Final report

Desmanthus legume in livestock grazing pastures and its role in methane emissions

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Prepared by: Ed Charmley
CSIRO

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Meat and Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

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Abstract

Methane is a greenhouse gas produced as a by-product of fermentation of feedstuffs in ruminants. *Desmanthus* is a tropical legume adapted to parts of northern Australia. Laboratory studies have demonstrated that *Desmanthus* can reduce the production of methane when incubated with rumen fluid. The objective of this project was to determine if methane production could be reduced by feeding *Desmanthus* to cattle and to provide data to support a methodology allowing the avoided emissions to be traded in the carbon market. Several cultivars developed by JCU and Agrimix Pastures Pty Ltd were tested in three cattle feeding trials. In the first trial, including *Desmanthus* up to 31% of the diet, methane reduced according to the relationship: *methane yield (g/kg dry matter intake) = 19.89 – 0.066 x % Desmanthus in the diet*. Based on these results, a diet comprising 30% *Desmanthus* would reduce methane yield by 10%. In two subsequent trials, there was no difference in methane yield when *Desmanthus* was included in the diet. The variation in response was attributed to the nutritive value of the companion grass being lower in trial one compared to trials two and three. The outcomes of this project will help in the development of a legumes methodology for the ruminant industries.

Executive summary

Background

Methane is a greenhouse gas produced as a by-product of fermentation of feedstuffs in ruminants. *Desmanthus* is a tropical legume adapted to parts of northern Australia. Laboratory studies have demonstrated that *Desmanthus* can reduce the production of methane when incubated with rumen fluid. The objective of this project was to determine if methane production could be reduced by feeding *Desmanthus* to cattle and to provide data to support a methodology allowing the avoided emissions to be traded in an emissions reduction scheme.

Objectives

The principal objective was to quantify the emissions of methane from growing cattle fed *Desmanthus* cultivars at a range of dietary inclusion levels. The hypothesis was that increasing the amount of *Desmanthus* in the diet would reduce the emissions of methane. Several Progardes® *Desmanthus* cultivars were evaluated. The data was used to provide evidence to the Clean Energy Regulator for the development and approval of a methodology under the Emissions Reduction Scheme; http://www.cleanenergyregulator.gov.au/ERF/About-the-Emissions-Reduction-Fund. The secondary objective of the research was to develop methods for quantifying the reduction in methane emissions under commercial conditions.

Methodology

There were three trials with cattle where freshly harvested *Desmanthus* was added to hay-based diets at levels varying from 0 to 45% of the diet. In two trials with steers, methane was measured using open path respiration chambers. In a third trial, methane was measured using a gas emissions monitoring (GEM) system that allows individual methane measurements from groups of cattle. A range of further measurements were taken that contributed to developing a method for quantifying methane emissions under commercial conditions.

Results/key findings

Desmanthus, included at 30% of the diet, can reduce methane emissions from cattle by up to 10% when the nutritive value of the diet is low (5 to 8% crude protein). This response depends, however, on the conditions under which *Desmanthus* is fed and no reduction in emissions was observed when the diets were of higher nutritive value.

Benefits to industry

This work will contribute to the development of a legumes methodology that will allow producers to earn revenue under the Emissions Reduction Scheme. It will further increase the adoption of *Desmanthus* as an adapted legume for areas of Queensland, the Northern Territory and northern New South Wales.

Future research and recommendations

The equivocal findings of this research suggest further broad-scale assessment of the variability in methane reduction from *Desmanthus* pastures is warranted. This could be achieved using a field-based system to achieve scale of measurement corroborated by controlled experimentation where the proportion of *Desmanthus* in the diet and quality of the non-legume component of the diet can be controlled. Establishing the cause of reduced methane production is also required.

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1. Background

1.1 *Desmanthus* in northern grazing systems

The northern beef industry is valued at approximately $7 billion a year (Chilcott et al, 2020). In northern Australia, livestock are typically fed high roughage diets with crude protein (CP) content less than 6% for approximately six months of the year (Hunt et al, 2014). The introduction of exotic legumes is an economically viable means of addressing the issue of low protein and improving animal productivity in the dry season. A range of legume species including stylos (*Stylosanthes* spp.), *Leucaena* and *Desmanthus*, have been evaluated over the years. The search for a legume adapted to low rainfall (<600 mm/yr) and cracking clay (vertisol) soils, however, has proven difficult.

Desmanthus is native to the Americas and is a legume that persists under heavy grazing on clay soils (Pengelly and Conway, 2000). The Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Queensland Department of Primary Industries (QDPI) have introduced numerous accessions of *Desmanthus* over the past 50 years (Pengelly and Liu, 2001). In the 1990s, Chris Gardiner at James Cook University (JCU), Townsville found that various *Desmanthus* accessions had persisted for more than two decades in abandoned trial sites across remote northern and central western Queensland, on semi-arid clay soils. The best of these varieties have been released by Agrimix Pastures Pty Ltd, JCU's commercialisation partner, as Progardes®. The three species that comprise the Progardes blend are *D. bicornutus* (JCU 4), *D. leptophyllus* (JCU 1 and 7) and *D. virgatus* (JCU 2, 3 and 5). These lines have been chosen to represent a range of climatic and edaphic tolerances and vary in maturity rate (Gardiner et al, 2016). Methods of establishment, nutrient requirements, and management techniques have been developed and Progardes® mixtures have been established across approximately 50,000 hectares in Queensland.

1.2 *Desmanthus* and animal performance

A number of studies have evaluated the animal response to including *Desmanthus* in the diet or pasture. Gardiner and Parker (2012) demonstrated that steers grazing a mixed buffel grass-Progardes® pasture during the dry season in central Queensland gained an extra 40kg liveweight (LW) over a 90-day period compared to steers on a Buffel grass-only pasture. Another central Queensland study has shown that cattle grazing paddocks containing Buffel grass and Progardes® with a population of seven plants/m^2 consistently gained and additional 40kg/head over several grazing seasons compared to steers grazing Buffel grass only (Collins et al., 2016). A 56-day feeding trial with growing goats compared the growth response to *D. bicornutus* with that from Lucerne when both were fed at 40% of the diet. Average daily gains were of 60.9 and 82.3g/day for Lucerne and *Desmanthus*, respectively (Kanani et al., 2006). Rangel and Gardiner (2009) showed the potential advantage of providing 30% *Desmanthus* to sheep on a Mitchell grass hay diet. They observed reduced weight loss, higher feed intake and wool growth over the six-week experimental period. In another study with sheep, weight gains were increased by supplementing *D. leptophyllus* cv JCU 1 to a Flinders grass diet (Ngo, 2017).

1.3 *Desmanthus* and GHG emissions

Poor quality grass-based diets are characterised by high rates of rumen methanogenesis due to the predominance of an acetate dominated fermentation in the rumen and low rates of digesta passage from the rumen. Under these conditions, the production of methane from the diet can account for up to 7% of dietary energy (Kennedy and Charmley, 2016). Forage legumes can decrease methane

emissions partly due to lower fibre content, faster rate of passage and in some cases, the presence of condensed tannins (Beauchemin et al., 2008, Eckard et al., 2010). A number of legume species have been shown to have antimethanogenic properties when fed to ruminants. These include both temperate species such as bird's foot trefoil (*Lotus conicularlus*) and sanfoin (*Onobrychis sativa*; Beauchemin et al. 2008) as well as tropical species such as Leucaena (*Leucaena leucocephala*) and *Acacia* species (Archimède et al. 2016). *In vitro* research with several Progardes® lines showed a reduction in methane gas production when the lines were incubated with rumen fluid in the laboratory (Vandermeulen et al. 2018). The research showed that antimethanogenic potential varied according to season and cultivar as shown in Figure 1.

Figure 1. *In vitro* methane gas production in *Desmanthus* cultivars relative to Rhodes grass (After Vandermeulen et al. 2016).

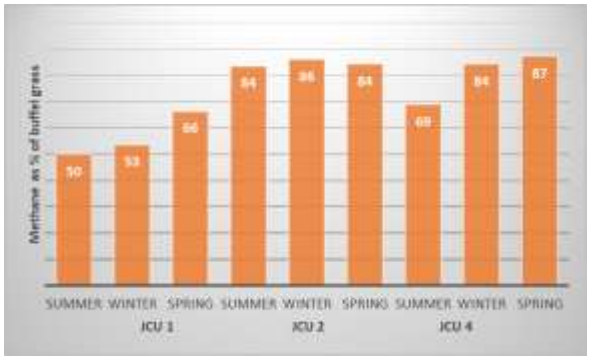
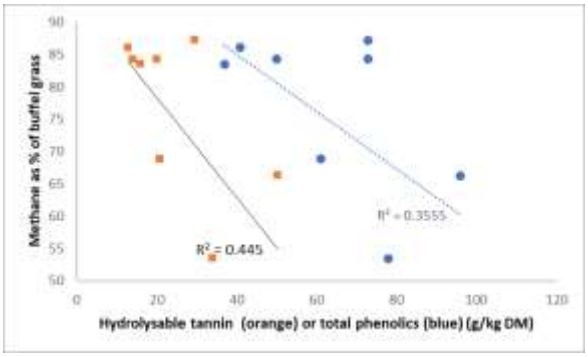
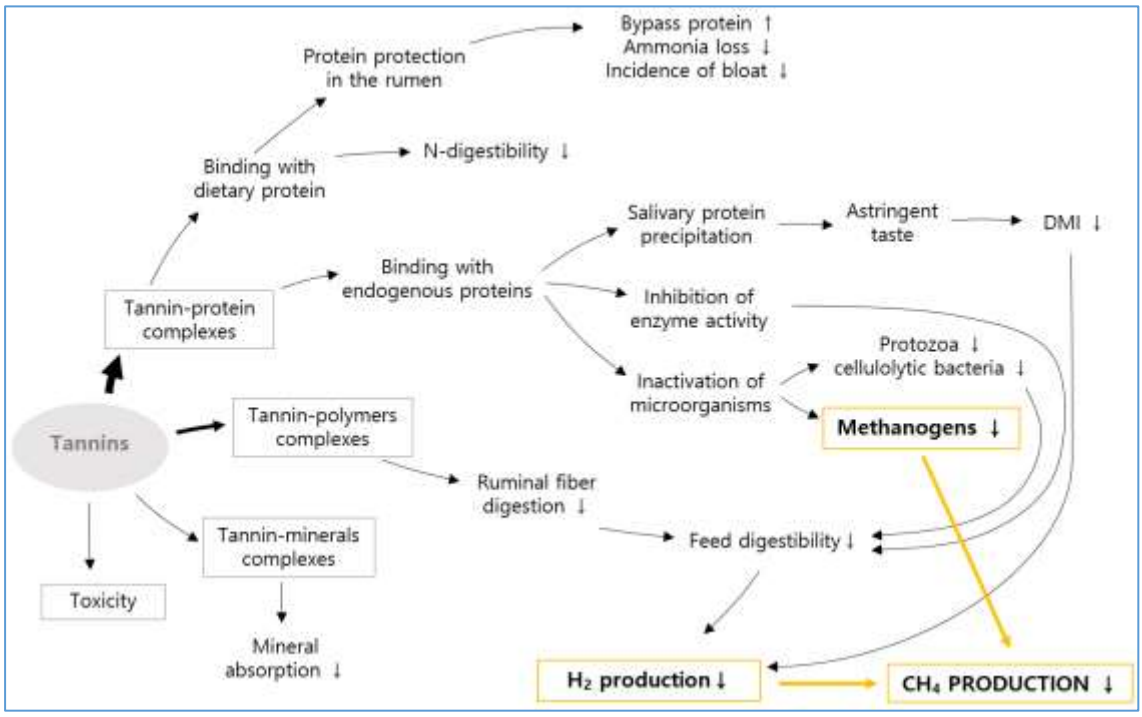


Figure 2. Relationship between tannin concentration and i*n vitro* methane gas production in *Desmanthus* cultivars relative to Rhodes grass (After Vandermeulen et al. 2016).



These researchers also noted a negative correlation between the level of tannins in the *Desmanthus* treatments and the reduction in methane gas production (Figure 2). The concentration of total phenolics and hydrolysable tannins accounted for 35 and 45% of the variation in methane production, respectively. Tannins are polyphenolic secondary plant products that are common in the plant kingdom. Figure 3 provides an overview of the potential modes of action for the effects of tannins on methane production in the rumen. As they bind to proteins, they are considered as "anti-nutritional" compounds which reduce protein digestibility. McMahon et al. (1999) reported that high tannin levels (exceeding 40 to 50g/kg dry matter) in forages may reduce protein and dry matter (DM) digestibility of the forages by ruminants. However, they showed that at low to moderate levels, condensed tannins increase the quantity of dietary protein, in particular, essential amino acids.

Figure 3. Simplified schema of tannin interactions and their mode of action on methanogenesis. DMI=dry matter intake; H_2=dihydrogen; CH_4= methane; N=nitrogen (after Stifkens 2019).



The evidence that tannins may reduce methanogenesis *in vivo* is equivocal and the mode of action not well understood (Tavendale et al. 2005). Feeding tanniferous plants has been shown to be associated with reduced methane production in some cases (e.g. Animut et al. 2008; Yang et al. 2017) but not in others (Soltan et al. 2013). Adding tanniferous compounds to the diet appears to be effective in reducing methane emissions but is often also associated with a reduction in protein and diet digestibility (Moate et al. 2014, Caetano et al. 2019). It would appear that modest levels of condensed tannins (20 to 40 g/kg DM) are associated with anti-methanogenic activity without the associated negative effects on diet digestibility.

