finalreport

ANIMAL PRODUCTION

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# What do cattle eat in tropical rangelands? – Implications for animal performance and grazing management

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# Abstract

Cattle in rangeland environments have access to a wide range of plant species and diet selection and foraging behaviour have a large impact on diet quality, animal production and rangeland condition. However, there is not a good understanding of what species are being consumed by free-ranging livestock. This project investigated whether innovative DNA techniques could be used for detecting species composition of the diet of grazing animals. The results clearly demonstrated it is possible to identify gene sequences in plant material in dung and match this to DNA of collected plant species. Although only exploratory techniques were used, about 70% of individual forage species were detected in the dung of cattle fed mixed diets containing 6-8 plant species. Further refinement of the technique should allow nearly all species in these complex rangeland environments to be differentiated. Such a technique would be a useful research tool for better understanding grazing behaviour with the ultimate aim of improving animal nutrition and grazing management in the rangelands.

# Executive summary

Cattle are predominantly bulk feeders and in grassy environments like the tropical rangelands that means grass makes up most of their diet. Despite the general dominance of grass in these environments, cattle have an extremely wide diet choice available to them which can have an impact on diet quality and animal production. Non-grass plants can make up a significant proportion of the diet and this can have a large effect on animal performance.

However, we don't have a good understanding of what species are being consumed and therefore contributing to this variation in diet quality. If we did understand better the species being selected this would be valuable information for formulating better grazing management strategies and adjusting supplementation regimes. This project investigated whether innovative DNA techniques could be used for detecting species composition of the diet of free-ranging livestock. If successful, such a technique would provide a new tool to help understand grazing behaviour which has direct links to improving grazing management. The approach may also help identify where variation between animals in diet composition creates variation in their performance, and consequently assist with targeting supplementation strategies and with animal selection for genetic improvement.

This initial exploration of DNA approaches to detecting plant species consumed by ruminants has proved promising. Fairly coarse universal primers were successful in differentiating a wide range of plant species typically found in north-east Queensland rangelands using standard genetic techniques i.e. polymerase chain reaction (PCR), which allows small genetic fragments to be amplified large enough to be detected visually in electrophoresis gels. Further refinement of primers used should allow nearly all species in these complex rangeland environments to be differentiated.

Initial testing of dung samples from the same paddocks where plant species were collected indicated that plant DNA fragments could be recovered and identified to species level.

On the basis of these initial encouraging results a pen study was undertaken where diets of differing numbers of component species were fed to cattle and the dung analysed to determine whether known dietary components could be detected. In the more complex diets, the majority of species (70%) could be detected. Given that this exploratory study used fairly coarse universal primers the fact that not all species could be detected is not a major concern.

This study also examined whether the relative amounts of species could be detected in the dung. The relative amount of DNA material in the dung was not at all consistent with that in the diet. These differences could not be explained by differential digestibility of species as it would be expected that digestibility of the species used would be in the range of 45%-65%. It is likely that the universal primers used in this study were differentially amplifying the DNA extracted from dung.

This study has clearly demonstrated it is possible to set up a genetic based technique which involves identification of gene sequences in plant material in dung and matching this to DNA of collected plant species. Such a technique would be a useful research tool for better understanding grazing behaviour with the ultimate aim of improving grazing management in the rangelands. It is recommended that the value proposition of the technique for industry be further developed before further investment in its development.

# Contents

# 1 Background

Cattle are predominantly bulk feeders and in grassy environments like the tropical rangelands that means grass makes up most of their diet. The quality of this grass, and hence diet quality, varies considerably through the year with crude protein and digestibility levels being high enough to sustain growth rates in steers of 700-1000 g/day in the early part of the wet season but in the dry season weight losses are frequent as crude protein concentrations drop below maintenance. The northern beef industry has responded to this dry season decline in grass quality by providing urea-molasses, dry loose licks or supplement blocks.

However, despite the general dominance of grass in these savanna ecosystems, cattle have an extremely wide diet choice available to them which can have an impact on diet quality and animal production. Non-grass plants can make up a significant proportion of the diet and this can have a large effect on animal performance.

However, we don't have a good understanding of what species are being consumed and therefore contributing to this variation in diet quality. If we did understand better the species being selected this could be valuable information for formulating better grazing management strategies and adjusting supplementation regimes.

Current techniques for identifying individual plant species consumed are unreliable and inefficient. However, recent discussions with molecular biologists in CSIRO indicate that it should be possible to set up a technique which identifies gene sequences in plant material in dung and matching this to DNA of collected plant species. Molecular biologists believe that the equipment and techniques are now so well advanced that once a methodology is established sample analysis would be quick and relatively inexpensive. Despite extensive literature searches of journals from the last four years there is no such application of DNA marker techniques for herbivore diet assessment.

This project aims to investigate whether innovative DNA techniques can be used for detecting species composition of the diet of free-ranging livestock. If successful, this project will provide a new to tool to help understand grazing behaviour which has direct links to improving grazing management. The approach may also help identify where variation between animals in diet composition creates variation in their performance, and consequently assist with targeting supplementation strategies and with animal selection for genetic improvement.

# 2 Project objectives

Research objectives for this study are:

1. Develop a DNA technique that will allow individual plant species to be identified in dung samples collected from a paddock.

2. Improve understanding of factors that contribute to individual variation in diet quality and use this understanding to develop strategies that better target nutritional management.

# 3 Methodology

Three experimental approaches were used to achieve the project objectives:

## 3.1 Phase 1 – Differentiating plant species

This phase determined whether a polymerase chain reaction (PCR) technique for DNA-based analysis of plant species in herbivore dung could be established to determine diet selection in rangeland situations. The first step was to determine whether plant species could be easily distinguished using currently available PCR primers. This involved collection of a substantial diversity of plant species and then PCR lab work to develop appropriate primers. It was then determined whether DNA plant fragments could be easily isolated from dung samples and multiplied using a PCR technique. It was envisaged that this might be possible using a group-specific technique that focuses on chloroplast DNA and therefore doesn't include DNA from the herbivore's gut or from associated stomach bacteria and fungi.

Experimental details

(a) Collection of plant and dung samples

Forty-three plant species were collected from Virginia Park Station near Charters Towers to carry out some initial tests on whether tropical plant species, typical of the northern Australian rangelands, could be detected using standard genetic techniques i.e. polymerase chain reaction (PCR), which allows small genetic fragments to be amplified large enough to be detected visually in electrophoresis gels. In the first instance fairly standard primers were used to see if they could amplify plant specimens from north Queensland. The forty-three plant specimens collected were from a range of plant groups including native perennial grasses, exotic grasses and legumes, native forbs, shrubs and one eucalypt species. This collection was not meant to be comprehensive of all plant species but representative of the main plant groups.

At the same time plant specimens were collected, fresh dung pats were collected from the same paddocks. Both plant and dung samples were frozen and sent to CSIRO in Canberra to the environmental biotechnology laboratory.

(b) DNA extraction from plants specimens

Fast Prep DNA kit (Q Bio gene) was used to extract DNA from plant specimens, following the manufacturer's instructions. Leaves were selected over stems or roots to have a better chance of getting some chloroplast DNA. Prior to placing the specimen in the homogenising matrix, the leaves were quickly chopped to make homogenising more effective.

