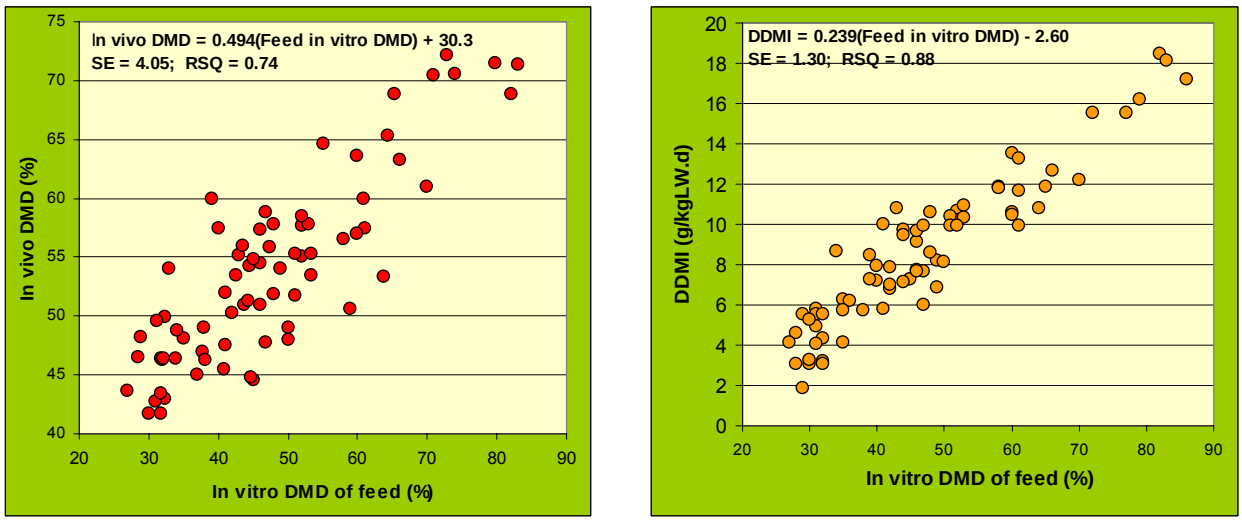

Figure 7. Relationships of *in vivo* dry matter digestibility (DMD) and digestible dry matter intake (DDMI) with *in vitro* DMD (data from CS.253 and NAP3.121 pen trials).

Faecal N concentration

The pre-project calibration equation was expanded by more than 400 samples and the existing equation provides accurate predictions of faecal N almost comparable to conventional chemical analysis.

Dietary non-grass proportions

Over 400 samples were added to the calibration set and the existing equation is much more robust than the pre-project equation. There are limitations to the accuracy of non-grass predictions due to a number of factors that have already been discussed. Despite errors which could be as high as 10%, predictions provide a very useful indication of the contribution of non-grass to dietary composition and are of considerable benefit in assessing and understanding overall diet quality.

Faecal P concentration

The calibration equation developed on a set of 250 samples indicated that faecal NIRS was not an appropriate analytical method for determining faecal P concentration. However, this was in accord with expectations.

Dietary NDF and ADF

As stated previously, no faecal NIRS calibration equations were developed for predicting dietary NDF and ADF. However, the work will be carried and reported separately.

Growth Rate

For various reasons the direct prediction of growth rate by means of faecal NIRS was no doubt the most ambitious of the project goals. This is because growth rate is influenced by so many factors other than nutrition and also because it is very difficult to obtain accurate reference values for pairing with faecal spectra. It now seems clear that a universal equation for general and beneficial use across all pasture types or communities and across all regions in northern Australia is unachievable. However, there are promising indications that useful equations can be developed for at least some regions and/or pasture types. This was demonstrated by the encouraging calibration statistics of the equation developed from the speargrass and Bothriochloa/Aristida monitor herds and especially by the calibration equation derived from the Lansdown and Swans Lagoon monitor herds.