1.4 Methane abatement and abatement incentives

Livestock methane emissions account for approximately 10% of Australia's national greenhouse gas inventory and approximately half of these are attributed to the northern cattle industry. The potential antimethanogenic activity in *Desmanthus* could form the basis of a legume methodology to allow producers to earn carbon credits from emissions avoided in *Desmanthus* grazing systems. Assuming a 10% reduction in methane emissions across the 50,000 hectares currently established with Progardes®, this would eliminate approximately 10,000 tonnes of GHGs (as CO_2 equivalents) a year. The *in vitro* evidence given above suggests that *Desmanthus* could reduce methane emissions by between 10 to 50%. Consumer acceptance of red meats is threatened by their relatively high GHG emissions per unit of meat, being between 10 and 12kg CO_2 equivalents per kg LW, depending on the production system (Wiedemann et al. 2016). Consequently, MLA have a goal for the red meat industry to become carbon neutral by 2030. Therefore, reducing enteric (produced in the digestive system) methane emissions could create a new revenue stream for farmers, reduce their risk of declining market share and maintain a viable and sustainable rural industry across Australia.

1.5 Requirements for an abatement methodology

To include *Desmanthus* in a methodology for producing carbon credits, a means of measuring the reduction in methane is required via a verifiable method to quantify the magnitude of methane reduction. With legumes and other plants, the antimethanogenic potential can vary depending on the conditions under which the plant is grown and consumed by grazing livestock. Under such circumstances a conservativeness factor is applied in recognition of the uncertainty around the expected reduction in emissions. The greater the uncertainty, the higher the discounting on the value of the carbon credit. Thus, to maximise the revenue from carbon it is incumbent on the developers of the methodology to understand the factors that affect the variation in response. For an antimethanogenic plant species in a grazing environment these include:

- The proportion of that plant in the pasture
- The selectivity with which ruminants graze that plant, i.e. how much do they eat
- The concentration and activity of the bioactive compound in the plant
- The conditions in the rumen that are influenced by the diet.

The proportion of *Desmanthus* in the pasture can be estimated from direct measurements on the ground or using remote monitoring from satellites or drones. The intake of the plant can be measured using markers in the faeces such as carbon isotope ratios. The activity of the bioactive compound(s) can be difficult to estimate but markers could be developed for specific compounds. For legumes the compounds most often implicated in methane reduction are tannins. Characterisation of factors that influence the mitigation response is a critical component of a legumes methodology.

1.6 Conclusions

The industry requirement for a legume adapted to low rainfall and clay soils and the apparent suitability of Progardes® *Desmanthus* cultivars to meet this need will lead to increasing inclusion of *Desmanthus* in pastures across many areas of Queensland, northern New South Wales and parts of the Northern Territory. With a range of species and cultivars to suit the variation in edaphic and climatic conditions across the north and proven establishment techniques, the area sown to *Desmanthus* will increase. A perennial, productive and persistent legume for the north will improve nutritive value of pastures, particularly in the dry season, and boost animal productivity. The potential bonus of reducing methane emissions will assist the industry in meeting its Carbon Neutral 2030 target and improve consumer acceptability for beef. The small financial gain at current carbon prices is expected to increase as the carbon price rises.

The results of this work will provide scientific evidence to support a pasture legume emissions methodology under the Australian Government's Emission Reduction Scheme; http://www.cleanenergyregulator.gov.au/ERF/About-the-Emissions-Reduction-Fund. This work will demonstrate a dose-dependent relationship between the proportion of *Desmanthus* in the diet and methane reduction and evaluate this response across several *Desmanthus* species and cultivars. Importantly, the experimental work will be corroborated with data from commercial plantings across properties in Queensland.

2. Objectives

The principal objective of this contract was to quantify the emissions of methane from growing cattle fed *Desmanthus* cultivars at a range of dietary inclusion levels. The hypothesis was that

increasing the amount of *Desmanthus* in the diet would reduce the emissions of methane. Several Progardes® cultivars were evaluated, these being JCU 1 (*D. leptophyllus*), JCU 2 (*D. virgatus*), JCU 4 (*D. bicornutus*) and JCU 7 (*D. leptophyllus*). The data will be used to provide evidence to the Clean Energy Regulator for the development and approval of a methodology under the Emissions Reduction Scheme, http://www.cleanenergyregulator.gov.au/ERF/About-the-Emissions-Reduction-Fund. The project also allowed for training of a PhD student who was seconded to the project, while funded through the CRC-Project *"New pastures to increase livestock productivity across the north"* (CRC-P58599). The secondary objective of the research was to demonstrate how the reduction in methane emissions can be quantified under commercial conditions. This scientific evidence would be used to allow for verification of mitigation potential.

Detailed objectives as outlined in the project schedule are given below.

Trial 1. Determining the antimethanogenic potential of the forage *Desmanthus* when fed at graded levels to beef cattle:

Determine the relationship between the proportion of *Desmanthus* in the diet and the reduction in methane emission (as g/day and g/kg Dry Matter (DM) intake) using closed circuit respiration chambers.

1. To determine the effect of cultivar on the reduction in methane emission (as g/day and g/kg DM intake) using open circuit respiration chambers.
2. Measure the changes in rumen microbiota and end products of rumen digestion in response to *Desmanthus* inclusion in the diet to ascertain mode of action.

Trial 2. Evaluating the anti-methanogenic potential of three *Desmanthus* cultivars when fed to cattle:

1. To determine the effect of substituting Lucerne hay with one of three *Desmanthus* cultivars on the reduction in methane emission (as g/day and g/kg DM intake) using open circuit respiration chambers.
2. Measure the changes in rumen end products of rumen digestion in response to *Desmanthus* inclusion in the diet to ascertain mode of action.

Trial 3. Methane emissions from a pen feeding trial with cattle fed varying levels of a mixture of three *Desmanthus* cultivars (JCU 2, 4 and 7).

1. To measure growth performance and carcase characteristics in growing steers fed a forage-based backgrounding diet where the percentage of *Desmanthus* in the diet is increased from 0 to 45%.
2. To measure methane emissions from growing steers fed a forage-based backgrounding diet where the percentage of *Desmanthus* in the diet is increased from 0 to 45%.

2.1 Success in meeting the objectives

Milestones related to the two research trials have all been met, with the exception that rumen microbiota samples were taken but results have not yet been received from the commercial provider.

Data is being used to support the development of a legume methodology co-ordinated by Tom Davison (University of New England) and Steve Wiedemann (Integrity Ag and Environment Pty Ltd) with support of Agrimix Pastures and MLA as part of an amendment to the current project (or a new MDC project). The new method development would be conducted in full consultation with the Department of Environment and Energy.

Additional research has been completed that was not in the original objectives on:

- Characterising the tannin content of *Desmanthus* and elucidating its role in reducing methane, and
- Evaluating methane emissions from a pen-based growth trial from gas emission measurements using GreenFeed™ technology. This work accompanied a feeding trial under the CRC project *"New pastures to increase livestock productivity across the north"* with Agrimix Pastures Pty Ltd as lead organisation.

3. Methodology

3.1 Methods common to all trials

3.1.1 Animal training

Cattle were trained to allow familiarization and adaptation to the methane chambers over a period of four weeks when hay was fed. Cattle were group housed for three to four weeks during which time they were moved individually to methane chambers and walked through the methane chambers with both back and front doors open, i.e. unrestricted. Following three to five passes the doors were closed to hold animals for four, then 18 then 22 hours. Training for use of the GEM monitoring system simply involved coaxing cattle to the units with a food reward.

3.1.2 Baseline measurements

Once cattle were adapted to a 23-hour duration in chambers and *ad libitum* hay intake established over a week, animals were sequentially rotated through the chambers to provide baseline measurements of methane emissions from hay only. When not in chambers, cattle were held in individual pens throughout the study. Cattle were weighed at the beginning and again at the end of hay feeding.

3.1.3 Feed analysis

Dry matter content of dietary components and experimental diets was determined by drying samples to a constant temperature at 60°C in a fan forced oven for 48 hours. The DM was calculated as the difference between the initial and final weights of samples expressed as a percentage. The oven dried samples were ground to pass through a 1-mm screen using a Tecator Cyclotec 1093 (FOSS, Hillerød, North Zealand, Denmark) for neutral (NDF) and acid detergent fibre (ADF) and total nitrogen analysis. NDF and ADF were measured using an ANKOM 200/220 Fibre Analyser (ANKOM Technology, Fairport, NY, USA). The analysis for total nitrogen was determined by combustion using a Leco CN628 N Analyser (Leco, St. Joseph, MI, USA; Sweeney et al. 1987) and the values multiplied

by 6.25 to give the crude protein (CP) percentage. *In vitro* dry matter digestibility (DMD) was determined using a modified pepsin-cellulase technique described by Clarke et al. (1982).

3.1.4 Tannin analysis

Desmanthus cultivars were freshly sampled every week. The samples were stored at -20°C, then freeze-dried at -50°C for three days in a freeze dryer (Labogene ScanVac CoolSafe freeze dryer, Bjarkesvej 5 DK-3450, Allerød, Denmark) and ground to pass a 1-mm screen using a Tecator Cyclotec 1093 (FOSS, Hillerød, North Zealand, Denmark) and stored at room temperature (Terrill et al. 1992). The freeze-dried material was passed through a 0.25 mm sieve before analysis. The tannin extraction from the samples was done according to the Terrill et al. (1982) method except that the supernatant was increased to 300µL total volume with distilled water.
Proanthocyanidin concentration (condensed tannins) was estimated by the Butanol-HCL-Fe^{III} method using purified *Desmanthus* condensed tannin as the standard with absorbance detection at 550 nm (Makkar, 2003; Porter el al. 1985). Condensed tannins (CT) were purified on Sephadex LH-20 as described by Wolfe et al. (2008). Total phenolics concentration was determined by the Folin-Ciocalteu method with catechin as the standard (Makkar, 2003).

3.1.5 Animal performance measures

The LW of each animal was recorded weekly prior to feeding to determine the daily LW gain. Individual dry matter intake (DMI) was determined by measurement of difference between offered feed and feed remaining after 23 hours. Individual daily intakes were recorded throughout the study to determine treatment group DMI. These values were used to calculate the DMI expressed as percent liveweight and to express the methane yield on a per kg DMI basis.

3.1.6 Rumen collection and rumen metabolite analysis

Rumen fluid samples were collected via oral stomach tubing using a reinforced plastic suction tube (approximately 3 cm diameter). A hand pump was used to extract 100-200 mL of rumen fluid from the ventral sac. The rumen fluid was collected three hours post-feeding following confinement in respiration chambers. Rumen fluid was measured for pH and a sub-sample taken, mixed with fresh 20% metaphosphoric acid (4:1) and frozen at -80°C for rumen volatile fatty acids (VFAs) and NH_3-N analyses.
Rumen fluid concentrations of short chain fatty acids (acetate, propionate, n-butyrate, iso-butyrate, iso-valerate, n-valerate and n-caproate) were measured by gas chromatography as described by Gagen at al. (2014). The rumen NH_3-N concentration was determined by the colorimetric method of Chaney and Marbach (1962).

3.1.7 Measurement of methane emissions

Four open-circuit respiration chambers were used to assess CH_4 production from individual steers as described by Martinez-Fernandez (2016). Briefly, methane emissions were measured using independent units (23.04 m^3, 3000 L/min airflow) equipped with drinking water and a feed bin containing the daily ration. The atmosphere inside the chambers was maintained at 2°C below ambient temperature, approximately -10 Pa and a relative humidity between 50 to 75%. The exact

flow rates of each chamber were corrected to measured conditions for temperature and pressure to calculate methane production. Steers remained in the chambers for 48 hours with CH_4 monitored continuously by infrared absorption (Servomex 4100, Servomex Group Ltd. Crowborough, UK). Methane production was calculated by averaging two 24 hour measurements. DMI in the chamber was also recorded daily to calculate the CH_4 emissions according to feed intake (CH_4 yield expressed as g/kg DMI).

3.1.8 Faecal sampling and analysis

Faeces were sampled by rectal palpation. The animal was restrained in a crush and the operator's hand inserted into the rectum where gentle massaging encourages muscular contraction and expulsion of a faecal sample. Faeces were analysed by near infrared spectrophotometry for determination of dietary nutritive value (Coates and Dixon, 2011) and by carbon isotope analysis for determination of the proportion of C3 and C4 plants in the diet (Jones et al. 1979).

3.1.9 Statistical analysis

Analysis of variance (ANOVA) was conducted to compare the chemical composition of forages and diets to determine significant treatment effects. A linear mixed-effects model was conducted to analyse the nature of the response to *Desmanthus* inclusion level (non-significant, linear, quadratic). Data was analysed either by Rstudio version 1.3 (Trial 1) or Genstat (Trials 2 and 3; 17th Edition VSN International, Hemel Hempstead, UK 2014). Significance was claimed at $P< 0.05$. The model was fitted with the residual maximum likelihood (REML) technique, with the percentage of *Desmanthus* in the diet as a fixed effect and individual animal nested in animal group as random effects.

3.2 Trial 1. Determining the antimethanogenic potential of the forage *Desmanthus* when fed at graded levels to beef cattle

3.2.1 Methods

The trial was conducted at the CSIRO Lansdown Research Station (19.59°S, 146.84°E) and was approved by the CSIRO Queensland Animal Ethics Committee (permit 2018-02). A group of 20 yearling steers were purchased and adapted to the conditions on the research station prior to commencement of the study. Observations of the weight, health and temperament of the animals were made during routine mustering and the 14 most suitable animals were selected for the study. The non-selected cattle were returned to the Lansdown herd.

Agrimix Pastures supplied the forages and CSIRO prepared the feeds and conducted the research. This process was refined over several months and worked well. Agrimix Pastures purchased a New Holland flail harvester (Crop chopper 38, New Holland, PA, USA) for harvesting both Rhodes grass and *Desmanthus*.