(c) Universal chloroplast PCR

The primers used were trnL-F and trnL-R, as described by Taberlet et al. (1991). Hotstartaq PCR mastermix (Qiagen cat no 203445) was used to prepare 50uL reactions with 4uL of template and 1uL of each primer (0.4uM). The $MgCl_2$ concentration was increased to 2.5mM from the orignal concentration in the mastermix (1.5mM). Hotstartaq contains 400uM of each dNTP.
PCR conditions:
95 deg 15 min
94 deg 1min
50 deg 1min15secs } 35cycles
72 deg 1min30sec
72 deg 10min
30 deg 5min

4 deg hold

(d) PCR Cleanup and Sequencing

QIAquick PCR Purification Kit (cat no. 28104) was used to clean-up PCR products according to the manufacturer's instructions (using the spin protocol rather than the vacuum protocol).Sequence reactions were prepared using a Beckman Coulter GenomeLab Dye Terminator Cycle Sequencing with Quick Start Kit (product no. 608120). For each reaction, 5 µL of template, 4µL DTCS, 4µL Better Buffer, 2µL primer (trn-L forward and reverse 1.7µM) and 5µL water. Two reactions were prepared per sample, one with forward primer and one with reverse primer. The thermal cycling program is:

96 degC 20 sec
50 degC 20 sec } 40cycles
60 degC 2 min
4 deg hold

Products from the sequence reactions were ethanol precipitated as described in the GenomeLab kit.

## 3.2 Phase 2 – Pen Studies

This phase involved feeding cattle housed in pens known diets of various complexities and collecting dung from the cattle. The lab component consisted of sequencing plant fragments, isolating plant fragments from dung samples and matching DNA to plant samples.

Experimental details

*(a) Selection of animals for pen trial*

Twenty-four Braham X steers were selected from a group of young cattle at Lansdown Research Station. Mean starting liveweight was 243kg (SD 25.7kg). Animals were held in the Lansdown cattle yards and fed mixed grass hay ad libitum for 2 days before being weighed and place in individual pens for the pen feeding trial on Monday Jan 22, 2007. Starting live weight data was used to determine *ad libitum* levels for each animal during the stabilisation period, based on 3% body weight.

*(b) The diet stabilisation period*

The stabilisation diet was chaffed local mixed grass hay (the same as fed in the yards prior to placement of animals in pens) containing around 70% *Chloris gayana* and the remainder mixed annual and perennial grasses and forbs. The hay was chaffed using the Lansdown PTO driven chaffcutter and placed in bulk bins for subsequent feeding out. Dry matter content (moisture %) was determined by oven drying chaffed feed samples of stabilisation and experimental diet components at 70$^{o}$C for 48 hours, in order to calculate quantities of chaffed hay required to meet the required dietary targets.

Stabilisation diet feed quantities were weighed out according to calculated requirements for each animal and fed out each morning to penned animals. Refusals were collected from feed bins and weighed to determine *ad libitum* diet levels for each animal. The size of refusals varied from day to day for some individual animals.

*(c) Collection and preparation of experimental diets*

The following tables list the eight component forages (Table 1) for the experimental diet mixes and the proportions in each of the experimental diets (Table 2).

**Table 1 - Experimental diet component details**

| Forage Code | Common name | Botanical name | Source |
|---|---|---|---|
| **G1** | Oaten chaff | *Avena sativa* | commercial |
| **G2** | Rhodes grass hay (chaffed) | *Chloris gayana* | commercial |
| **G3** | Millet hay (chaffed) | *Echinochloa* sp. | commercial |
| **G4** | Guinea grass hay (chaffed) | *Panicum maximum* | local |
| **L1** | Lucerne chaff | *Medicago sativa* | commercial |
| **L2** | Verano hay (chaffed) | *Stylosanthes hamata* | local |
| **B1** | *Leucaena leaf/stem (chaffed)* | *Leucaena leucocephala* | local |
| **B2** | Acacia leaf/stem (chaffed) | *Acacia salacina* | local |

**Table 2 - Experimental diet mixes**

| **CODE ID** | **% composition for each forage in mix** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Feed Mix code** | **G1** | **G2** | **G3** | **G4** | **L1** | **L2** | **B1** | **B2** |
| **2W** | 90 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| **2X** | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| **2Y** | 90 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| **2Z** | 90 | 0 | 0 | 0 | 0 | 10 | 0 | 0 |
| **6** | 50 | 10 | 0 | 0 | 10 | 10 | 10 | 10 |
| **8** | 30 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Commercially available oaten chaff formed the base for each of the six experimental diets, comprising either 90%, 50% or 30% of each diet. Other commercially sourced forages included Lucerne chaff, Rhodes grass hay and millet hay. Verano stylo was sourced as commercial hay grown locally and comprised >90% verano. Guinea grass was sourced from standing dry material grown in large pots for another pen feeding trial. Both Leucaena and Acacia was sourced from local wild supplies, from which leaf and soft green stem (<1cm thick) material was harvested, mulched green and air or oven dried at 40$^{o}$C for 48 hours before chaffing.

Forages already sourced as commercial chaff (oats and lucerne) did not receive any further treatment. Commercial hays were chaffed using a PTO driven chaffcutter. Leucaena and Acacia were chaffed after mulching and drying using a small electric chaff cutter at Lansdown Research Station. The objective was to ensure all component forages were similar in particle size and consistency as far as possible, to enable even mixing of diets and discourage selective consumption of diet components.

Experimental diets were prepared daily in a large plastic mixing container according to the proportions listed in Table 2. Material for the four animal replicates in each diet treatment were mixed as one batch, and then weighed out according to the quantities calculated for each animal using the pre-determined 85% of *ad libitum* threshold for feed ration offered.

*(d) Feeding of experimental diets*

Feeding of experimental diets commenced on day 8 of the pen trial after animals had been on the stabilisation diets for 7 days. Experimental diets continued for 7 days. Feeding took place each morning after pens had been cleaned and refusals collected.

*(e) Animal management*

All pens were swept and hosed out each morning throughout the 7 day stabilisation and 7 day experimental diet feeding period. Water provided in 100l water containers in each pen was topped up daily and changed completely every 2 days (or more regularly if soiled by forage or faecal material). Animals were treated for buffalo fly during the pen trial and monitored for any evidence of illness, especially 3 day sickness. All animals remained healthy and injury free throughout the trial period and though feed consumption varied a little from day to day, all animals ate well throughout the trial period and consumed the vast majority of feed offered in all diets. No obvious selection of diet components was evident in terms of composition of refusals compared with diets offered.

*(f) Diet and faecal Sampling*

Samples of each individual diet component (8 samples) and also the 6 prepared diets were taken at time of feed preparation each day during the experimental feeding period. These samples were bulked across days, as per experimental schedule. Samples were placed in large zip-lock plastic bags and stored in a large esky prior to transport back to CSIRO Davies Laboratory, where they were placed in a cool room at 2$^{o}$C.

Faecal samples were collected from all individual animals on days 6 and 7 of the experimental diet feeding period, as per experimental schedule. Representative samples were collected from recently dropped faecal samples in each pen, using as large plastic spoon, cleaned between each sample. Samples were placed in individual zip-lock plastic bags, placed in a cooled esky and transported back to the CSIRO Davies Laboratory as soon as possible after collection. Samples were initially placed in a cool room at 2$^{o}$C then in a freezer at -40$^{o}$C.

Each of the 48 faecal samples was subsequently split into two replicates, one of which was sent to CSIRO Entomology, Black Mountain, ACT by TNT overnight express courier, in a foam container with dry ice. The remaining replicate faecal samples remain in the deep freeze facilities at Davies Laboratory. Replicates of the 8 individual forage components were also despatched to CSIRO Entomology, Black Mountain, ACT by TNT overnight express courier.

*(g) Extraction of DNA from cattle dung*

A total of 48 dung samples (collected at day 6 and day 7 of the experiment from each of 24 individual cows) were available for extraction of DNA. Samples (0.5 – 1.0 g) were cut from the centre of each of the 48 frozen dung samples using a new sterile surgical blade for each sample. DNA was then extracted from the samples using the QIAamp® DNA stool mini kit (Qiagen: Cat. no. 51504), following the manufacturer protocols for larger stool samples. Buffer ASL was added to each stool sample at the rate of 10 mL/g of sample.