Impact on industry

Faecal NIRS in Australia was first used by a small number of producers in 1999 and since then the number of clients has steadily increased. 156 new clients submitted samples during 2003 and another 80 new clients have submitted samples to May 2004 making a total of over 650 producers who have submitted samples for analysis at one time or another. Feedback from most producers has been positive.

The potential benefits of faecal NIRS as an educational tool, as a decision support tool for the nutritional management of grazing cattle, and as an aid to forward marketing have been published in journals (Coates 1999, 2000) and publicised at seminars, conferences (Coates 2000, 2001), workshops (Coates 2002b) and Meat Profit Days. There have also been numerous newspaper articles and radio interviews. Apart from the educational value, most producers probably use the faecal NIRS analytical service as a decision support tool for making supplementation decisions. Herein lies a problem because the reliable prediction of diet quality in grazing cattle is just one component of the technology package needed for the cost effective supplementation and nutritional management of grazing cattle. Other important components of the technology package include (a) nutritional modeling (the knowledge of realistic and authentic nutrient requirements in relation to class of animal and level of production including the ability to interpret diet quality in terms of cattle productivity and the identification of limiting nutrients) and (b) supplementation technology with respect to determining what, when and how much to feed to meet production targets **in relation to** the quality of the basal forage diet. Even if faecal NIRS estimates of diet quality are entirely accurate the information will be of limited use to producers without decent support technologies in nutritional modeling and supplementation strategies. My great concern at present is that the support technologies are found to be wanting and there is an urgent need to improve these technologies so that the potential benefits of faecal NIRS can be realised. My view is that the impact of faecal NIRS in the northern beef industry in five years time will be largely dependent on advancements that need to be made in the support technologies.

Faecal NIRS is also a powerful research tool and it is being used in many research projects involving grazing cattle as an aid to more effective research. It has also shown potential in the identification of hitherto unknown aspects of grazing behaviour and diet selection in cattle.

Conclusions and recommendations

Conclusions

1. Work conducted in NAP3.121 has resulted in the significant expansion and improvement of faecal NIRS technology for the northern beef industry. Calibration equations for predicting dietary CP and digestibility are sufficiently robust to be of practical use as a decision support technology in the nutritional management of grazing cattle. There are, however, some common diet types that are not represented in the calibration set. As such, a question mark hangs over the predictive accuracy in regard to these diet types which include those with a substantial (>20%) browse content, diets with a substantial (>25%) forb content, and spinifex diets.

2. The most recent faecal NIRS calibration equation for predicting dietary non-grass proportions is a robust equation that can be used to enhance the value of dietary CP and digestibility predictions by providing additional insights into the composition of the diet and the drivers of diet quality. Non-grass predictions provide new and valuable information on diet selection patterns and the contribution of grass and non-grass components of the vegetation resource to cattle diets and to productivity.

3. Faecal NIRS predictions of faecal N are almost comparable to conventional chemical analysis in terms of accuracy. Data from right across northern Australia for different pasture types and regions have demonstrated the deficiencies of faecal N concentration as an indicator of dietary protein and as a guide to identifying whether cattle are likely to respond to non-protein N supplementation. However, faecal N predictions can be helpful in assessing whether dietary CP predictions may be in error, and especially helpful in detecting faulty diet CP and digestibility predictions in faecal samples contaminated with soil.

4. Faecal NIRS predictions of growth rate at the current stage of development are of questionable value due to their unreliability for many pasture types. A universal calibration equation for predicting growth rate now appears to be unachievable and the goal should be to develop a series of equations for use in defined regions or for defined pasture types.

5. The recent MLA survey indicated a surprisingly low level of producer awareness of the technology despite publicity via written, oral and live presentations. Nevertheless, faecal NIRS has generally met with ready industry acceptance where producers are aware of the technology.

6. Faecal NIRS has the potential to make an important and continuing contribution to efficiency, profitability, and sustainability within the beef industry through its application as a research tool.