The protocol was modified to account for a required change from using fresh Rhodes grass as the basal ration to Rhodes grass hay. In the original protocol it was agreed that cattle would be supplied with fresh forage to mimic as close as possible the typical grazing situation (Table 1). However, the supply of fresh Rhodes grass was exhausted by August. The Rhodes grass was also of very poor quality, averaging only 4.2% CP in the dry matter. MLA were contacted, and it was agreed to switch to a Rhodes grass hay purchased from a grower near Tully in Queensland. Originally it was agreed to source grass of poor to medium feed quality (~8% CP). However, given the scarce supply in the

prevailing drought conditions, quality of hay was less than planned with a crude protein content averaging only 5.8%

3.2.2 *Desmanthus* study

The selected steers were familiarised with routine experimental procedures by familiarization with housing in individual pens and entry, exit and holding in respiration chambers. Once adapted there were four periods of 21 days each. Seven steers were allocated to cultivar JCU 1 and a further seven to cultivar JCU 4. Steers remained on the same cultivar for the duration of the study to eliminate nutritional perturbations associated with changeover designs. This allowed for shorter adaptation periods thus reducing the duration of the trial. The percent *Desmanthus* was planned to increase in each period from 0 % in period one through 12, 24 and 36 % in subsequent periods. Animals were held in individual pens from day one to 19, transferred to respiration chambers on day 19 and removed from respiration chambers on day 21 (after 48 hours). Rumen and faecal samples were collected on day 21, three hours after the AM feeding. Day 22 would be the first day of the next treatment period. Following the last of the four periods, animals were to be held on the 36 % *Desmanthus* diet for a further 14 days and half dosed with polyethylene glycol (PEG) to remove the "tannin effect" of the *Desmanthus*. The PEG was fed at a ratio of PEG:tannin content of 2:1. Using data from Vandermeulen et al. (2018), PEG was fed at 150g/head/d. As a result of quality and supply issues with Rhodes grass, the trial was modified to account for a change from fresh grass to hay as the basal dietary component (Table 1). In brief, when the basal diet was changed from grass to hay, levels of *Desmanthus* inclusion were chosen to cover a range of *Desmanthus* intake from 0 to 31% of diet dry matter, whereas when fresh grass was fed the *Desmanthus* inclusion levels varied from 0 to 22%.

Table 1. Details of changed protocol

Original protocol			Revised protocol		
	Basal ration	% *Desmanthus*		Basal ration	% *Desmanthus*
23/05/18 (start)	Fresh Rhodes	0	23/05/18 (start)	Fresh Rhodes	0
14/06/18	Fresh Rhodes	12	14/06/18	Fresh Rhodes	10
05/07/17	Fresh Rhodes	24	05/07/17	Fresh Rhodes	22
26/07/18	Fresh Rhodes	36	08/08/18	Rhodes hay	15
06/09/18	Fresh Rhodes	36% plus PEG	23/08/18	Rhodes hay	31
27/09/18 (end) [1]	Fresh Rhodes		13/09/18	Rhodes hay	22 % plus PEG
06/09/18	End date for group 3		29/09/18	Rhodes hay	0
			18/10/18 (end)[1]		
			27/10/18	End date for group 3	

[1] Note, as there are only four chambers, the 12 animals enter the chambers in groups of four, one week after each other. Thus, the end date for group 1 (as indicated in the table) is actually two weeks before the end date of the trial

3.2.3 PEG feeding period

Immediately following the 31% Desmanthus period, cattle remained on the same treatments but with polyethylene glycol (PEG) added to the diet at 160 g/kg legume DM to half the cattle for 21 days. Measurements were as detailed above. This completed the study and cattle were returned to pasture.

3.2.4 Feeding details

A mineral vitamin supplement was provided in the form of a lick block. Fresh Rhodes grass was harvested from Reidies Hay Farm on Mondays, Wednesdays and Fridays and transferred to Lansdown Research Station 20 km away and stored in a cold room at 5^{o}C until feeding. Rhodes grass hay was purchased for the trial with an average crude protein (CP) content of 5.8% of dry matter. All grass and hay was fed chopped to 50 mm. *Desmanthus* cultivars were mechanically harvested three days a week (Monday, Wednesday and Friday) and stored in a cold room at 5^{o}C until feeding. Immediately prior to feeding, legumes were mixed with chopped hay and offered to allow for 10% uneaten feed after 22 hours. Once *ad libitum* feed intake had been established (usually five to seven days), the allowance was reduced to 90% of *ad libitum.*

3.3 Trial 2. Evaluating the anti-methanogenic potential of three *Desmanthus* cultivars when fed to cattle

3.3.1 Methods

The trial was conducted at the CSIRO Lansdown Research Station (19.59°S, 146.84°E) and was approved by the CSIRO Queensland Animal Ethics Committee (permit 2019-32). Sixteen animals were ranked according to weight and blocked into four blocks of similar weight (Table 2). Block A was allocated to Lucerne while Blocks B, C and D were allocated to a different *Desmanthus* cultivar in each of three periods.

Table 2. Allocation of blocks to treatments (Trial 2)

	Block			
Background (hay; 3 wk)	A	B	C	D
Desmanthus comparisons	Lucerne	JCU2	JCU4	JCU7
Period	**Block**			
1 (3 wk)	A	B	C	D
2 (2 wk)	A	D	B	C
3 (2 wk)	A	C	D	B
PEG (3 wk)	A	D	D	B

To facilitate rotation of animals through the methane chambers, one animal from each block was assigned to a different group (groups 1 to 4). Each group followed the previous group (Table 3).

Table 3. Methane chamber scheduling (Trial 2)

Group Comprised of four treatments: one animal from each block	Measurement week in each period	Days in chamber
1	Week 1	Monday, Tuesday
2	Week 1	Wednesday, Thursday
3	Week 2	Monday, Tuesday
4	Week 2	Wednesday, Thursday

3.3.2 *Desmanthus* study

Cattle were allocated to treatments at a planned 30% inclusion rate on the assumption that all legumes (Lucerne and *Desmanthus* cultivars) would be of equal CP %. Ongoing analysis showed this not to be the case, and the proportion of Lucerne was reduced to 20% of the diet to equilibrate CP in the diets. Cattle cycled through three periods, one of 21 days and two modified to be 14 days as a result of Covid-19 restrictions, with chamber measurements on days 20 and 21 (or 13 and 14) and rumen and faecal sampling on day 21 (or 14) four hours after removal from chambers. Cattle receiving *Desmanthus* were given a different cultivar in each of the three periods, whereas cattle receiving Lucerne were fed Lucerne throughout all periods, to act as a positive time dependent control. This was introduced to account for possible changes in composition of *Desmanthus* throughout the trial. Cattle were weighed at the end of each period to monitor performance (three-week intervals).

Due to a recommendation to shut down research to meet Covid-19 restrictions, the trial was suspended after the first *Desmanthus* period. Following this decision, permission was granted to resume the trial beginning 20th April. To ensure the project finished while *Desmanthus* was available (June 30th), the remaining three periods of the trial were reduced by seven days, from 21 days to 14 days. However, there was a seven-day readaptation period to diets following the shutdown.

3.3.3 PEG feeding period

Immediately following the third *Desmanthus* period, cattle remained on the same treatments but with polyethylene glycol (PEG) added to the diet at 160 g/kg legume DM to half the cattle for 21 days. Measurements were as detailed above.

3.3.4 Feeding details

A mineral vitamin supplement was provided in the form of a lick block. Rhodes grass hay was purchased for the trial with a minimum crude protein (CP) content of 8% of dry matter. Lucerne hay was purchased for the trial with a planned CP content similar to that in *Desmanthus* (15%). All hay was fed chopped to 50 mm. *Desmanthus* cultivars were mechanically harvested three days a week (Monday, Wednesday and Friday) and stored in a cold room at 5°C until feeding. Immediately prior to feeding legumes were mixed with chopped hay and offered to allow for 10% uneaten feed after 22 hours. Once *ad libitum* feed intake has been established (usually five to seven days) the allowance was reduced to 90% of *ad libitum.*

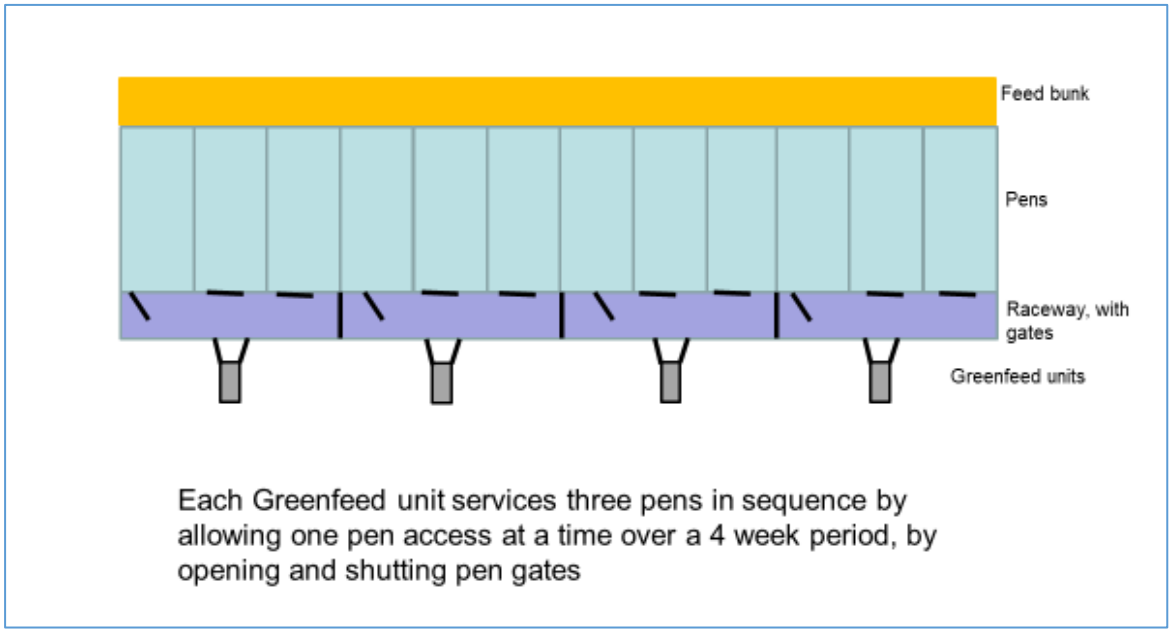
3.4 Trial 3. Pen feeding trial where Lucerne was substituted with a mixture of JCU 2, JCU 4 and JCU 7 at four levels of inclusion in the diet

Fifty Droughtmaster cross steers were purchased for the trial and conditioned at Lansdown Research Station on 21st February 2020. The trial was approved by the CSIRO Queensland Animal Ethics Committee (permit 2019-38). Forty-eight were selected for the study and housed and managed in feedlot-like conditions to replicate commercial practice on 9th March 2020 (Figure 4). Following adaptation to diets, the trial measurement period began 31st March and continued for 124 days. Four GF units were installed adjacent to the animal pens, such that each unit serviced three pens in rotation (Figure 4, 5). Cattle in each pen had access to GF units for 14 to 28 days. Cattle were attracted to the units with feed pellets and daily methane emissions were calculated based on at least four visits of >2 minutes per visit. Due to operational problems with units, not all units were functioning the entire duration of the trial.

Cattle entered the trial at 330kg and were fed a forage-based diet where legumes were supplied by various combinations of either Lucerne or a mixture of three cultivars of *Desmanthus,* these being the same as Trial 2; JCU 2, JCU 4 and JCU 7. These strains were chosen based upon the optimal blend suited to northern growing conditions. The 124-day trial duration allowed sufficient time for animals to express their growth response (weight and fat composition) to the treatments and take cattle to a growth point where they would normally enter a fattening phase prior to slaughter. A feature of this study was to replicate a normal grower phase in rearing steers for slaughter.

Figure 4. Location and layout of new cattle pens in relation to existing cattle handling facility

Figure 5. Schematic showing location of Greenfeed units



On entry to the pens, all cattle were fed Rhodes grass hay (11% crude protein). The legume content of the diet was gradually increased to the desired percentage of the dry matter over seven to 14 days. Legumes fed were Lucerne and *Desmanthus*. Lucerne was included to ensure the crude protein of the diet remains constant and was substituted with *Desmanthus* to achieve four levels of *Desmanthus* inclusion (0, 15, 30 and 45% of the diet dry matter; Table 4).