*(h) PCR of DNA extracts*

HotStarTaq® Master Mix (Qiagen: Cat. no. 203445) was used to prepare 25 µL PCR reactions. The final $MgCL_2$ concentration of the Master Mix was 1.5 mM. The primers used in the reaction were the forward fluorescent primer trnL-F (5' -FCgAAATCggTAgACgCTACg-3') and a reverse primer, either trnL_internal1R (5' -ACggATTTggCTCAggATT-3') or trnL_internal2R (5' -TTCCATTgAgTCTCTgCASC-3'). In each reaction 2 µL of sample was used and 0.5 µL of each

primer (0.4uM). The PCR was performed on a thermal cycler with initial denaturation at $95^0$C for 10 minutes followed by 35 cycles of $94^0$C for 1 minute, $58^0$C for 1 minute, and $72^0$C for 10 seconds. This was followed by a final extension period of $72^0$C for 10 minutes, and then $30^0$C for 5 minutes. The presence of product was verified for several samples on a 1% TBE agarose gel with GelPilot 100bp Plus Ladder (Qiagen: Cat. no. 239045).

Two PCR products were obtained for each sample, one using primer trnL_internal1R and the other using primer trnL_internal2R. All PCR products were purified using the QIAquick PCR Purification Kit (Qiagen: Cat. no. 28104) according to the manufacturer spin protocol. DNA concentrations (ng/µL) were measured for all purified PCR products using an Eppendorf BioPhotometer.

*(i) Digests of PCR products*

PCR products from primer trnL_internal1R were digested using the enzyme FastDigest™ MvaI (Fermentas Life Sciences: Cat. no. FD0554). The following reaction components were combined: 17–23 µL water (nuclease free); 2 µL of 10x FastDigest™ buffer; 5-10 µL of PCR product (volume adjusted to be roughly equivalent to 0.2ug of DNA); 1 µL of MvaI enzyme. Digests were incubated at $37^0$C for 5 minutes.

*(j) Fragment Analysis*

Fragment analysis was performed on all uncut PCR products from primer trnL_internal2R and all MvaI enzyme digests using the CEQ™ 8000 Genetic Analysis System (Beckman Coulter$_{TM}$). Load quantities of products were determined using measures of DNA concentration and were approximately equivalent to 10 ng of DNA. To each well with uncut PCR product were added 38 µL of Genome Lab™ Sample Loading Solution (SLS) and 1 µL of Genome Lab™ DNA Size Standard 400 marker. To each well with digest were added 38 µL of Sample Loading Solution and 1 µL of Genome Lab™ DNA Size Standard 80 marker. Each sample was overlain with a drop of mineral oil. Uncut products were run at Frag 3 and resultant fragments were analysed using size-standard 400 parameters. Digests were run at SNP-1 for 30 minutes and resultant fragments were analysed using size-standard 80 parameters.

Presence of the different feed contents in dung samples was determined by the occurrence of fragment signals at sizes that corresponded with those expected for the respective plant species (Table 3). In uncut PCR products G3 and G4 could not be distinguished and B2 and L1 could not be distinguished. Digests only distinguished between the presence of G1 and all other feed contents. For each PCR product, a measure of the relative ratio of the different feed contents was calculated from measures of fragment signal height (rfu).

**Table 3 - The expected fragment size (nt) of each of the feed contents determined by sequencing PCR products from specimens of each of the plant species**

| Feed Code | Plant species | Fragment size (nt) Uncut | Fragment size (nt) MvaI digests |
|---|---|---|---|
| G1 | *Avena* sp | 188 | 38 |
| G2 | *Chloris gayana* | 192 | 85 |
| G3 | *Echinochloa* sp | 193 | 85 |
| G4 | *Panicum maximum* | 193 | 85 |
| B1 | *Leucaena leucacephala* | 197 | 85 |
| B2 | *Acacia salacina* | 196 | 85 |
| L1 | *Medicago sativa* | 196 | 85 |
| L2 | *Stylosanthes hamata* | 200, 210 | 85 |

*(j) Using NIRS to detect individual species in the diet*
The pen trial provided the opportunity to undertake NIRS analyses on dung samples from the pen trial to see if the variation in spectral signal could be used as a diagnostic for species consumed in the diet. Faecal samples have been analysed and David Coates is examining the spectral profiles to see if individual dietary species have an identifiable spectral signal in the dung.

### 3.3 Phase 3 - Exploring individual animal variation in foraging strategy through NIRS

Using two moderate size paddocks (450ha) at Belmont Research Station that are representative of diverse plant communities, samples of faeces were collected from individual animals on five occasions over a 12 month period. This data will allow us to determine how much diet varies between individuals using NIRS predictions of dietary crude protein.

These two paddocks were surveyed for species composition and abundance in May 2007 using a modified BOTANAL technique to start matching diet variation in NIRS analyses to forage species available in the paddock, especially grass and non-grass components.

## 4 Results and discussion

***Phase 1 - Differentiating plant species***

All 43 plant specimens that were collected from Victoria Park Station for analysis were amplified by polymerase chain reaction (PCR) using the universal chloroplast DNA primer pair trnL-F and trnL-R. Of the 43 plant specimens collected, 38 were successfully amplified using these standard primers at a fairly coarse scale. The PCR product sizes ranged from 450 base pairs to 750 base pairs. Gene sequences were obtained for the products of 32 of the 38 species that were successfully amplified by PCR (Table 4 and Figure 2).

As an exploration of the technique we were not concerned about not being able to detect all plant species as we believe this is simply an issue of finding more suitable primers for the species that currently can't be detected using standard large base pair primers.

**Table 4 - PCR Products obtained from plant specimens collected from Victoria Park Station using the trn-L primer pair**

| Genus | Species | Main functional group | Preferred species | Estimated product size from gel |
|---|---|---|---|---|
| Aristida | calycina | native_perennial_grass | | 650bp* |
| Bothriochloa | pertusa | exotic_perennial_grass | y | 550bp |
| Bothriochloa | ewartiana | native_perennial_grass | y | 550bp |
| Chrysopogon | fallax | native_perennial_grass | y | 550bp |
| Dichanthium | sericeum | native_perennial_grass | y | 550bp |
| Enneapogon | polyphyllus | native_perennial_grass | | 600bp |
| Heteropogon | contortus | native_perennial_grass | y | 550bp |
| Heteropogon | triticeus | native_perennial_grass | y | 550bp |
| Panicum | decompositum | native_perennial_grass | y | 600bp |
| Melinis | repens | exotic_perennial_grass | | 650bp |
| Sorghum | plumosum | native_perennial_grass | y | 550bp |
| Sporobolus. | australicus | annual_grass | | 680bp |
| Themeda | triandra | native_perennial_grass | y | 550bp |
| Urochloa | mosambicensis | exotic_perennial_grass | y | 650bp |

| | | | | |
|---|---|---|---|---|
| Stylosanthes | hamata | exotic legume | y | 600 bp |
| Stylosanthes | scabra | exotic legume | y | 650bp |
| Cajanus | scarabeoides | native_legume | | 450bp |
| Crotalaria | novae-hollandiae | native_legume | | 650bp |
| Crotalaria | verrucosa | native_legume | | 650bp |
| Indigofera | colutea | native_legume | | 600bp |
| Indigofera | linifolia | native_legume | | |
| Indigofera | linnaei | native_legume | y | |
| Tephrosia | rosea | native_legume | | 650bp |
| Rostellularia | acendens | forb | y | 610bp |
| Gomphrena | cellusoides/canescens | forb | y | 750bp |
| Pterocaulon. | redolens | forb | | |
| Salsola | kali | forb | | 600bp |
| Phyllanthus | maderspatensis | forb | | 630bp |
| Melhania | oblongifolia | forb | | |
| Sida | subspicata | forb | | 630bp |
| Boerhavia | schonburgkiana | forb | | 600bp |
| Portulaca | oleracea | forb | y | 750bp |
| Spermacoce | brachystema | forb | | 600bp |
| Grewia | retusifolia | forb | | 600bp |
| Solanum | dianthophorum | forb | | 620bp |
| Bursaria | incana | tree/shrub | | 600bp |
| Maytenus | cunninghamii | tree/shrub | | 550bp |
| Corymbia | erythrophloia | tree/shrub | | 600bp |
| Eucalyptus | crebra | tree/shrub | | |
| Atalaya | hemiclauca | tree/shrub | | 600bp |
| Acacia | farnesiana | tree/shrub | | 600bp |
| Acacia | bidwillii | tree/shrub | | 630bp |
| Carissa | ovata | tree/shrub | | 550bp |