Recommendations

Although faecal NIRS has already been adopted by a good number of northern beef producers and by sections of the research community, the technology is in its infancy and it has a number of identifiable deficiencies and limitations that can only be resolved by further research. Whether these deficiencies/limitations are addressed will of course depend on the priority given to the continuing development of the technology by research institutions and funding bodies. No doubt there are also additional capabilities and applications of faecal NIRS that are currently unidentified but which will emerge over time. The NIRS research team has identified a number of research topics that need to be addressed to maintain and improve the technology *per se* and to enhance the beneficial application of the technology. These are presented below.

1. Performance monitoring. As with all applications of NIRS there is a critical and essential requirement to monitor the performance of the technology to ensure that predictive accuracy is maintained within certain defined limits. Performance monitoring depends on the conduct of planned validation procedures so that NIRS predictions can be compared with reference

values determined by a recognised primary method of analysis. This is a simple procedure when reference values and NIR spectra can be determined on the same material. With faecal NIRS this situation exists with regard to faecal N and dietary non-grass proportions, the latter being calculated from faecal $\delta^{13}C$ measurements as previously described. For the other attributes, however (dietary CP, digestibility, NDF, ADF, DMI, DDMI and growth rate), reference values cannot be determined from faecal analysis and validation exercises require specially conducted experiments for obtaining valid reference values. The conduct of such experiments will be a necessary adjunct to maintaining a useable faecal NIRS technology. The necessary frequency of validation trials will, however, depend on the ongoing performance of faecal NIRS as determined by such validation exercises – poor performance requires more frequent monitoring; good performance less frequent monitoring. **To reduce the cost burden of validation experiments every effort should be made to ensure that every relevant sample and every bit of relevant information from experiments designed for other purposes is acquired for the purpose of maintaining and building up faecal NIRS technology**. In addition, it might well be that some experiments can be modified/expanded at little additional cost to allow the acquisition of samples/information beneficial to faecal NIRS.

2. Effect of cattle breed. The possibility of faecal NIRS predictions being affected by cattle breed has recently come to light. The evidence came from pen experiments at Lansdown where Droughtmaster and high grade Brahman steers were fed the same diets. There was an obvious trend for predicted dietary CP and digestibility to be higher for the Droughtmaster steers (Table 23). Predicted CP averaged 0.7% higher while predicted digestibility averaged 1.1% higher for the Droughtmaster steers. With regard to making nutritional decisions based on faecal NIRS predictions, the breed difference in predicted dietary CP is of more concern than the difference in digestibility and it is important to confirm whether the results obtained in the experiments at Lansdown represent a true breed effect and to investigate whether *Bos Taurus* cattle differ yet again. Because the technology is already being used by a substantial number of producers this matter needs to be resolved with urgency.

Table 23. The effect of cattle breed on faecal NIRS predictions of dietary CP and digestibility as demonstrated in pen trials at Lansdown Research Station.

Diet	Predicted diet CP%		Predicted digestibility%	
	Brahman	Droughtmaster	Brahman	Droughtmaster
Blue couch hay	6.7	8.4	52	54
Bl. couch + Mol 0.3[1]	7.1	7.7	53	55
Bl. couch + Mol 0.6[2]	7.4	7.7	54	54
Indian couch hay	7.0	8.6	52	55
Ind. Couch + Mol 0.3	7.3	7.9	53	54
Ind. Couch + Mol 0.6	7.5	8.8	54	55
Urochloa hay	7.4	7.7	54	55
Urochloa + Mol 0.6	8.9	8.6	56	55
Forage sorghum hay	6.1	6.3	51	51
For. sorghum + Mol 0.6	7.6	8.5	52	54

[1] Molasses plus urea fed at 0.3% of liveweight; [2] Molasses plus urea fed at 0.6% of liveweight