Table 4. The ingredient composition of the diets, % of diet dry matter

	0 % *Desmanthus*	15 % *Desmanthus*	30 % *Desmanthus*	45 % *Desmanthus*
No of animals (n)	12	12	12	12
No of pens (n)	3	3	3	3
Rhodes grass hay	70	65	60	55
Fresh *Desmanthus*[1]	0	15	30	45
Lucerne hay	30	20	10	0

[1] *Desmanthus* comprised equal proportions of the strains JCU 2, JCU 4 and JCU 7

Cattle were fed daily in the morning at a level of feeding to ensure uneaten feed did not exceed 5% of the feed offered. Uneaten feed was removed daily. Cattle were weighed at the beginning and end of the trial and at two or four weekly intervals throughout the study. At the beginning, mid-point and end of the study cattle were sampled for:

- Faeces for near infrared analysis of legume content of the diet
- Rumen fluid for rumen pH, ammonia and volatile fatty acids
- Methane was estimated from breath sample taken from cattle in 12 pens using the GreenFeed system

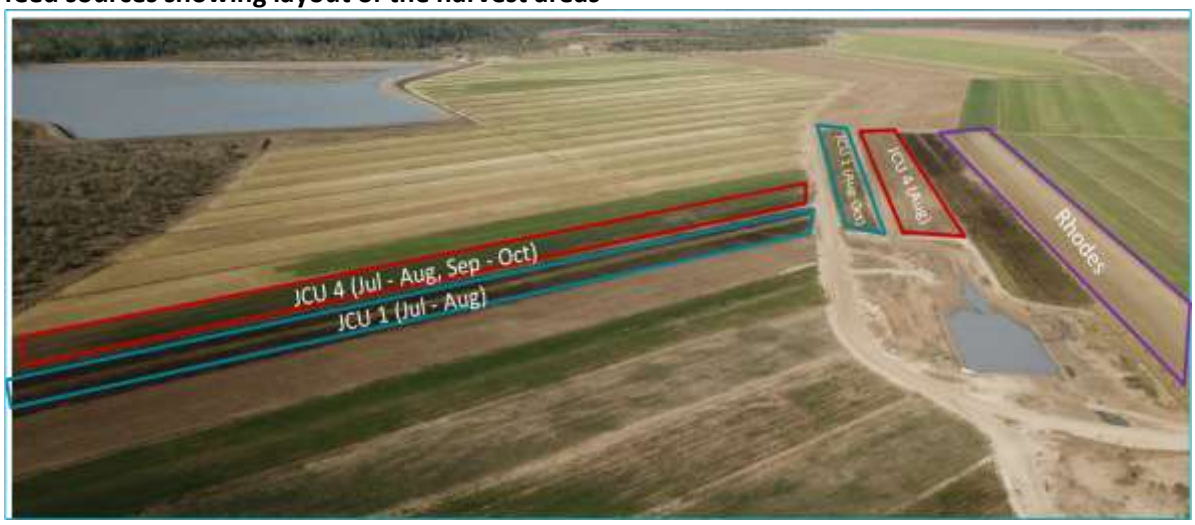
4. Results

4.1 Trial 1. Determining the antimethanogenic potential of the forage *Desmanthus* when fed at graded levels to beef cattle

4.1.1 Forages

Progardes® cultivars were established in January/February 2018 on irrigated plots at Reidies Hay Farm, located approximately 18km south of Lansdown Research Station (19°40'40"S 147°00'39"E). Harvesting occurred between July and October 2018. The harvest area is shown in Figure 6. To simulate as close as possible a grazing situation, Rhodes grass was harvested fresh in the same way as the *Desmanthus*. The available block was identified, and initial feed testing indicated a CP of approximately 7% which was considered adequate and representative of the quality of grass under dry season grazing conditions in northern Australia. However, ongoing senescence in the stand resulted in CP dropping to below 5% and cattle were losing excessive body weight (BW) over the first 63 days of the trial. The Animal Ethics Approval stipulated that excessive weight loss should be avoided. Consequently, the fresh Rhodes grass was replaced by a higher quality Rhodes grass hay on Day 64 of the trial. *Desmanthus* harvesting began in the blocks on the left of the figure. As material advanced in maturity or became exhausted, harvesting switched to other blocks as shown in Figure 6.

Figure 6. Arial photograph (2/9/18) of the *Desmanthus* and Rhodes Grass areas selected as the feed sources showing layout of the harvest areas



Both cultivars were characterized by quite rapid changes in growth stage that proved difficult to manage (Figure 7). Figure 8 shows the variation in crude protein of the two *Desmanthus* cultivars. The CP content of JCU 1 was initially 15% and dropped in the early weeks of the trial to reach levels below 10% CP by early August. These levels were maintained throughout the remainder of the trial. JCU 4 was characterized by a binomial pattern with crude protein content being over 20% at the beginning of the trial, dropping to 10% mid trial and increasing again to approximately 15% at the end of the trial. These fluctuations correspond with decreasing quality as the crop matured followed with a marked increase in quality when harvesting was switched to a new block. This was attributed to the need to harvest out of the normal growing season for *Desmanthus*.

Figure 7. Bay C2 photographed on 28/6/18 and 10/8/18 showing senescence

Figure 8. Changes in crude protein content of *Desmanthus* and hay over the trial period

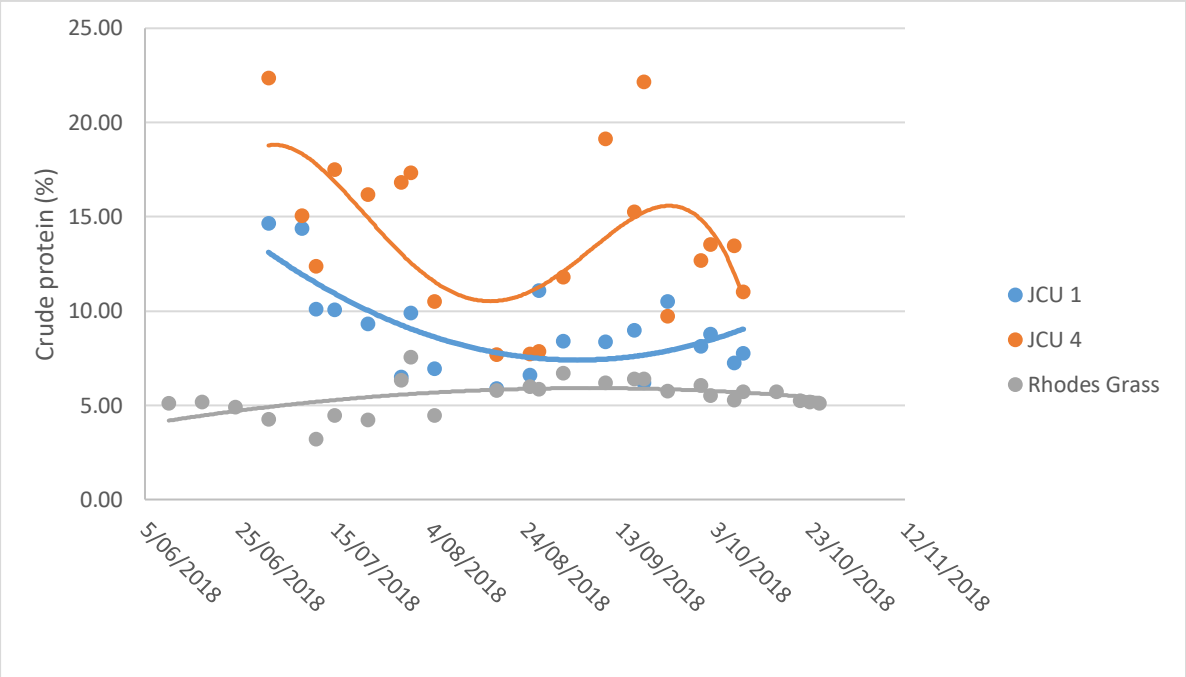


Table 5 details the composition of forages used up until the basal diet was changed from grass to hay. The grass was of typical dry season quality for Rhodes grass having a CP content of 5% and high fibre content. There was a marked quality difference between JCU cultivars, with JCU 4 having higher CP and lower fibre that JCU 1.

Table 5. Mean chemical composition (±SE) of the dietary components when the basal diet was fresh Rhodes grass (% DM unless otherwise stated)

	Rhodes grass	JCU 1	JCU 4
Crude protein	5.09±0.459	10.5±2.90	14.6±2.54
Neutral detergent fibre	74.2±1.374	63.6±3.05	54.4±1.60
Acid detergent fibre	42.2±2.23	43.2±2.95	33.4±0.600

Table 6. Mean chemical composition (±SE) of the dietary components when the basal diet was Rhodes grass hay (% DM unless otherwise stated)

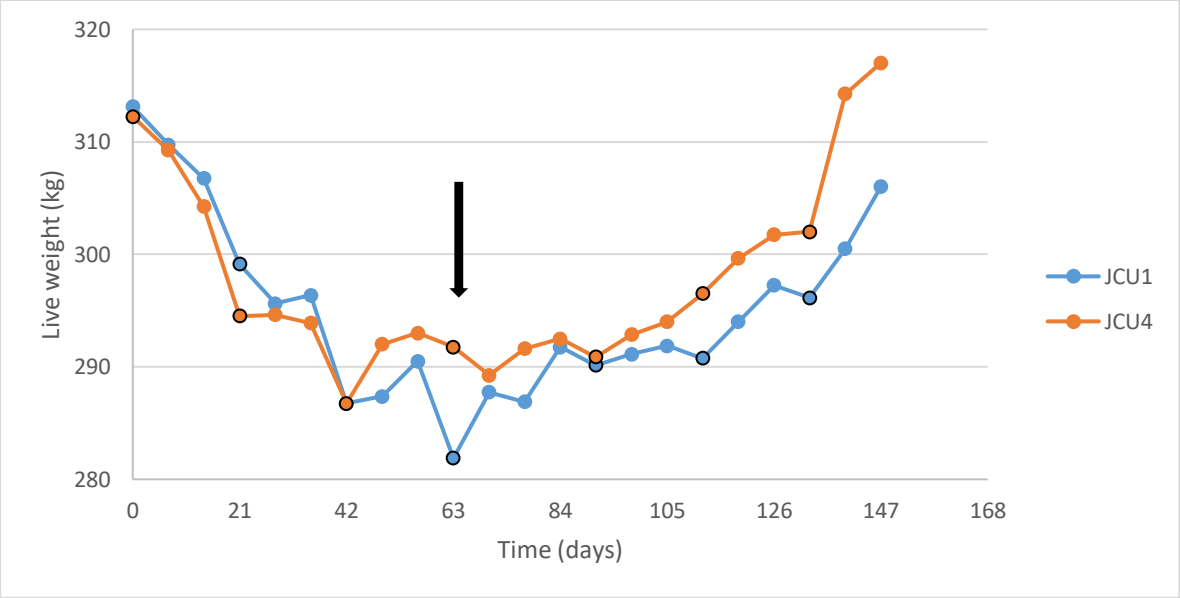
	Rhodes grass	JCU 1	JCU 4
Crude protein	8.23 ± 0.349	11.0 ± 0.479	14.6 ± 1.71
Neutral detergent fibre	76.2 ± 0.602	67.4 ± 0.291	58.3 ± 2.08
Acid detergent fibre	45.0 ± 0.359	46.3 ± 0.606	36.8 ± 1.98
Total phenolics (% DM as catechin equivalent)	0.3 ± 0.027	1.7 ± 0.12	2.3 ± 0.19
Condensed tannins (% DM)	ND	3.5 ± 0.19	3.7 ± 0.30

Table 6 describes the forages fed following the switch from grass to hay as the basal ingredient. The hay was of higher protein content than the grass (8.2% versus 5.1%) but the fibre was similar for both hay and grass. As seen in the grass period of the trial, JCU 4 was again higher in nutritive value than JCU 1.

4.1.2 Animal performance

Figure 9 shows the BW of cattle throughout the study. As a result of the poor quality of Rhodes grass and the low inclusion rates of *Desmanthus* (maximum 22%) over the first 63 days of study, steers lost between 0.5 and 1.0 kg/day. When better quality hay replaced the grass and as the proportion of *Desmanthus* increased throughout the trial, BW change became positive and at the end of the study steers were gaining over 0.5 kg/day. It was also apparent that cattle fed JCU 4 gained more weight than those fed JCU 1. This was attributed to the higher quality of JCU 4.

Figure 9. Pattern of weight change over the duration of the study. The black arrow indicates the point at which the basal forage was changed from grass to hay and the circles outlined in black indicates changes in the *Desmanthus* proportion of the diet



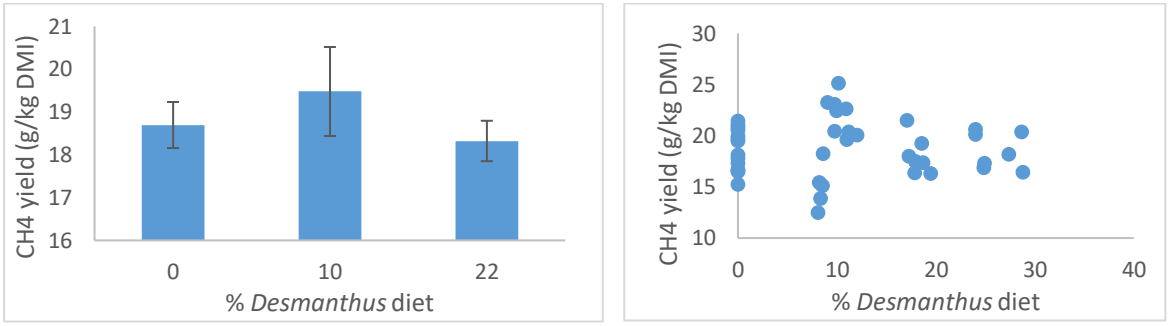
The impact of changing from fresh grass to hay was such that the analysis of data was best described by considering the data from periods when fresh grass was fed separately to the data when hay was fed. Intakes recorded during methane measurements when grass was fed are given in Table 6 and show that on these generally poor quality diets, intakes were low at between 11 and 17 g/kg LW. Intake was significantly higher for JCU 4 than JCU 1 ($P<0.05$).

4.1.3 Feed intake and methane emissions when the basal ingredient was fresh grass

Dry matter intake was low on all diets averaging just 13.8 g/kg LW (Table 7). Intake and intake relative to LW was higher for steers fed JCU 4 compared to JCU 1 ($P = 0.05$ and $P = 0.024$, respectively). There was an interaction between *Desmanthus* cultivar and level ($P < 0.001$) for methane production, with methane decreasing with increasing the proportion of *Desmanthus* in the diet for JCU 1 but the opposite was observed for JCU 4. However, there were no treatment effects for methane yield (g/kg DMI).

Figure 10 shows the methane yield as bars for the treatment means and the individual animal data. There was no significant (P> 0.05) linear reduction in methane yield as the proportion of *Desmanthus* in the diet increased.

Figure 10. Relationship between methane yield (g/kg DMI) and percentage of *Desmanthus* in the diet (a) by averaging the values in each period and (b) by taking all the individual animals. Data when fresh grass was the basal dietary ingredient



(**a**) (**b**)

4.1.4 Rumen fermentation parameters when the basal ingredient was fresh grass

Increasing the proportion of *Desmanthus* in the diet increased rumen ammonia concentration (P < 0.001) and the acetate: propionate ratio (P = 0.04) as a result of reduced propionate proportions (P = 0.027; Table 8).