** = base pair*

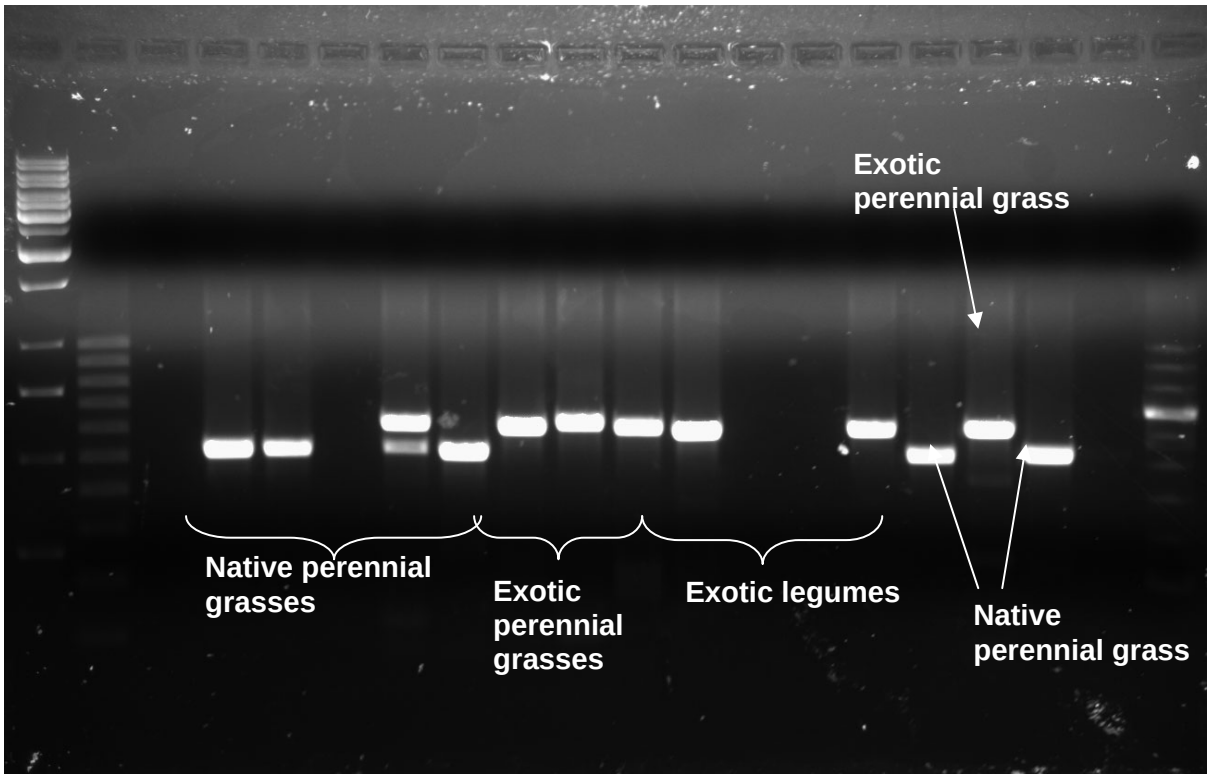



**Figure 1 - Electrophoresis in a 1.0% gel of PCR products using the trn-L primer pair obtained from plant specimens collected from Victoria Park Station**

DNA extracts of dung samples from Virginia Park Station were amplified by PCR with the trn-L primer pair and identifiable products were successfully obtained. An initial sequencing test identified three of the collected plant species in the dung samples i.e. plant chloroplast DNA was detected and sequenced. This detection of some plant species using quite coarse primers was quite surprising and encouraging as it wasn't expected that base pair fragments of 450-750 base pairs would be present in dung material that had been through a ruminant digestive tract.

Primers that detect base pair sizes in the order of 200-300 base pairs were obtained and they successfully increased the number of plant species that could be detected in the dung.

Based on these early encouraging results of being able to amplify DNA of plant species typical of northern Australian rangelands using universal chloroplast DNA primers and being able to detect most species in plant dung it was decided to move on to Phases 2 and 3 of the experiment.

***Phase 2 – Pen Studies***

The results from the pen study indicate that some DNA fragments from forage sources could be detected in the faeces using PCR techniques in 43 of the 48 dung samples (89.6%) tested.

When pooled across all animals and sampling days each of the component species could be detected in all four of the diets with 2 components. For the diets with six species and eight species not all species could be detected because of an inability to discriminate between feeds L1 and B2 (Lucerne and Acacia) in the faeces of the six species diet and between G3 and G4 (Echinochloa and Panicum) in the eight species diet. When pooled across all animals and days the five detectable species from the six species diet could be found in dung samples and similarly the six detectable species in the eight species diet could be found in the dung.

However, not all plant species could be detected in the dung from individual animals or from one day to the next (Table 5, Figure 2). For example, in only one of the four diets with two component species were the two species detected in all four individuals. In the six and eight species component diets, at least four species could be detected in all animals and in the eight species component diet, six species were detected in 75% of the animals.

**Table 5 - Percentage of individual animals in which individual dietary species could be detected**

| Diet | No of component diet species detected in faeces | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5/6 | 7/8 |
| 2W | 100 | 50 | | | | |
| 2X | 100 | 100 | | | | |
| 2Y | 100 | 50 | | | | |
| 2Z | 75 | 25 | | | | |
| 6 | 100 | 100 | 100 | 100 | 0 | |
| 8 | 100 | 100 | 100 | 100 | 75 | 0 |

The PCR technique used allows the relative amount of DNA of different plant species occurring in dung to be quantified. For all six feed mixes, the relative ratios of each of the feed contents present in dung samples (calculated from the signal strength of fragments) differed from the ratios of the contents fed to cows (Table 6, Figure 2). Generally, G1 (oaten chaff) was present in dung samples at lower ratios than what was present in feed, while B1 (*L. leucacephala*) and B2 (*A. salacina*) were present in higher ratios. Signal strengths of L1 (lucerne) and L2 (verano), when present in dung samples, were on average low compared with those of other feed contents (Figure 3).

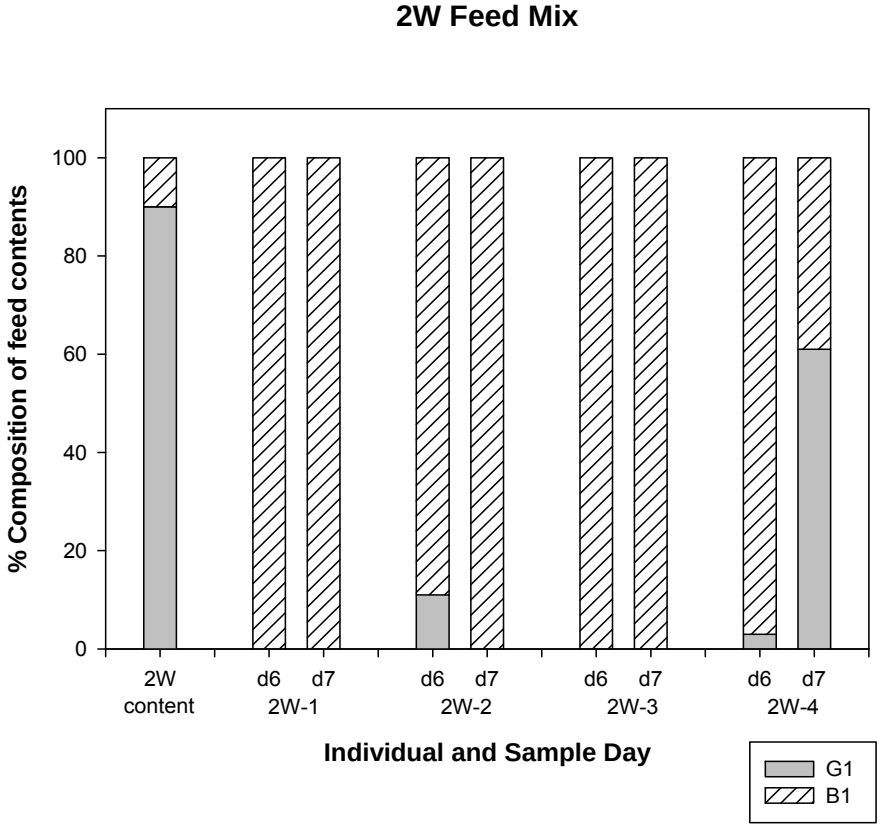
Although differences in digestibility could account for some of the difference in relative amounts of DNA in dung compared with feeds, the very large differences in relative composition in dung suggests that the amount of DNA either appearing in dung or which could be detected varies greatly from that ingested. It is more likely that it is a detection problem i.e. the primers used in this study did not amplify the DNA of the main dietary component (oaten chaff) as well as other species in the diet. More refined primers or probes could overcome this problem.