3. Diets not represented in the calibration set. Three types of forage diets have been identified as needing special attention to determine the reliability of diet quality predictions using current calibration equations. If the current equations are found to be wanting a decision then needs to be made whether to conduct the work necessary to improve predictive accuracy to within designated limits. The diets identified are those with a significant component of native browse, those with a significant component of non-leguminous forbs, and those with a significant component of spinifex. There are special difficulties associated with obtaining the necessary diet faecal pairs for such diets, difficulties that also contributed to their not being represented in the calibration set at this point in time. One difficulty is the time and resources needed to obtain sufficient material for feeding to cattle in pens. For example, hand harvesting (of the browse component) is necessary for pen trials with browse diets. Situations rarely exist where forbs can be mechanically harvested in sufficient quantity to typify the non-grass proportions often seen in selectively grazed cattle diets. Another difficulty with regard to high browse diets, and possibly to spinifex diets as well, is that the cattle in pens need to be adapted to the species being offered. This virtually means that the feeding trials have to be conducted adjacent to the areas where the species are abundant and these rarely coincide with the presence of suitable research facilities. However, portable pen feeding facilities are easy enough to fabricate and the conduct of such feeding trials will depend of the availability of funding and the commitment of research staff to tackle a challenging problem.

4. Digestible dry matter intake (DDMI).. The calibration equation statistics for predicting DDMI (Table 15, diet means used as reference values, math treatment 2,4,4,1) are surprisingly good and surpassed all expectations. Despite these most encouraging results, the predictive performance of the existing equation is not likely to compare at all favorably with the calibration statistics because of the relatively small number of diets represented in the calibration set. However, since DDMI is such a critical and useful nutritional measure, the apparent potential of faecal NIRS to predict this attribute with accuracy would seem to justify generating more diet-faecal pairs for DDMI with the ultimate aim of achieving a robust calibration equation for predicting this dietary property.

5. Predicting growth rate. It is evident that useful faecal NIRS predictions of growth rate will require separate calibration equations for different regions and/or pasture types. The calibration equation developed for the speargrass-Borthriochloa/Aristida pasture regions of coastal and sub-coastal Queensland from Injune to Charters Towers is without doubt more robust and accurate than the equations for buffel grass pastures and for Mitchell grass pastures. The buffel grass and Mitchell grass calibration sets are small in comparison with the speargrass-Bothriochloa/Aristida calibration set and they need to be expanded by many more monitor herd-years of data (ADG-faecal pairs). Data are still being collected from monitor herds at Swans Lagoon and Brian Pastures (NE and SE speargrass regions), at Toorak and Longreach (Mitchell grass region), and at South Galway in the Channel Country. The Brian Pastures and Swans Lagoon data will strengthen the already quite robust calibration equation for the eastern speargrass region. The Toorak and Longreach data will strengthen the Mitchell grass equation but more monitor herd-years from existing and additional sites will be needed to build an equation of sufficient predictive accuracy to be of benefit to the industry. Similarly, more data would be needed to develop useful equations for the brigalow belt of central and southern Queensland, for the south-western corner of the state, and for regions in the Northern Territory and the top half of Western Australia. Obviously the development of robust calibration equations to serve all regions/pasture-types across northern Australia would be an enormous undertaking. I envisage that further development of this aspect of faecal NIRS will be an ongoing process with the pace of development driven by demand, by funding opportunities, and by the relative success in achieving targets for predictive accuracy.

There are other research needs that relate not to the development of better faecal NIRS calibration equations but rather to the use of faecal NIRS technology in the nutritional management of grazing cattle.

Given that faecal NIRS predictions of growth rate have serious limitations in terms of predictive accuracy, faecal NIRS at its current stage of development is primarily a technology for predicting the protein and energy contents of grazed diets. This information is of little or no benefit unless it can be interpreted in terms of animal performance, protein and/or energy limitations to performance, and cost-effective supplementation strategies for improving performance or meeting production targets. My assessment of the technologies available with which to make such interpretations is that they are not adequate to allow the potential benefits of faecal NIRS to be fully exploited. Therefore, I do not believe that faecal NIRS will provide major benefits to the northern beef industry until substantial progress is made in nutritional modelling for northern Australia and in the area of responses to supplements in relation to the quality of the basal diet. Since the inefficient use of natural resources can no longer be responsibly tolerated my strong recommendation is that research aimed at improving nutritional modelling and responses-to-supplements technologies for northern Australia be accorded high priority and be stimulated as a matter of urgency.