Table 7. Effect of *Desmanthus* inclusion in the diet and cultivar on intake and methane emissions when the basal ingredient was fresh Rhodes grass

	JCU 1			JCU 4			SE	Probability		
	0	10	22	0	10	22		Level	Cultivar	L x C
DM intake (kg/d)	4.07	3.65	3.64	4.11	4.15	4.99	0.149	0.294	0.054	0.005
DM intake (g/kg LW)	13.6	12.8	13.0	13.4	13.9	16.3	0.468	0.126	0.053	0.024
CH4 (g/d)	76.1	69.1	67.5	74.7	74.8	89.0	1.921	0.197	0.101	<0.001
CH4 (g/kg DMI)	19.0	19.9	18.6	18.4	19.1	18.1	0.0422	0.677	0.358	0.875

Table 8. Effect of *Desmanthus* inclusion in the diet and cultivar on rumen ammonia, pH, total volatile fatty acid concentration and molar proportions when the basal ingredient was fresh Rhodes grass

	JCU 1			JCU 4			SE	Probability		
	0	10	22	0	10	22		Level	Cultivar	L x C
Ammonia (mg/dL)	2.74	2.05	4.62	2.07	2.61	4.78	0.338	<0.001	0.980	0.506
pH	6.92	6.77	6.97	6.97	6.78	7.07	0.0406	0.336	0.504	0.764
Total VFA (mg/dL)	70.4	71.3	61.1	71.8	71.1	62.9	1.684	0.251	0.139	0.522
Acetate (molar %)	76.7	77.8	77.5	76.7	76.6	76.9	0.186	0.251	0.139	0.522
Propionate (molar %)	15.0	14.6	14.4	14.8	14.7	14.3	0.109	0.027	0.825	0.718
Iso butyrate (molar %)	0.449	0.414	0.488	0.455	0.480	0.465	0.117	0.374	0.520	0.510
Acetate/Propionate	5.13	5.34	5.41	5.20	5.24	5.37	0.0491	0.040	0.870	0.644
n-Butyrate (molar %)	6.56	6.07	6.28	6.71	6.80	6.90	0.0986	0.847	0.014	0.510
Iso valerate (molar %)	0.600	0.511	0.605	0.613	0.617	0.564	0.0217	0.652	0.631	0.537
n-Valerate (molar %)	0.545	0.537	0.645	0.600	0.689	0.712	0.0222	0.009	0.023	0.957
n-Caproate (molar %)	0.122	0.105	0.099	0.146	0.113	0.121	0.0055	0.036	0.174	0.971

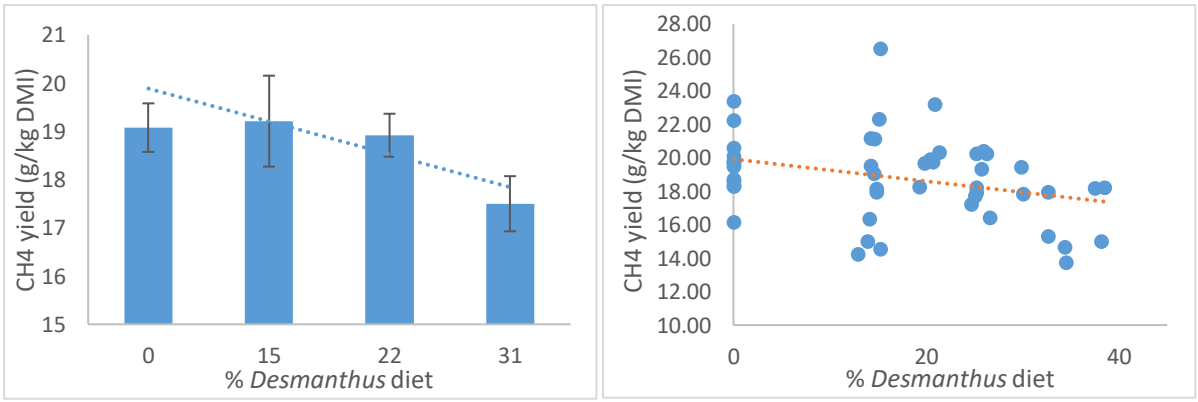
4.1.5 Feed intake and methane emissions when the basal ingredient was hay

Dry matter intake was similar when hay was fed (13.9 g/kg LW) compared to when fresh Rhodes grass constituted the basal ingredient (13.8 g/kg LW). The level of *Desmanthus* inclusion in the diet increased DM intake (P<0.001) but there was no effect of cultivar, with DMI being 13.7 and 14.1 g/kg LW for JCU 1 and JCU 4, respectively (Table 9). In contrast to the data when fresh grass was fed, methane production (P = 0.030) and methane yield (P < 0.010) were reduced by increasing the proportion of *Desmanthus* in the diet (Figure 11). This linear effect can be defined by the equation:

$$Y = 19.89 - 0.066X,\ R^2 = 0.2,\ P = 0.009$$

where Y = methane yield (g/kg DMI) and X is the percent *Desmanthus* in the diet. Thus, for every percent increase in *Desmanthus* in the diet, the yield of methane drops by 0.066 g/kg DMI. A diet consisting of 30% *Desmanthus* for example would be expected to reduce methane emissions by 10%. Cultivar influenced CP intake (P < 0.001) and methane production in g/day (P = 0.050) as a result of higher CP in JCU 4 and higher intake of JCU 4, respectively. Significant interactions for intake indicated that the response to *Desmanthus* inclusion level between the two cultivars was different. However, the response in methane production and yield was the same for both cultivars.

Figure 11. Relationship between methane yield (g/kg DMI) (y) and (X) percentage of *Desmanthus* in the diet (a) by averaging the values in each period (y = 19.89 – 0.066X, R^2=0.2, *p* = 0.009) and (b) by taking all the individual animals (y = 19.92 – 0.066X, R^2=0.2, *p* = 0.0097). Data when hay was the basal dietary ingredient.



(**a**) (**b**)

4.1.6 Rumen fermentation parameters when the basal ingredient was hay

Increasing the proportion of *Desmanthus* in the diet had significant effects on many parameters of rumen function (Table 10). Rumen ammonia N concentrations were very low for the hay only diet, as expected based on the low CP of this diet. Moderate increases in rumen ammonia were observed as the *Desmanthus* proportion in the diet increased and the linear effect was significant (P = 0.028) Total volatile fatty acid (VFA) concentration varied between 50 to 80 mg/dL. Rumen ammonia (P = 0.02) and the concentration of VFA in rumen liquor (P < 0.001) both increased in response to *Desmanthus* inclusion level. Molar proportions of acetate increased (P < 0.001) while propionate decreased (P < 0.001) thus influencing the acetate: propionate ratio (P < 0.001) in favour of a more acetogenic fermentation. Among the longer chain VFAs, iso-butyrate (P = 0.012) and iso-valerate (P = 0.048) proportions were decreased, whereas valerate (P < 0.001) was increased by increasing the inclusion of *Desmanthus* in the diet. There was no effect of cultivar or the level x cultivar interaction

on any of the rumen fermentation parameters, except for valerate, that increased its molar proportion with increasing *Desmanthus* ($P < 0.001$) level in the diet, especially for JCU 4 (interaction $P < 0.001$). The increase in rumen VFA concentration and the change in the acetate: propionate ratio is consistent with an increase in N supply to the rumen promoting microbial activity in general when a high fibre diet is offered.

Table 9. Effect of *Desmanthus* inclusion in the diet and cultivar on intake and methane emissions when the basal ingredient was Rhodes grass hay

	JCU 1				JCU 4				SE	Probability		
Desmanthus level	0	15	22	31	0	15	22	31		Level Linear	Cultivar	L x C
DM intake (kg/d)	3.79	3.56	4.28	4.72	3.90	3.72	5.14	4.93	0.128	<0.001	0.189	0.047
DM intake (g/kg LW)	12.7	12.3	14.7	15.2	12.4	12.2	16.5	15.3	0.436	<0.001	0.330	0.048
CH4 (g/d)	72.9	67.3	71.1	84.3	83.9	76.8	70.2	92.8	1.904	0.016	0.050	0.016
CH4 (g/kg DMI)	19.5	19.2	16.8	18.1	19.8	19.6	18.3	19.4	0.0353	0.014	0.388	0.773

Table 10. Effect of *Desmanthus* inclusion in the diet and cultivar on rumen ammonia, pH, total volatile fatty acid concentration and molar proportions

	JCU 1				JCU 4				SE	Probability		
Desmanthus level	0	15	22	31	0	15	22	31		Level Linear	Cultivar	L x C
Ammonia (mg/dL)	6.42	6.94	9.69	7.12	6.30	6.19	10.1	8.97	0.412	0.028	0.453	0.318
pH	7.11	6.95	7.14	6.90	6.90	6.92	7.06	6.83	0.0309	0.256	0.135	0.645
Total VFA (mg/dL)	48.9	68.3	56.1	71.4	50.9	69.4	61.9	77.2	1.980	<0.001	0.132	0.156
Acetate (molar %)	70.3	74.1	71.6	74.0	70.9	74.7	71.4	73.8	0.260	<0.001	0.378	0.098
Propionate (molar %)	19.6	16.8	18.1	16.6	19.1	16.0	18.1	16.4	0.192	<0.001	0.048	0.862
Acetate/Propionate	3.60	4.43	3.97	4.45	3.73	4.71	3.94	4.50	0.0618	<0.001	0.106	0.865
Iso butyrate (molar %)	0.523	0.481	0.495	0.387	0.511	0.512	0.533	0.477	0.0141	0.012	0.286	0.365
n-Butyrate (molar %)	8.22	7.16	8.39	7.76	8.24	7.34	8.37	7.73	0.0967	0.565	0.971	0.599
Iso valerate (molar %)	0.662	0.613	0.588	0.472	0.653	0.677	0.639	0.686	0.0225	0.048	0.971	0.132
n-Valerate (molar %)	0.534	0.675	0.641	0.667	0.509	0.687	0.792	0.812	0.0169	<0.001	<0.001	<0.001
n-Caproate (molar %)	0.132	0.107	0.176	0.128	0.127	0.120	0.171	0.129	0.0053	0.320	0.932	0.927

4.1.7 Polyethylene glycol to investigate the effect of tannins on methane production when the basal ingredient was hay

The effect of tannins on protein digestion and methanogenesis has been demonstrated (e.g. Hess et al; 2006) and tannins in the diet can reduce intake. To ascertain if tannins were having such effects in the current trial, polyethylene glycol (PEG) was added to the diet of half the steers receiving 22% *Desmanthus*. The addition of PEG to the 22% *Desmanthus* treatments had no influence on methane yield, intake, daily liveweight gain and VFA (Table 11) except for an increase in iso-butyrate ($P < 0.05$) and iso-valerate ($P < 0.05$). PEG also significantly increased the concentration of NH_3-N ($P < 0.05$). The response in ammonia N and iso-acids are consistent with an increase in rumen available N as a result of PEG counteracting the protein binding capacity of tannins.

Table 11. Effect of adding polyethylene glycol to the diet on intake, methane missions and rumen ammonia and volatile fatty acids of steers fed 22% *Desmanthus* in the diet and hay was fed as the basal ingredient

	JCU 1			JCU 4		
	No PEG	With PEG	P	No PEG	With PEG	P
Dry matter intake (kg/d)	4.33	5.21	NS	4.77	5.15	NS
Methane (g/d)	74	87	NS	85	95	NS
Methane (g/kg DMI)	17.1	16.8	NS	18.2	18.6	NS
Ammonia N (mg/dL)	12.4	17.7	0.05	14.0	15.9	0.05
pH	7.19	7.07	NS	7.12	6.98	NS
Total VFA (mg/dL)	51.3	62.3	NS	58.6	66.3	NS
Acetate (molar %)	71.9	71.2	NS	71.9	70.7	NS
Propionate (molar %)	17.9	18.3	NS	18.0	18.3	NS
Acetate/Propionate	4.02	3.90	NS	3.99	3.88	NS
Iso butyrate (molar %)	0.454	0.549	0.05	0.434	0.664	0.05
n-Butyrate (molar %)	8.38	8.41	NS	8.16	8.64	NS
Iso valerate (molar %)	0.527	0.671	0.05	0.503	0.821	0.05
n-Valerate (molar %)	0.629	0.658	NS	0.795	0.788	NS
n-Caproate (molar %)	0.181	0.169	NS	0.183	0.155	NS

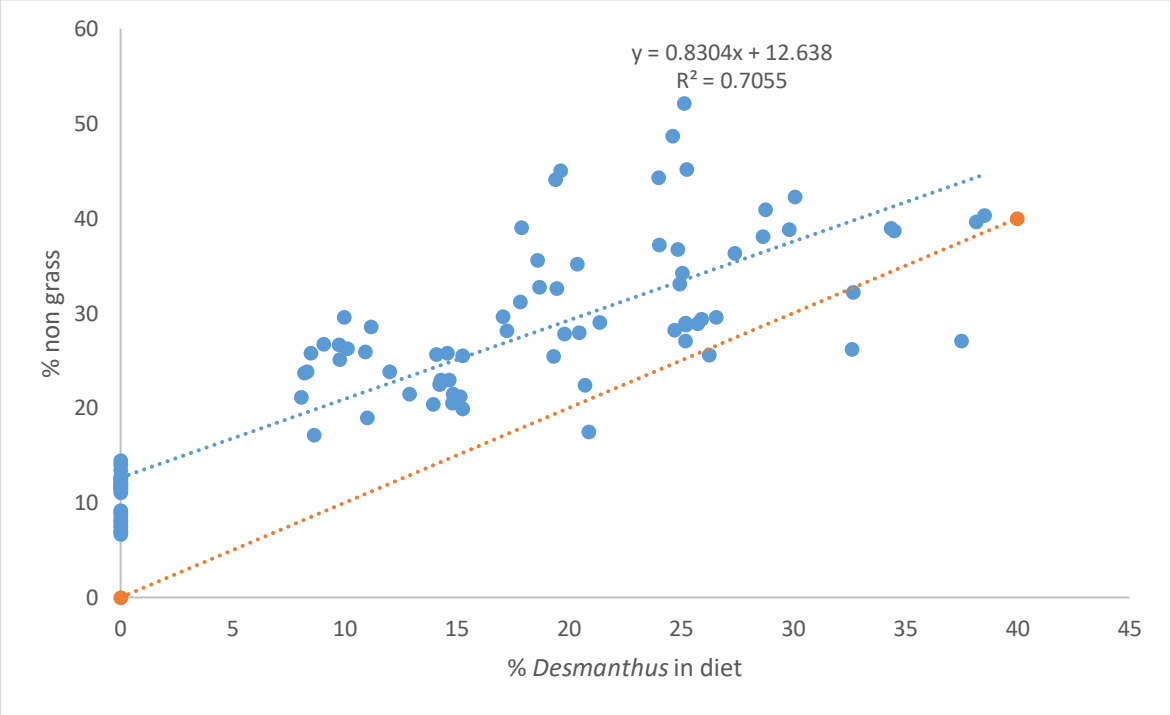
4.1.8 Estimation of *Desmanthus* intake from ^{12}C:^{13}C ratios

Faecal samples collected immediately after animals were confined to chambers were collected and analysed for delta carbon ratios. Figure 12 shows the relationship between observed *Desmanthus* proportion in the diet and predicted non-grass (i.e. C3 plants, which in a tropical context include legumes). The origin of the relationship did not pass through zero, indicating that this method may not be appropriate for diets with less than 10% legume present. Nevertheless, there was a clear linear relationship (R^2 = 0.7) with the slope of the line being 0.83. The relationship can be represented by the equation:

$$Y = 12.6 + 0.830x$$

Where Y = percent non grass and X = % *Desmanthus* in the diet.