**Table 6 - Percent composition of eight feed contents**

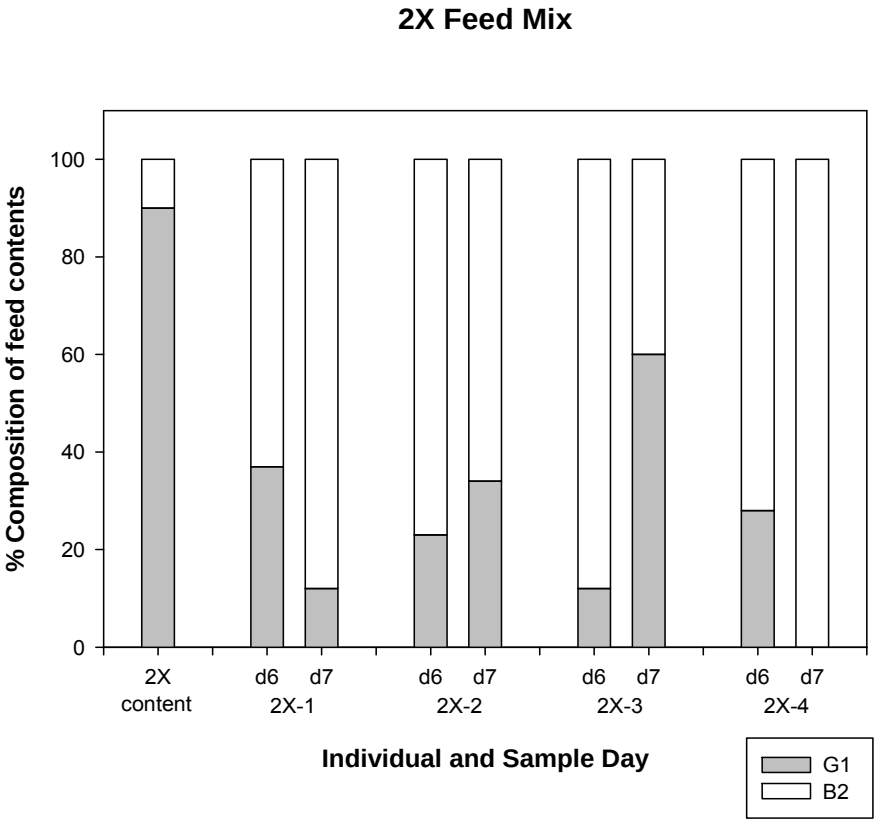
| Feed Mix | Contents | % Composition in Feed Mix | % Occurrence in Stool | |
|---|---|---|---|---|
| | | | Mean | SE |
| 2W | G1 | 90 | 9.37 | 7.46 |
| | B1 | 10 | 90.63 | 7.46 |
| 2X | G1 | 90 | 25.82 | 6.50 |
| | B2 | 10 | 74.18 | 6.50 |
| 2Y | G1 | 90 | 37.50 | 18.30 |
| | L1 | 10 | 37.50 | 18.30 |
| 2Z | G1 | 90 | 50.00 | 18.90 |
| | L2 | 10 | 12.50 | 12.50 |
| 6 | G1 | 50 | 15.37 | 4.62 |
| | G2 | 10 | 2.65 | 1.02 |
| | L1 and B2 | 20 | 7.39 | 2.71 |
| | L2 | 10 | 0.07 | 0.07 |
| | B1 | 10 | 74.52 | 6.49 |
| 8 | G1 | 30 | 7.85 | 1.89 |
| | G2 | 10 | 2.89 | 1.89 |
| | G3 and G4 | 20 | 15.25 | 3.43 |
| | L1 and B2 | 20 | 12.70 | 6.70 |
| | L2 | 10 | 2.09 | 1.86 |
| | B1 | 10 | 59.22 | 5.23 |

Percent coposition of eight feed contents (G1: *Avena* sp, G2: *Chloris gayana*, G3: *Echinochloa* sp, G4: *Panicum maximum*, B1: *Leucaena leucacephala*, B2: *Acacia salacina*, L1: *Medicago sativa,* L2: *Stylosanthes hamata* cv verano) in 6 different feed mixes fed to 24 cows; and mean (±SE) percent occurrence of the same feed contents in dung samples collected on day 6 and 7 of the experiment. Ratios of feed contents in dung were measures of fragment signal strength of PCR products.

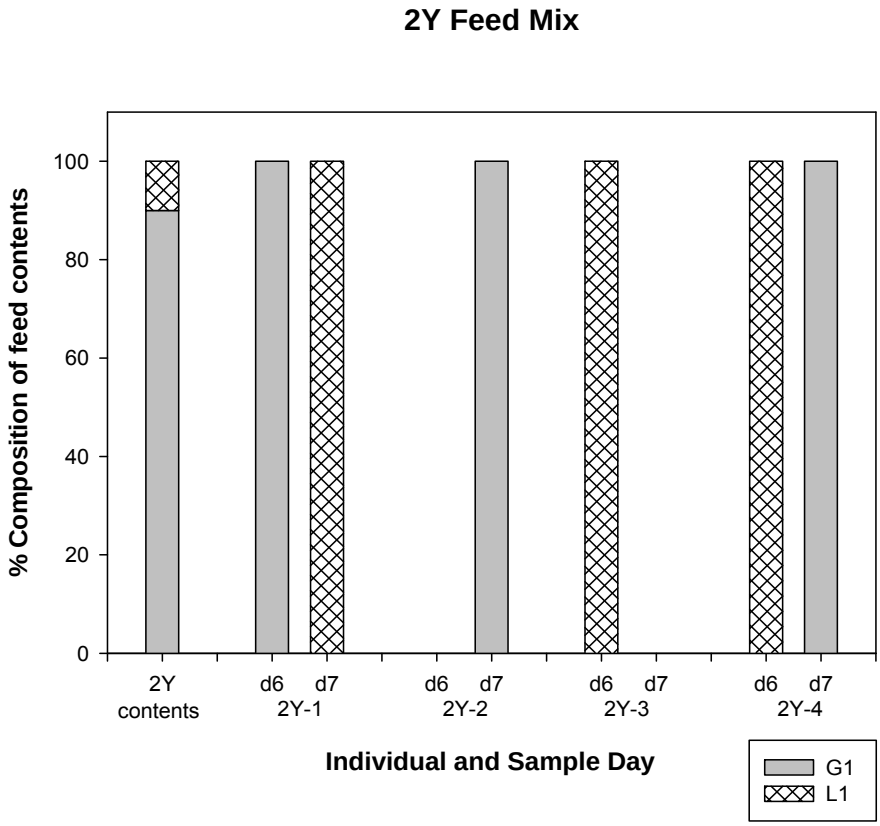
a)