Another obvious research topic of importance to much of northern Australia is the nutritive quality of diets containing browse, especially browse containing high concentrations of condensed tannins. Cattle usually start browsing when the protein content of the grass falls to low levels and while dietary protein concentrations are elevated by browsing, the availability of the protein is reduced by the presence of condensed tannins. The difficulty is to determine the amount of available protein in such diets and so assess the overall nutritive value of the diet. This is not an NIRS research topic *per se* but NIRS may well be the research tool for studying and resolving this problem. It would depend on whether faecal NIRS could be used to determine dietary tannin concentrations. Faecal NIRS analaysis of many thousands of samples submitted from properties across northern Australia has highlighted the magnitude and importance of browse or top-feed in the diet of cattle during the dry season or in drought. The contribution of top-feed to cattle diets justifies an active research program in this field. In particular this research area may well lend itself to PhD or other student projects.

Acknowledgements

The research work carried out in NAP3.121 involved many people from various research institutions and private enterprise and I would like to acknowledge the contribution and team spirit of enthusiasm and cooperation of all those who participated in this work.

Rob Dixon and David Hurst for pen trials conducted at Swans Lagoon and for Rob Dixon's support in many ways throughout the project.

Neil MacDonald and Rebecca Mather-Brown for pen trials conducted at Katherine Research Station.

Joanne Akeroyd and Brunchilly Station management for the pen trials conducted at Brunchilly.

Jim Gibbs for the tremendous amount of work that he accomplished in his PhD project, for additional pen feeding work that he conducted at Mt Cotton just for the project, and for organising the OF sampling work on speargrass and Bothriochloa pastures at Brian pastures. Also to staff at Brian Pastures for the hands on work with the OF sampling program.

Michael Jeffery for conducting a pen trial with mulga diets at Croxdale and for establishing a monitor herd at Croxdale.

Peter Smith for conducting a spinifex grazed diet trial at Mallina in the Pilbara.

Maree Bowen for access to samples from her PhD project at Brian Pastures.

Ron Hendricksen for samples and data from *in vivo* digestibility trials that he conducted at Brian pastures.

Rob Dixon, Mick Sullivan, Steve O'Connor and Dave Smith for samples and data from monitor herds at

Swans Lagoon and Toorak Research Station.

Peter Venamore for running the monitor herd at Berrigurra.

Felicity Hill for organising the monitor herd at Morungle and Dick Cribb for doing the work of weighing and faecal sampling.

John and Helen Rickertt for running the monitor herd at South Galway.

Peter Finlay for running the monitor herd at Fletcheview.

John Brownson for providing land to conduct the experiment at Forest Home.

Clare Hill and Jocelyn Coventry for samples and data from the monitor herd at Alice Springs.

I would especially like to thank research support staff at the CSIRO Davies Laboratory, particularly my full time technician, Kylee Verry, and also Mike Nicholas, Lindsay Whiteman and David Poppi.

Finally the generous funding provided by MLA is gratefully acknowledged.

Bibliography

Coates, D.B., Schachenmann, P. and Jones, R.J. (1987) Reliability of extrusa samples collected from steers fistulated at the oesophagus to estimate the diet of resident animals in grazing experiments. *Australian Journal of Experimental Agriculture* **27**: 739-745.

Coates, D.B. (1999) The use of faecal $\delta^{13}C$ values to improve the reliability of estimates of diet quality when sampling tropical pastures with oesophageal fistulated cattle. *Australian Journal of Experiment Agriculture* **39**:1-7.