Figure 12. Relationship between estimation of non-grass in the diet from delta carbon and observed proportions of *Desmanthus* in the diet



4.2 Trial 2. Evaluating the anti-methanogenic potential of three *Desmanthus* cultivars when fed to cattle

4.2.1 Forages

The cultivars JCU 2, JCU 4 and JCU 7 were established under centre-pivot irrigation in November 2019, 5 km south of the Lansdown Research Station (Griggs Farming, Elliott Creek). Each cultivar was established in parallel blocks across half of the pivot circle. A fourth block of JCU 2 was established approximately two months later due to seed contamination in the original JCU block. Within each block, prior to the feeding trial, sections were sequentially harvested, and the material discarded to ensure a supply of *Desmanthus* of similar growth stage as the trial progressed.

The mean composition of forages fed throughout the study is shown in Table 12. These samples were taken from individual forages at weekly intervals and analysed in a commercial laboratory using near infrared reflectance.

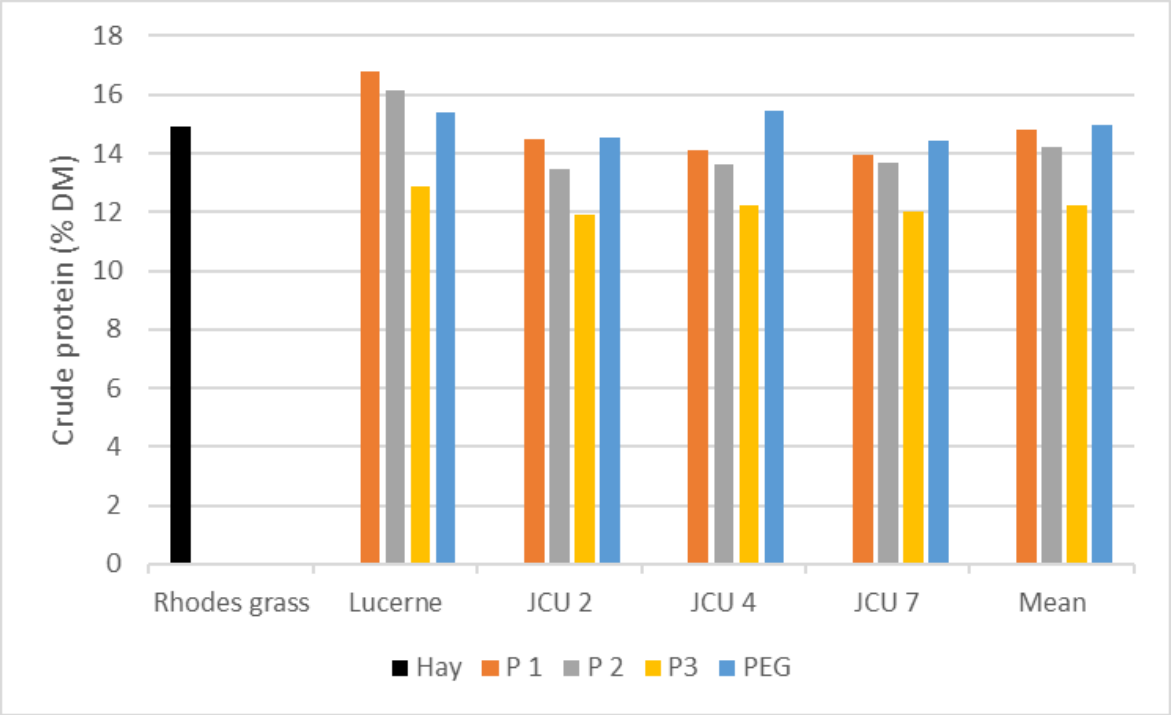
Table 12. Chemical composition (mean ± standard error) of forages (NIR analysis)

	Hay	Lucerne	JCU 2	JCU 4	JCU 7
CP (% DM)	10.8 ± 0.392	19.5 ± 1.173	14.2 ± 1.320	16.2 ± 1.034	12.6 ± 0.664
NDF (% DM)	67.4 ±0.738	42.8 ± 1.453	64.5 ±2.559	58.4 ± 2.446	66.2 ±1.696
ADF (% DM)	41.2 ±0.701	35.2 ±1.323	41.7 ± 2.051	39.4 ± 1.843	45.7 ± 1.011

Figure 13. *Desmanthus* cultivars grown under centre pivot irrigation for Trials 2 and 3. JCU 4 is on the left, JCU 2 on the right.

One of the challenges of the trial was the need to keep all diet isonitrogenous when the CP content of the forages was only known approximately one week after they had been fed. It was expected that all legumes would be of similar CP. However, the CP of Lucerne was noticeably higher than the *Desmanthus* cultivars. Therefore, the proportion of Lucerne in the Lucerne diet was periodically reduced following confirmation of the nutritive value at the end of each week. Furthermore, the CP of *Desmanthus* cultivars also changed over time depending on growth stage and the stand being harvested. The variation in diet composition, as measured by faecal NIR, over the five periods of the trial is shown in Figure 14. The mean CP of all diets over the whole trial was 14.3% and varied between 12 and 16% across periods. Within a period, CP of the Lucerne diet was generally approximately two percentage units higher than the *Desmanthus* diets, which were all similar in CP content.

Figure 14. Change in crude protein concentration of diets offered over the five periods of the trial as measured by faecal NIR. Hay was the sole dietary ingredient during the background period.



Forage samples were also taken to correspond with feed offered over the two days when steers were in methane chambers (Table 13). These were analysed following trial completion by CSIRO in Perth using NIR and cross referenced with wet chemistry methods. Overall, there were differences in values between the two laboratories. This can be explained by laboratory methods using different NIR calibrations and by the fact that sampling periods were different. The CP bias was consistent within *Desmanthus* cultivars, with CSIRO data being 81, 71 and 92% of commercial values for JCU 2, JCU 4 and JCU 7, respectively. For hay, CP data from the CSIRO lab was 8% higher. The large discrepancy for Lucerne can be explained by very high values of samples taken before cattle were in chambers.

Table 13. Chemical composition (mean ± standard error) of forages fed during chamber measurements (NIR analysis by CSIRO)

	Hay	Lucerne	JCU 2	JCU 4	JCU 7
CP (% DM)[A]	8.95 ± 0.226	15.4 ± 0.590	11.6 ± 1.01	14.4 ± 0.82	11.6 ± 2.70
NDF (% DM)[B]	73.8 ± 0.397	49.3 ±0.702	56.6 ± 1.53	52.1 ± 1.45	58.0 ± 3.22
ADF (% DM)[C]	42.8 ±0.412	37.3 ±0.666	43.1 ± 1.49	39.3 ± 1.05	42.4 ± 3.05
DMD (%)[D]	50.7 ±0.551	65.6 ±0.991	50.3 ± 2.35	54.4 ± 1.65	50.7 ± 4.54

[A]CP = crude protein, [B]NDF = neutral detergent fibre, [C]ADF = acid detergent fibre, [D]DMD = dry matter digestibility

Faecal samples can be used to estimate the chemical composition of consumed diets (i.e. the combined nutritive value of both the basal hay ration and the added legume). These data are shown in Table 14 and confirm that across all periods the diets were similar in CP (isonitrogenous), particularly among the three *Desmanthus* diets. Estimated dry matter digestibility (DMD) and DMI was higher for hay and Lucerne diets compared to the three *Desmanthus* diets.

Table 14. Estimated nutritive value (mean ± standard error) of diets fed during chamber measurements (faecal NIR analysis by CSIRO)

	Diet				
	Hay only	Lucerne	JCU 2	JCU 4	JCU 7
CP (% DM)[A]	14.9 ± 0.236	15.3 ± 0.444	13.6 ± 0.354	13.8 ± 0.386	13.5 ± 0.277
DMD (%)[B]	60.2 ±0.349	59.4 ± 0.541	55.4 ±0.792	56.6 ± 0.684	55.9 ±0.870
Non grass (% DM)	-2.6 ± 1.11	16.4 ± 2.59	31.0 ± 3.04	31.1 ± 3.22	32.3 ± 3.43
DMI (g/kg LW)[C]	25.3 ± 0.226	24.2 ± 0.276	22.4 ± 0.317	22.7 ± 0.328	22.5 ± 0.292

[A]CP = crude protein, [B]DMD = NIR predicted dry matter digestibility, [C]DMI = dry matter intake

4.2.2 Animal performance

Steers on all treatments gained weight throughout the trial (Figure 15). When Rhodes grass hay was fed alone during the first period of the trial LW gain averaged 0.51 kg/day. Steers fed the hay/Lucerne diet throughout the study gained 0.65 kg/day. For steers fed the *Desmanthus* diets, the cultivar offered changed in every period, so it was not possible to sensibly measure a treatment response in LW gain. However, the overall gain of steers fed the diets containing *Desmanthus* cultivars was 0.39 kg/day.

Figure 15. Change in live weight during the trial for steers fed diets containing Lucerne or *Desmanthus*

4.2.3 Feed intake and methane emissions

Feed intake was higher (kg/d $P < 0.001$, g/kg LW $P < 0.10$) for steers fed Lucerne compared with other diets, which were not significantly different to one another (Table 15). Methane production (g/day) was also higher for steers fed the Lucerne diet, compared to other diets ($P < 0.001$).

However, when methane was expressed relative to LW, there was no differences between any of the diets. Methane yield averaged 21.1 g/kg LW.

4.2.4 Rumen fermentation parameters

Within the *Desmanthus* treatments there were no differences in any of the rumen fermentation parameters (Table 16). In relation to steers fed hay only those fed *Desmanthus* treatments had a higher acetate: propionate ratio (P = 0.002) as a consequence of higher acetate (P < 0.001) and lower propionate (p = 0.002) proportions. When comparing steers fed Lucerne or *Desmanthus* diets, those with *Desmanthus* in the diet had lower total VFA concentration (P = 0.010) and lower iso acid proportions (P = 0.005 and 0.016 for iso-butyrate and iso-valerate, respectively). These differences were probably related to the higher DM intake of steers fed Lucerne stimulating rumen activity. Rumen ammonia and pH were not influenced by diet.

4.2.5 Polyethylene glycol to investigate the effect of tannins on methane production

During the final period of the trial, PEG was added to the diet of half the steers. Comparisons were made between all steers in the final period (no PEG vs PEG) or between the steers in Period 3 and Period 4 that had received PEG in Period 4 (Table 17). The clearest comparison was between the same steers that had not received PEG in Period 3 but had received PEG in Period 4 and this showed a significant increase (P = 0.007) in methane yield in response to PEG for all diets including Lucerne. This implied that the increase in methane was unlikely to be due to tannins (because Lucerne is low in tannins). Table 18 demonstrates that within *Desmanthus* treatments, the only effect of PEG on rumen fermentation was to increase the molar proportion of the iso-acids, iso-butyrate and iso-valerate (P <0.05 or greater) apart from an increase in rumen pH for JCU 2 (P = 0.001). In the Lucerne diet, PEG increased rumen ammonia and iso-valerate (P < 0.001).

Table 15. Effect of *Desmanthus* cultivar on intake and methane emissions.

	Hay	Lucerne	JCU 2	JCU 4	JCU 7	SE	P
DM intake (kg/d)	5.38a	6.46c	5.77b	5.78b	5.80b	0.241	< 0.001
DM intake (g/kg LW)	19.9	21.1	19.8	20.0	19.6	0.642	0.094
CH_4 (g/d)	117a	137b	122a	121a	122a	2.20	< 0.001
CH_4 (g/kg DMI)	21.5	21.1	21.0	20.8	21.0	0.560	0.927

Table 16. Effect of *Desmanthus* cultivar on rumen fermentation parameters.