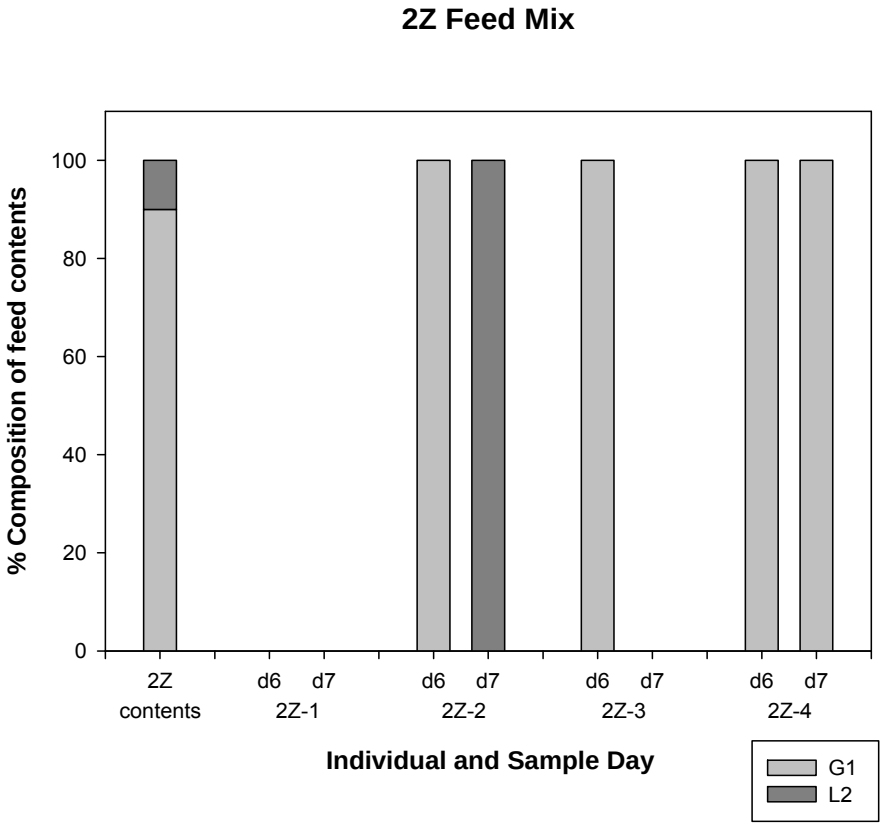
b)


2X Feed Mix
% Composition of feed contents
100
80
60
40
20
0
2X content
d6
d7
2X-1
2X-2
2X-3
2X-4
Individual and Sample Day
G1
B2

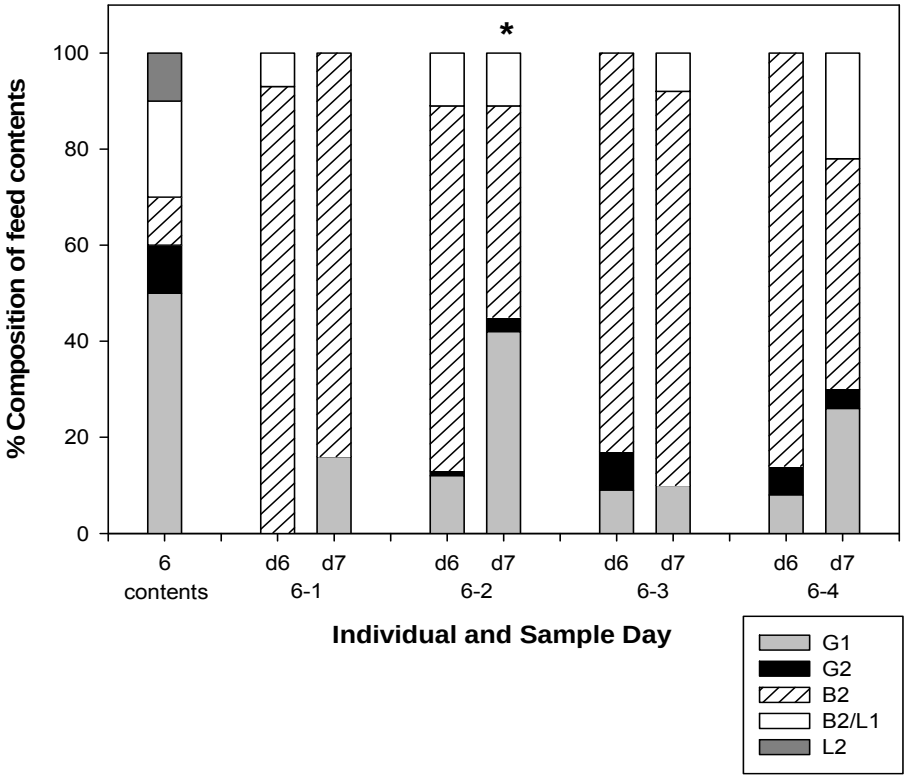
c)


2Y Feed Mix
% Composition of feed contents
100
80
60
40
20
0
2Y contents
d6
d7
2Y-1
2Y-2
2Y-3
2Y-4
Individual and Sample Day
G1
L1

d)


2Z Feed Mix
% Composition of feed contents
100
80
60
40
20
0
2Z
contents
d6
d7
2Z-1
2Z-2
2Z-3
2Z-4
Individual and Sample Day
G1
L2

e)


6 Feed Mix
*
% Composition of feed contents
100
80
60
40
20
0
6
contents
d6
d7
6-1
6-2
6-3
6-4
Individual and Sample Day
G1
G2
B2
B2/L1
L2

f)