Coates, D.B. (1999) Faecal spectroscopy (NIRS) for nutritional profiling of grazing cattle. Proceedings of the *VI International Rangeland Congress* Townsville, Australia. July 19-23 Vol. **1**:466-467

Coates, D.B. (2000) Faecal NIRS – what does it offer today's grazier? *Tropical Grasslands* **34**:230-239.

Coates, D.B. (2001) Faecal NIRS – opening the door to a better understanding of nutrition. *Proceedings of the Northern Australia Beef Industry Conference 2001*, 8 & 9 November, Kununurra, Australia. pp 101 –105.

Coates. D.B. (2002a) An empirical approach to the question "Is NIRS only as good as the laboratory reference values?" *Spectroscopy Europe* 14 No. 4:24-26

Coates, D.B. (2002b) Potential and limitations in relation to the practical application of faecal NIRS technology in the northern beef industry. *BIA Workshop 2002*, 8 & 9 August, Longreach, Australia.

Lyons, R.K. and Stuth, J.W. (1992) Fecal NIRS equations for predicting diet quality of free-ranging cattle. *Journal of Range Management*, **45**, 238-244.

Naes, T., Isaksson, T., Fearn, T. and Davies, T. (2002) A user-friendly guide to multivariate calibration and classification. *NIR Publications*, Chinchester, p. 124.

Shenk, J.S. and Wetserhaus, M.O. (1991) *Crop Science* **31**: 469-474.

Appendix 1. Pen Feeding Trials

SITE	DATE	DIET
LANSDOWN	May 1999	Speargrass (*Heteropogon contortus*)(hay)
		Speargrass plus urea
		Speargrass plus Albizia (*A. lebbeck*) fallen leaf
		Speargrass plus Albizia pods
		Speargrass plus Albizia fallen flowers
		Speargrass plus current bush (*Carissa lanceolata*) leaf
		Speargrass plus prickly pine (*Bursaria spinosa*) shoots
		Speargrass plus green oaten hay (*Avena sativa*)
	Feb 2001	Rhodes (*Chloris gayana*) (fresh)
		Urochloa (*U. mosambicensis*) high N (fresh)
		Urochloa low N (fresh)
		Buffel (*Cenchrus ciliaris*) (fresh)
	May 2001	Urochloa (fresh)
		Rhodes (fresh)
		Stylo (*Stylosanthes scabra*) (fresh)
		Para grass (*Brachiaria mutica*) (fresh)
	Aug 2001	Rhodes (fresh)
		Indian couch (*Bothriochloa pertusa*) (fresh)
		Indian couch/Albizia (fresh)
		Indian couch/Leucaena (*Leucaena leucocephala*) (fresh)
	Feb 2002	Buffalo couch (dried)
		Urochloa (fresh)
		Urochloa/Leucaena (75/25) (fresh)
		Urochloa/Leucaena (50/50) (fresh)
	Mar 2002	Rhodes (fresh)

LANSDOWN	Mar 2002	Buffel (fresh)
		Para (fresh)
		Indian couch (fresh)
	Jun 2002	Buffel (fresh)
		Rhodes (fresh)
		Rhodes/Leucaena (75/25)(fresh)
		Rhodes/Leucaena (50/50) (fresh)
	Sep 2002	Native grass (harvested dry)
		Native grass & lucerne (*Medicago sativa*) hay
		Native grass & green oaten hay
		Native grass & Cavalcade (*Centrosema pascuorum*) hay
		Buffalo couch (dried)
	Mar 2003	Blue couch (*Digitaria didactyla*) (hay)
		Indian couch (hay)
		Blue couch plus molasses @ 0.3% BW
		Indian couch plus molasses @ 0.3% BW
		Blue couch plus molasses @ 0.6% BW
		Indian couch plus molasses @ 0.6% BW
	May 2003	Urochloa hay
		Forage sorghum hay
		Urochloa plus molasses @ 0.6% BW
		Forage sorghum plus molasses @ 0.6% BW

Appendix 1 (Continued)