Variable	**RG Hay**	**Lucerne**	**JCU2**	**JCU4**	**JCU7**	**SEM**	***p-V*alue**		
							Hay vs. Des	**Lucerne vs. Des**	**Des**
NH_3-N (mg/dL)	15.7	17.6	15.5	16.4	15.6	0.375	0.859	0.321	0.725
pH	7.0	7.0	7.0	7.1	7.2	0.0255	0.198	0.104	0.127
Total VFA[1] (mg/dL)	61.1	65.2	60.2	57.0	51.5	1.32	0.086	0.010	0.113
Acetate (molar %)	74.4	75.4	76.5	75.9	76.8	0.234	<0.001	0.173	0.391
Propionate (molar %)	15.2	14.5	13.9	14.5	14.0	0.134	0.002	0.340	0.320
Acetate/propionate ratio	4.9	5.2	5.5	5.3	5.5	0.0680	0.002	0.343	0.391
Iso-Butyrate (molar %)	0.91	0.97	0.81	0.76	0.80	0.0203	0.034	0.005	0.736
n-Butyrate (molar %)	7.5	7.0	6.8	6.9	6.6	0.107	0.003	0.630	0.582
Iso-Valerate (molar %)	0.95	1.0	0.87	0.83	0.86	0.0227	0.271	0.016	0.807
n-Valerate (molar %)	0.75	0.95	0.89	0.93	0.81	0.0331	0.333	0.646	0.660
n-Caproate (molar %)	0.25	0.15	0.16	0.17	0.17	0.0200	0.471	0.875	0.872

[1] VFA = volatile fatty acids

Table 17. Effect adding polyethylene glycol to diets containing Lucerne or one of three *Desmanthus* cultivars on methane yield.

	Lucerne		**JCU 2**		**JCU 4**		**JCU 7**			P	
PEG	No	Yes	No	Yes	No	Yes	No	Yes	SE	TRT	PEG
CH_4 (g/d) P3 v P4	22.8	24.7	20.6	21.9	21.7	24.4	22.0	24.0	0.853	0.007	0.005
CH_4 (g/kg DMI) With or without PEG in P4	25.4	24.7	24.4	21.9	21.6	24.4	22.3	24.0	1.191	0.374	0.858
Response to PEG (P3 vs P4)	1.09		1.06		1.12		1.09				
Response to PEG (With or without PEG in P4	0.97		0.90		1.13		1.10				

Table 18. Effect of adding polyethylene glycol to diets containing Lucerne *Desmanthus* cultivars on fermentation parameters (period 3 vs period 4).

Variable	Lucerne				JCU 2				JCU 4				JCU 7			
	No PEG	PEG	SEM	P	No PEG	PEG	SEM	P	No PEG	PEG	SEM	P	No PEG	PEG	SEM	P
NH_3-N (mg/dL)	14.5	17.0	1.82	0.001	12.6	14.5	1.60	0.663	20.9	14.6	2.48	0.361	15.4	17.2	0.700	0.225
pH	7.0	7.0	0.157	0.730	6.9	7.2	0.109	0.001	7.3	7.2	0.062	0.617	6.9	7.2	0.125	0.334
Total VFA[1] (mg/dL)	57.3	52.3	6.53	0.353	51.6	36.0	5.10	0.337	40.7	34.8	5.26	0.538	48.7	48.9	4.74	0.861
Acetate[2]	74.5	77.5	0.945	0.685	76.6	77.6	1.05	0.627	77.3	79.3	0.379	0.642	77.0	78.0	1.40	0.430
Propionate[2]	13.4	12.9	0.400	0.640	13.4	13.3	0.383	0.345	12.9	13.1	0.215	0.654	13.1	12.8	0.770	0.444
Ac/prop ratio[3]	5.8	6.0	0.249	0.707	5.7	5.8	0.268	0.54	6.0	6.1	0.121	0.836	6.0	6.2	0.464	0.509
Iso-Butyrate[2]	0.88	0.90	0.064	0.887	0.63	0.88	0.060	0.003	0.78	0.61	0.084	0.001	0.74	0.90	0.045	0.001
n-Butyrate[2]	7.0	6.4	0.330	0.467	7.3	6.0	0.530	0.267	6.8	5.3	0.177	0.129	6.9	6.1	0.530	0.185
Iso-Valerate[2]	0.82	1.1	0.066	0.001	0.64	0.97	0.072	0.005	0.88	0.67	0.083	0.021	0.89	1.1	0.056	0.001
Valerate[2]	1.0	1.1	0.098	0.814	1.2	1.1	0.174	0.781	1.1	0.83	0.033	0.123	1.1	0.95	0.093	0.583
Caproate[2]	0.31	0.16	0.058	0.383	0.21	0.19	0.024	0.721	0.18	0.15	0.033	0.317	0.22	0.16	0.033	0.362

[1] VFA = volatile fatty acids, [2]molar %. [2]acetate:propionate ratio

4.2.6 Estimation of *Desmanthus* intake from ^{12}C:^{13}C ratios

In Trial 2, the relationship between observed legume intake and predicted non-grass intake is shown in Figure 16. It shows a very good relationship, with the intercept passing through the origin and a slope close to unity. These data confirm the mean values in Table 14, which predicted *Desmanthus* intake at close to 30% and a mean Lucerne intake of 16%. Figure 17 shows the relationship from Trials 3. Again, the intercept is close to zero, but in this case the slope is less than unity.

Figure 16. Relationship between estimation of non-grass in the diet from delta carbon and observed proportions of legume (*Desmanthus* and Lucerne) in the diet (Trial 2)

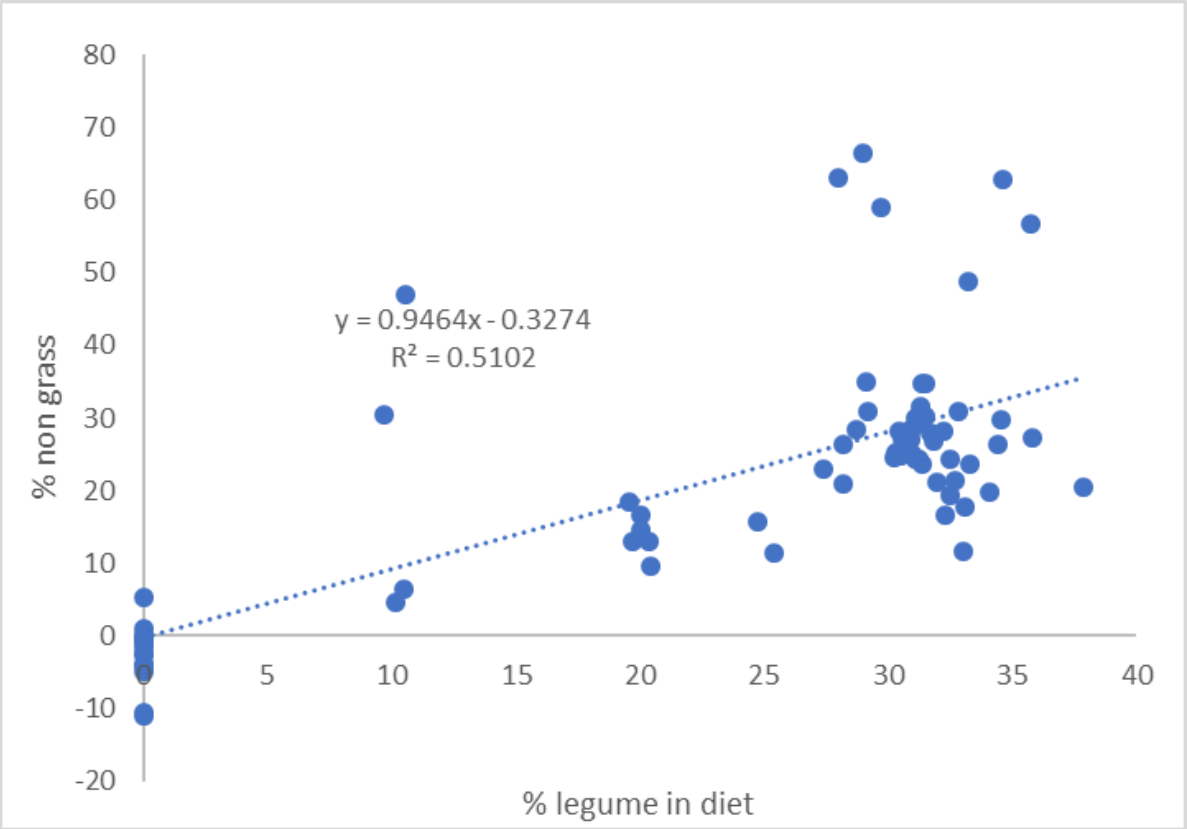
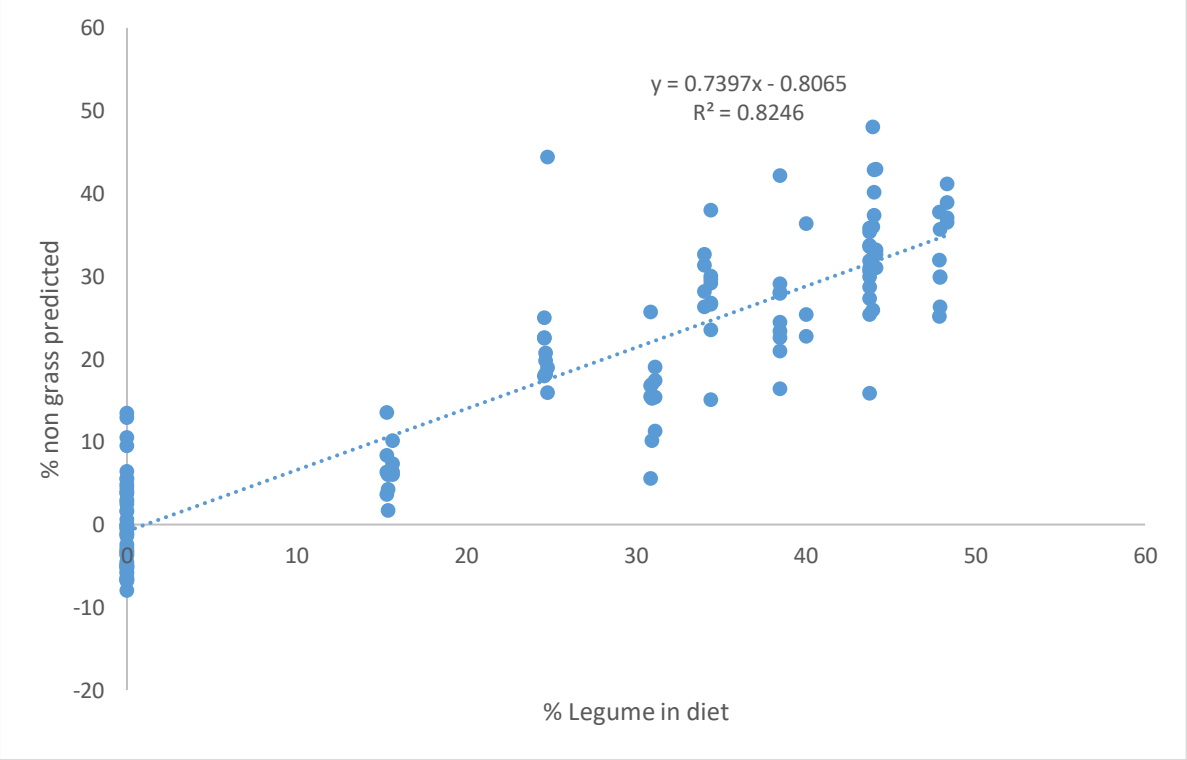


Figure 17. Relationship between estimation of non-grass in the diet from delta carbon and observed proportions of legume (*Desmanthus* and Lucerne) in the diet (Trial 3)



4.3 Trial 3. Pen feeding trial where Lucerne was substituted with a mixture of JCU 2, JCU 4 and JCU 7 at four levels of inclusion in the diet.

4.3.1 Performance and methane emissions

All diets were very similar in CP which ranged from 14.2 to 15.3% DM when estimated from faecal NIR (Table 19). Predicted DM digestibility and intake were also similar among the four diets.

Table 19. Effect of substituting Lucerne with a mixture of three *Desmanthus* cultivars on nutritive value of diets predicted from faecal NIR

	Percent *Desmanthus* in the diet (mean ± SEM)			
	0	15	30	45
CP (% DM)[A]	15.3 ± 0.193	14.8 ± 0.174	14.4 ± 0.110	14.2 ± 0.171
DMD (%)[B]	60.0 ± 0.271	58.9 ± 0.414	57.9 ± 0.398	58.0 ± 0.559
DMI (g/kg LW)[C]	21.3 ± 0.185	22.6 ± 0234	23.5 ± 0.314	24.4 ± 0.430

[A]CP = crude protein, [B]DMD = dry matter digestibility, [C]DMI = Dry matter intake

Measured DM intake during periods when steers had access to GreenFeed units was similar across diets; however, when expressed relative to LW intake was lower for cattle fed the 45% *Desmanthus* diet (Table 20). Over the whole trial, DM intake decreased linearly as % *Desmanthus* in the diet increased. LW gain was treatment influenced with the 15% *Desmanthus*-fed cattle growing faster than the 45% *Desmanthus*-fed cattle, however there was no effect on feed/gain ratio. Overall, increasing the proportion of *Desmanthus* up to 30% of the diet had no negative effect on performance, demonstrating that *Desmanthus* is of similar nutritive value to Lucerne. Methane production and yield were higher for cattle fed *Desmanthus* compared those fed Lucerne. There were no meaningful differences between the level of *Desmanthus* inclusion.

Table 20. Effect of substituting Lucerne with a mixture of three *Desmanthus* cultivars on intake, methane production and yield

	Percent *Desmanthus* in the diet					
	0	15	30	45	SEM	P <
Greefeed periods only						
DM intake (kg/d)	8.65	8.17	7.70	7.54	0.239	ns
DM intake (g/kg LW)	22.3b	21.7b	21.4ab	20.0a	0.583	0.05
Methane (g/d)	186a	233b	219b	212b	8.34	0.05
Methane (g/kg DMI)	20.6a	26.2c	24.2b	24.9bc	0.913	0.05
Whole trial data						
DM intake (kg/d)	8.87	8.55	8.28	7.69	0.04	<0.001 (L)
LW gain (kg/d)	0.63ab	0.71b	0.64ab	0.55a	0.037	0.05
Feed/gain	14.0	12.0	12.0	13.7	0.580	0.117

Figure 17 shows the relationship between the measured intake of legumes in Trial 3 and predictions of legume intake using the carbon isotope ratio. In this trial, there was a clear linear relationship but with the intercept passing through the origin. However, the slope was 0.7, different to unity observed in Trial 2. This may have been due to an overestimation of measured legume intake by these group-fed steers. Weighbacks averaged 10% and we were unable to separate out refused legume from refused grass. It was observed however, that cattle tended to select against fresh

Desmanthus when it heated in the bunk although this cannot be substantiated at this time as we are awaiting results on refusal samples.