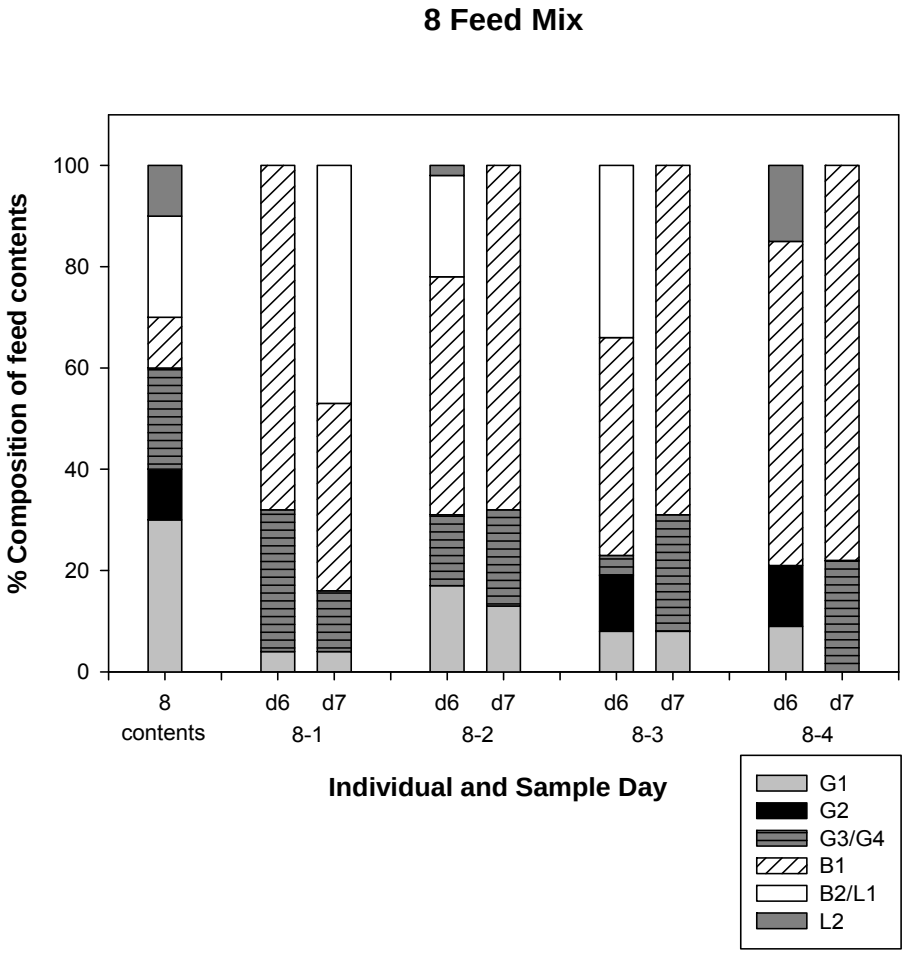



**Figure 2 -** Percent composition of eight feed contents (G1: *Avena* sp, G2: *Chloris gayana*, G3: *Echinochloa* sp, G4: *Panicum maximum*, B1: *Leucaena leucacephala*, B2: *Acacia salacina*, L1: *Medicago sativa,* L2: *Stylosanthes hamata* cv verano) in six different feed mixes: a) 2W, b) 2X, c) 2Y, d) 2Z, e) 6, and f) 8, each fed to 4 individual cows; and the ratios of the same food contents in stool samples collected from individual cows on day 6 and day 7 of the experiment (as determined by CEQ fragment analyses of PCR products). * L2 present at <1%.

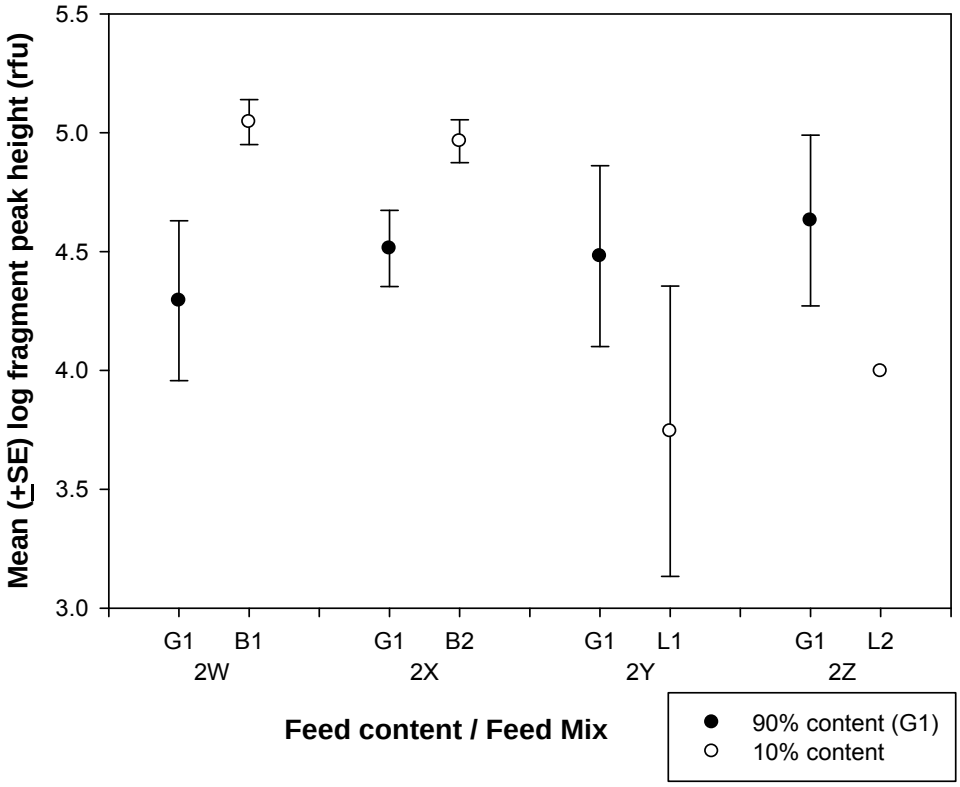
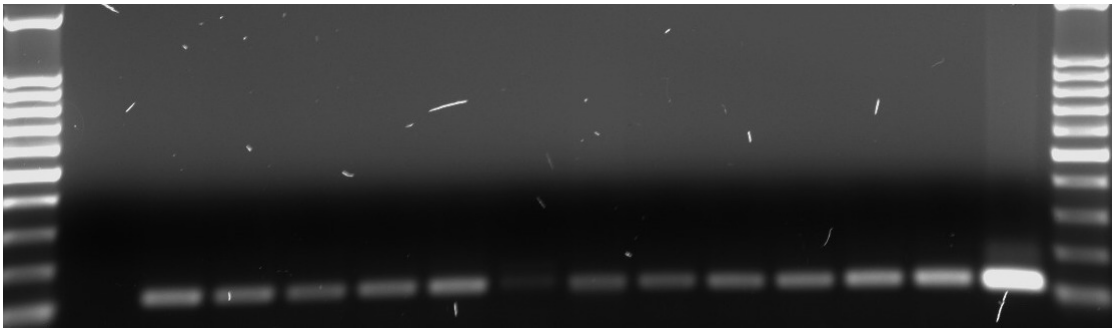



**Figure 3 -** Logarithmic mean (±SE) values of fragment peak heights (rfu) of feed contents (G1: *Avena* sp, B1: *Leucaena leucacephala*, B2: *Acacia salacina*, L1: *Medicago sativa,* L2: *Stylosanthes hamata* cv verano) in PCR products of stools from cows fed dual feed mixes (2W, 2X, 2Y and 2Z). Fragment signal strengths for each feed mix group are averages of 8 measures from stool samples collected from 4 cows on day 6 and day 7 of the experiment.

Fragment analyses of MvaI digests supported the results for the uncut PCR products, indicating the presence of feed contents other than the known dietary components occurring in nearly all individual dung samples and in every diet (Figure 4). The G1 feed (Oaten chaff) was common to all diets and the detection of this "foreign" forage component suggests a contaminant in the oaten chaff. These unknown DNA fragments could be identified as coming from the Solanum genus indicating it as the contaminant source in the oaten chaff.

a)



b)

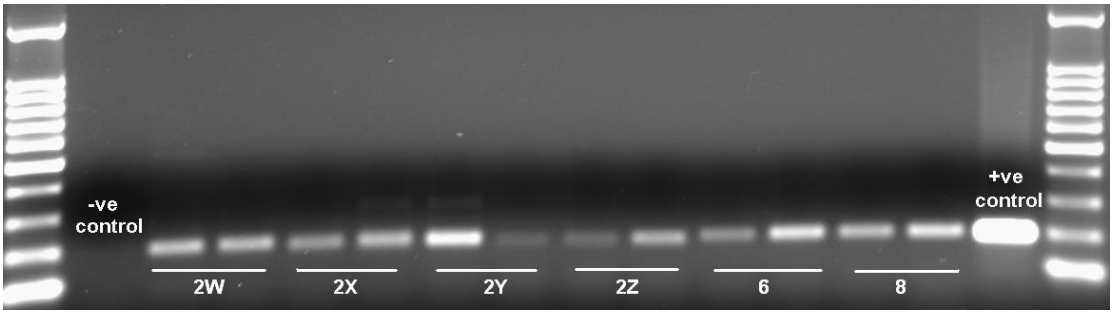



**Figure 4 -** 1% TBE agarose gel of cow stool PCR products using the forward primer trnL_F and a) the reverse primer trnL_internal1R; and b) the reverse primer trnL_internal2R. Two separate samples from all six feed groups (2W, 2X, 2Y, 2Z, 6 and 8) are represented and bands at 160 and 200 for the respective 1R and 2R reverse primers indicate the presence of product.

### *Phase 3 – Paddock sampling for individual diet quality*

It took quite a bit of time to locate suitable paddocks that were large enough to have appropriate plant diversity and where it was possible to muster individual animals on a regular basis. Eventually two paddocks, approximately 450 ha in size, at Belmont Research Station were found to be suitable for this phase of the experiment. However, individual animal sampling did not commence until February 2007. Dung sampling is still in progress and while initial samples have undergone NIRS analysis it is too early for trends to be evident.