SITE	DATE	DIET
KATHERINE	Feb 2001	Buffel (fresh)
		Speargrass (fresh)
		Urochloa (fresh)
	Mar 2001	Buffel (fresh)
		Speargrass(fresh)
		Urochloa (fresh)
	May 2001	Buffel (fresh)
		Speargrass (fresh)
		Urochloa (fresh)
	Jul 2001	Buffel (fresh)
		Speargrass (fresh)
		Urochloa (fresh)
	Dec 2001	Buffel (fresh)
		Urochloa (fresh)
	Jan 2002	Buffel (fresh)
		Speargrass (fresh)
		Urochloa (fresh)
	Mar 2002	Buffel (fresh)
		Speargrass (fresh)
		Urochloa (fresh)
	Apr 2002	Buffel (fresh)
		Speargrass (fresh)
		Urochloa (fresh)

Appendix 1 (Continued)

SITE	DATE	DIET
BRUNCHILLY	Aug 2000	Mitchell grass (*Astrebla spp*.)(fresh)
		Flinders (*Isolema fragile*)(fresh)
	Oct 2000	Mitchell (fresh)
		Flinders (fresh)
	Dec 2000	Rained out (fresh)
	Apr 2001	Mitchell (fresh)
		Flinders (fresh)
		Red country – native grasses (fresh)
	Jul 2001	Mitchell (fresh)
		Flinders (fresh)
		Red country (fresh)
	Mar 2002	Weeping Mitchell (fresh)
		Bull Mitchell (fresh)
		Barley Mitchell (fresh)
JANIBEE	Jul 2001	Sorghum stubble (fresh)
		Blue Grass (fresh)
PENROSE	Apr 2001	Leucaena (fresh)

Appendix 1 (Continued)

SITE	DATE	DIET
SWANS LAG	Dec 2001	Native grass pasture (fresh)
	Jan 2002	*Bothriochloa spp.* (fresh)
	Mar 2002	Native grass pasture(a) (fresh)
		Native grass pasture (b) (fresh)
		Native grass pasture (c) (fresh)
		Native grass pasture (d) (fresh)
	Apr 2002	Native grass pasture (a) (fresh)
		Native grass pasture (b) (fresh)
		Native grass pasture (c) (fresh)
		Native grass pasture (d) (fresh)
	Aug 2002	Native grass pasture (fresh)
		Native grass pasture + Lucerne hay I
		Native grass pasture + Lucerne hay II
		Native grass pasture + Lucerne hay III
		Native grass pasture + Lucerne hay IV
		Native grass pasture + Lucerne hay V
	Apr 2003	Native grass pasture(a) (fresh)
		Native grass pasture (b) (fresh)
		Native grass pasture (c) (fresh)
		Native grass pasture (d) (fresh)
		Native grass pasture (e) (fresh)
		Native grass pasture (f) (fresh)

Appendix 1 (Continued)

MT COTTON		Signal grass (*Brachiaria decumbens*) (fresh)
		Rye grass (*Lolium perenne*) (fresh)
		Green Panic (*Panicum maximum*) (fresh)
		Buffel grass (hay)
		Rye grass A (hay)
		Rye grass B (hay)
		Pangola grass (*Digitaria decumbens*) A (hay)
		Pangola grass B (hay)
		Rhodes grass (hay)
		Mitchell grass A (hay)
		Mitchell grass B (hay)
		Setaria (*Setaria sphaselata*) (hay)
		Speargrass (hay)
CAMDEN PK	Jul 2002	Kikuyu A (*Pennisetum clandestinum*) (fresh)
		Kikuyu B (fresh)
BRIAN PAST	Apr 2000	Buffel grass – young (hay)
		Buffel grass – old (hay)
CROXDALE	Jul 2003	Forage sorghum (hay)
		Forage sorghum plus 25% mulga (*Acacia aneura*)
		Forage sorghum plus 50% mulga
		Forage sorghum plus 75% mulga