Full results from this phase of the study won't be available until later in 2007 when a larger sequence of individual animal data has been collected. Paddock BOTANAL analyses undertaken in late May 2007 are not yet available.

# 5 Success in achieving objectives

## 5.1 Achievements

Objective 1. Develop a DNA technique that will allow individual plant species to be identified in dung samples collected from a paddock.

This initial exploration of DNA approaches to detecting plant species consumed by ruminants has proved promising. Fairly coarse universal primers were successful in differentiating a wide range of plant species typically found in north-east Queensland rangelands using standard genetic techniques i.e. polymerase chain reaction (PCR), which allows small genetic fragments to be amplified large enough to be detected visually in electrophoresis gels. Further refinement of primers used should allow nearly all species in these complex rangeland environments to be differentiated.

Initial testing of dung samples from the same paddocks where plant species were collected indicated that plant DNA fragments could be recovered and identified to species level.

On the basis of these initial encouraging results a pen study was undertaken where diets of differing numbers of component species were fed to cattle and the dung analysed to determine whether known dietary components could be detected. In the more complex diets, the majority of species (70%) could be detected. Given that this exploratory study used fairly coarse universal primers the fact that not all species could be detected is not a major concern.

This study also examined whether the relative amounts of species could be detected in the dung. The relative amount of DNA material in the dung was not at all consistent with that in the diet. These differences could not be explained by differential digestibility of species as it would be expected that digestibility of the species used would be in the range of 45%-65%. It is likely that the universal primers used in this study were differentially amplifying the DNA extracted from dung.

Interestingly, the technique in this study was able to detect a contaminant in all diets. Oaten chaff was the only species common to all diets so it is likely that the contaminant, identified as from the Solanum genus by the PCR technique, was present in the oaten chaff. This result highlights the potential for using this sort of technique for identifying contaminants in commercial hays and weed contaminants in pasture seed.

The goal of being able to identify all species in the diet and the relative contributions of dietary components could be achieved using new DNA microarray approaches, which allow much better targeting of species through “designer” probes. The microarray itself is a small chip (Fig. 5), about the size of a finger nail. Its surface, usually made of glass or perspex, has thousands of spots and in each of these spots are thousands of DNA probes. A set of targets, in this case DNA in dung samples that has been extracted and amplified and labelled with fluorescence, can be applied to the chip and where there is a match between the probe and the target, the gene is expressed as being fluorescent and the chip can be scanned by a computer to identify target species.


**Figure 5 - Example of a microarray gene chip**

With these new approaches it is envisaged that the technique explored in this pilot trial could be applied quite inexpensively if appropriate microarray chips are developed. The application of such a technique would not be confined to just free-ranging livestock in the rangelands but would have much wider potential application in wildlife herbivore studies.

Objective 2. Improve understanding of factors that contribute to individual variation in diet quality and use this understanding to develop strategies that better target nutritional management.

Only modest progress was achieved on this objective due to difficulties associated with obtaining individual animal faecal samples on a regular basis. Two paddocks were used at Belmont Station to sample individual animals (3 to 4 per paddock) over a 12 month period to observe individual animal variation in diet quality.

Figure 6 shows how dietary crude protein varied between individual animals throughout a 12 month period. Even with a small sample size of four animals per paddock there were sampling periods when there was quite a large range amongst individuals in NIRS-predicted dietary crude protein, at times over 3% units different. Some of this variation would be due to prediction error in the NIRS method. However, assuming at least some of the variation represents variation amongst animals, it could have implications for animal performance especially when dietary crude protein is near maintenance levels.

The amount of C3 plant species in the diet can explain some of the individual animal variation (approximately 40%) in diet quality but other factors contribute most of the individual animal variation. There was relatively little difference in diet quality between the two paddocks, which is not surprising as both were dominated by perennial grasses, though there were some significant differences in the make-up of perennial grass components (Table 7).

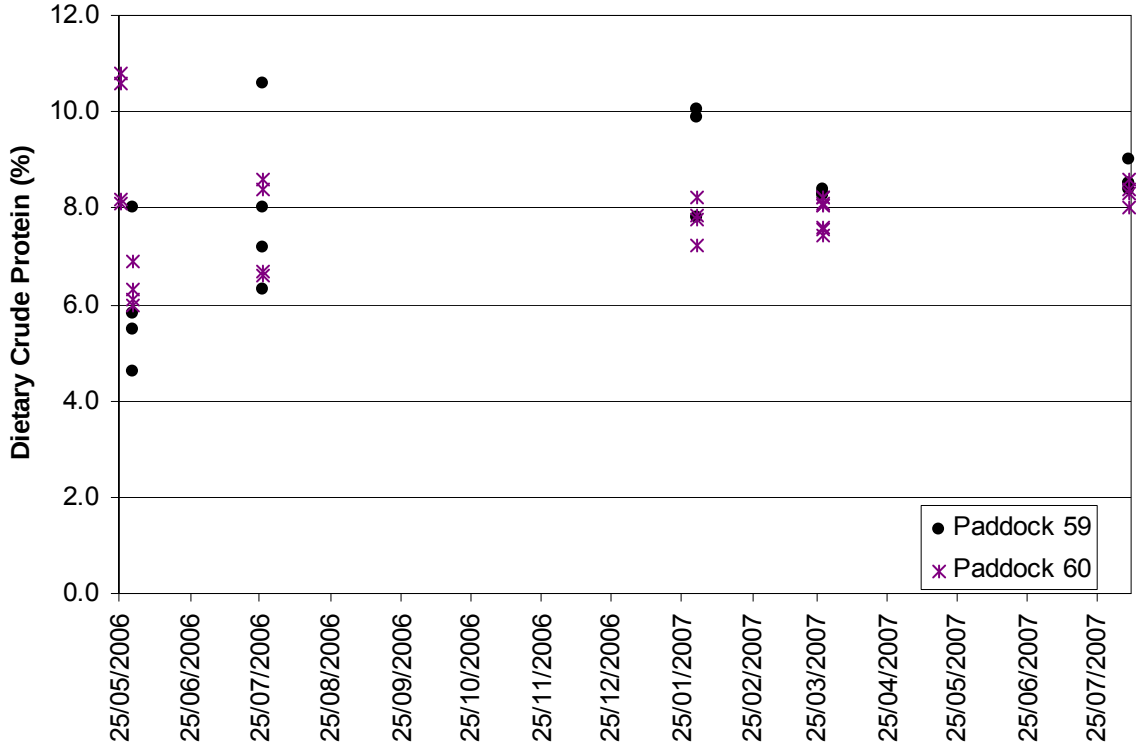



**Figure 6 - Dietary crude protein of individual animals over a 12 month period in two paddocks at Belmont Research Station**

**Table 7 - Botanical characteristics of the two paddocks at Belmont Research Station where individual animal faecal samples were collected**

| | Paddock 59 | Paddock 60 |
|---|---|---|
| Above-ground standing biomass (kg DM/ha) | 2069 | 1728 |
| Cover (%) | 88.2 | 84.8 |
| Defoliation (%) | 56.9 | 57.6 |
| Decreaser native perennial grasses (%) | 22.9 | 36.3 |
| Increaser native perennial grasses (%) | 37.9 | 9.6 |
| Exotic perennial grasses (%) | 26.5 | 46.3 |
| Annual grasses (%) | 0.0 | 0.0 |
| Native legume (%) | 0.8 | 0.0 |
| Forbs (%) | 11.9 | 7.2 |
| Stylosanthes (%) | 0.2 | 0.0 |

# 6 Conclusions and recommendations

This study has demonstrated that it is possible to identify gene sequences in plant material in dung and match this to DNA of collected plant species. It is recommended that further effort be devoted to refining the approaches developed in this pilot study to develop a practical and effective research technique for identifying species in the diet of rangeland livestock. Such a technique would be a useful research tool for better understanding grazing behaviour with the ultimate aim of improving both animal nutrition and grazing management in the rangelands.