4.3.2 Slaughter data

Half the cattle were slaughtered at the completion of the pen feeding trial while the remainder were transferred to a commercial feedlot (DAVCO, Reid River) on 22nd July and slaughtered on 26th October after 96 days on a commercial feedlot ration. Cattle with the lowest LW gain in the pen trial tended ($P<0.01$) to have the highest LW gain in the finishing trial (45% *Desmanthus* inclusion). Hot carcass weight averaged 220 kg for steers slaughtered at the end of the pen trial and 273 kg for those slaughtered following a further finishing period. There were no treatment effects in either period. Dressing percentage was similar across all treatments and slaughter times, averaging 53.4%. Value of steers slaughtered at the end of the pen trial averaged $1,244, whereas those slaughtered at higher weight and P8 fat thickness averaged almost $500 more at $1,712. It was never expected that cattle would reach finishing weight or grade after the pen trial where an all-forage diet was fed. However, the purpose of this was to determine if treatment had any effect on carcase characteristics and to determine if any potential differences were still apparent after cattle were taken to finishing. Apart from a treatment effect on LW gain, no other differences were observed in the pen trial and this difference was lost over the finishing period.

Table 21. Slaughter data for cattle at completion of the pen trial on different levels of *Desmanthus* or the finishing trial on a standard commercial finishing ration

	Pen period				Finishing period					Probability =		
	0	15	30	45	0	15	30	45	SEM	TRT	Period	Interaction
LW gain (kg/d)					1.31	1.37	1.40	1.70	0.110	0.097	-	-
HCW (kg)[A]	225	219	216	220	267	275	274	275	6.60	0.984	<0.001	0.638
Dressing %	52.8	52.0	53.1	53.6	54.5	54.3	53.3	53.6	0.471	0.868	0.042	0.306
P8 fat (mm)	3.92	4.34	3.10	2.47	9.92	8.50	7.20	8.43	0.182	0.247	<0.001	0.485
Value ($)	1,279	1,248	1,209	1,239	1,684	1,733	1,730	1,736	49.1	0.975	<0.001	0.670

[A] HCW = hot carcass weight

5. Conclusion

Including *Desmanthus* legume in the diet of cattle can reduce methane emissions. However, the response appears to vary depending upon the conditions under which it is fed. In the first trial a linear reduction in methane emissions was observed as *Desmanthus* inclusion in the diet increased from zero to 31% of the diet:

Y= 19.89 – 0.066X

where Y = methane yield (g/kg DMI) and X is the percent *Desmanthus* in the diet.

Thus, for every percent increase in *Desmanthus* in the diet, the yield of methane drops by 0.066 g/kg DMI. A diet consisting of 30% *Desmanthus* for example would be expected to reduce methane emissions by 9%. This effect was independent of cultivar, with two species (*D. leptophyllus* cv. JCU 1 and *D. bicornutus* cv. JCU 4) exhibiting the same response. In two subsequent trials with *D. virgatus* cv. JCU 2, *D. bicornutus* cv. JCU 4 and *D. leptophyllus* cv. JCU 7, no reduction in methane emissions was observed.

5.1 Understanding the methane response to *Desmanthus* inclusion

Several factors can be postulated as causative reasons for this observed variability in methane reduction. While tannins are implicated in reducing methane production in the rumen, the evidence from Trial 1 did not support this supposition. Polyethylene glycol (PEG) was added to the diet during the final period of methane measurement. The PEG can deactivate tannins thus removing any methane inhibitory effect. Thus, in the presence of PEG an increase in methane production would be expected if tannins were the causative agent. This was not the case in Trial 1. In Trial 2, where no methane inhibition was observed, when Lucerne (a legume purported not to have antimethanogenic activity) was substituted with *Desmanthus*, an increase in methane production was observed in the presence of PEG, for all diets including the Lucerne diet. This apparent contradiction may be explained through an indirect effect of tannins on protein availability in the rumen.

The major difference between trials 1 and 2 was the nutritive value of the basal ration. In Trial 1 a conscious effort was made to replicate the nutritive value of a northern dry season pasture (low CP grass). However, under these feeding conditions DM intake was low (~13 to 16 g/kg LW) and cattle initially lost weight (Figure 6). Thus, in Trial 2 a higher quality hay was sourced, and all diets were formulated to be iso-nitrogenous at approximately 15% CP. Intake and weight gain were higher in Trial 2 and rumen ammonia concentration was more than doubled. We conclude that feeding conditions may have an important overriding effect on the methane response to *Desmanthus*. Under conditions typical of dry season grazing, an effect can be anticipated. However, under better feeding conditions, such as in the wet season, there may be no methane reduction response to including *Desmanthus* in the diet.

The interaction between N availability in the rumen and methanogenesis appears to be implicated in the methane response to *Desmanthus*. The response to PEG provides some insight into the dynamics of the rumen. In Trial 1, adding PEG increased VFA and rumen ammonia concentrations but did not change methane yield per kg DMI. The associated increase in N availability due to PEG inclusion promoted overall rumen activity and DM intake but did not promote methanogenesis. This confirms that the antimethanogenic effect observed in Trial 1 was unlikely due to the presence of tannins. In Trial 2 however, PEG inclusion had no effect on VFA or rumen ammonia concentration in *Desmanthus* diets but did increase methane yield in all diets, including the Lucerne diet. This response was in spite of the fact that methane yield was not reduced when *Desmanthus* was fed. Given that the methane response was apparent in the low tannin Lucerne and hay diets it is again

unlikely that the response could be tannin related. We conclude that under conditions of both low and high nutritive value, tannins were unlikely to influence methanogeneisis. Under conditions of low nutritive value, reducing methane yield is most likely attributed to changes in C:N dynamics in the rumen.

5.2 Implications for industry

The increasing area established with Progardes® attests to its suitability as a legume for large areas of the north. The benefit in animal response appears to be in the dry season when quality of grasses has fallen and cattle select for *Desmanthus* and respond to the additional CP provided by the legume. Under these conditions is exactly when a reduction in methane can be anticipated (as evidenced by the results from Trial 1 when the grass protein was low). As a component of a backgrounding diet (Trial 3), it was apparent that Desmanthus was as good as Lucerne and could readily replace this high quality legume under these conditions. However, in this situation methane emissions may not be affected. It will take further work to fully understand the mechanisms involved in the methane reduction story. However, this work, the first anywhere with cattle feeding trials, has provided the initial evidence in support of a carbon methodology. As carbon prices are predicted to continue to increase, the antimethanogenic activity of *Desmanthus* will become increasingly important for an industry striving for carbon neutrality.

The variability in methane mitigation in response to *Desmanthus* has major implications for industry. It complicates the broad adoption of a simple dose response algorithm for calculating avoided emissions in relation to a predicted *Desmanthus* intake. The expectation that tannins were playing a major role was not apparent in these trials but cannot be ruled out in other situations. The quality of the non-legume component of the diet appears to be important. While this research has indicated that N availability, particularly in relation to an energy source such as rumen fermentable carbohydrate, is probably important, further research is needed to fully explore this interaction. It is necessary to conduct grazing studies across a range of conditions and measure the variation in emissions response across these conditions. Further work along these lines is anticipated. Studies should be designed where the inclusion rate of *Desmanthus* and the nutritive value of the companion grasses are manipulated. This can be achieved by investigating methane emission across season and under different inclusion rates of *Desmanthus* in the pasture. Over time a response curve can be developed that accurately accounts for both diet quality and *Desmanthus* intake.

Further work is also required in developing accurate markers for *Desmanthus* intake and potentially tannin intake. Again, a proposed project for NLMP2 is focussed on this aspect. It is proposed to test the utility of using carbon isotopes in faeces, to differentiate between C3 and C4 plants consumed by grazing cattle. Under tropical conditions, C4 plants will include tropical grasses, while C3 plants are the non-grass species, which in most cases where a methodology is to be applied, should be the target species, *Desmanthus*. The tropical C4 grasses, as a result of their photosynthetic pathway, take up proportionally more ^{13}C than C3 plants. Thus, the ^{12}C: ^{13}C can be used to discriminate between the two types of plants. Data collected in this research has shown a clear relationship between the known *Desmanthus* intake and carbon ratios. However, further research is required to further refine this technique across multiple grazing environments.

5.3 Key findings

- Inclusion of *Desmanthus* in the diet of cattle can reduce methane yield (g methane/kg DM intake) by up to 10%.
- The response is independent of cultivar/species.
- The response is variable, depending upon the conditions.
- The nutritive value of the non-legume component of the diet influences the magnitude of the methane reduction.
- The studies in this project suggest that tannins were not primary determinants of methane reduction.
- Carbon isotope ratios in faecal samples offer a potential method for measuring intake of *Desmanthus.*

5.4 Benefits to industry

Desmanthus species offer the potential of delivering an adapted legume for the vertisol clay soils in drier parts of northern Australia. The benefit of an adapted legume to increase animal productivity in the dry season has been demonstrated and can increase annual production per hectare by 10 to 20%. We have demonstrated that under dry-season conditions, there is a dose-response in methane yield to the proportion of *Desmanthus* in the diet. There is an estimated 50,000 hectares currently established with Progardes®. Assuming a 10% reduction in methane emissions during six months of the year this would eliminate approximately 6,000 tonnes of GHGs (as CO_2 equivalents) a year. Finally, the potential for a N-fixing legume to increase soil carbon is also being investigated. Taken together, the animal production benefit, soil carbon sequestration and methane avoidance make the establishment of *Desmanthus* attractive to producers. The challenge of establishment under variable climatic conditions is being addressed through on farm and research evaluation. The potential for the *Desmanthus* area to increase is real and can be grown as far south as northern New South Wales and across large parts of western Queensland. The current project adds valuable information to the development of an adapted legume for drier parts of the north. Meat and Livestock Australia is committed to the goal of a carbon neutral red meat industry by 2030. Given that approximately 4% of Australia's total GHG emissions come from the northern beef herd, addressing the problem will have positive environmental and marketing benefits. Approximately 90% of red meat emissions are derived from pasture-based systems. Thus pasture-based methodologies for methane avoidance can be applied to large fractions of the industry, even though the abatement potential may only be in the 10% range. A 10% reduction in methane across 90% of the industry may be a stretch goal by 2030 but the impediments to adoption are less than many other options. An agronomically adapted legume species with proven productivity and GHG reduction potential offers a significant step towards carbon neutrality of the red meat industry. The research in this project will feed directly into the development of a generic legume methodology that will have benefits for other legumes such as Leucaena.

6. Future research and recommendations

This work reports on the first studies with animals into the antimethanogenic potential of *Desmanthus*. The results are mixed, with evidence that *Desmanthus* can reduce methane emissions but that the response varies according to nutritive value for the accompanying forages. Further work is required to characterise the conditions that influence the degree of methane inhibition. These could include measurements of methane emissions from grazing cattle under paddock-scale conditions. This can be achieved using the C-Lock gas emissions monitors (Greenfeed). Measurements should be conducted in swards containing a range in proportions of *Desmanthus* and also across a range of pasture quality. Accompanying data on carrying capacity and seasonal gain per head and per hectare should also be recorded. This work could be envisaged within producer demonstration sites with several properties contributing. Further detailed studies, where the nutritive value of the non-legume component of the diet can be accurately controlled are also required. Research to date would suggest less emphasis is needed on individual cultivars or *Desmanthus* species. No cultivar effects have been observed across two trials with four cultivars.

The use of carbon isotopes to predict the non-grass contribution to mixed diets in tropical conditions shows promise and further work is required to develop robust algorithms to predict non-grass intake from ^{13}C:^{14}C isotopic ratios. We recommend collection of faecal samples from any legume study where the intake of the diet and proportion of the legume in the diet is known.

The primary practical implication for the industry is the opportunity to develop a legumes methodology for avoided methane emissions from grazing livestock. Considering the importance of pastures in Australian red meat industries, any methodology that can account for:

- type of legume,
- legume intake, and
- dose response in methanogenesis to legume intake

Even with abatement potential in the 5 to 20% range the potential to generate a new revenue stream from grazing livestock is significant. Such an outcome would play a major role in allowing the Australian red meat sector to meet the goal of carbon neutrality.

A program to develop a methodology, building on the findings of the current project is essential and holds real promise of delivering a methodology within two years. Discussions are ongoing to fund the research to achieve this outcome.

Two papers on research from this project has already been published in scientific journals and papers presented at the Northern Beef Research Update Conference and the International Greenhouse Gases in Animal Agriculture conference.

- Suybeng, B., Charmley, E., Gardiner, C.P., Malau-Aduli, B/S. and Malau-Aduli, E.O. (2020). Supplementing northern Australian beef cattle with *Desmanthus* tropical legume reduces in vivo methane emissions Animals, 10,2097; doi 10.3390/ani10112097.
- Suybeng, B., Charmley, E., Gardiner, C.P., Malau-Aduli, B/S. and Malau-Aduli, E.O. (2019). Methane emissions and the use of *Desmanthus* in beef cattle production in northern Australia. Animals 9,542; doi 10.3390/ani9080542.
- Suybeng, B., Charmley, E., Gardiner, C.P., Malau-Aduli, B/S. and Malau-Aduli, E.O. (2019). *Desmanthus*: a tropical legume for reducing methane emissions in northern Australia. In: Proceedings of the Northern Beef Research Update Conference, 19th – 22nd August 2019, Brisbane.
- Suybeng, B., Charmley, E., Gardiner, C.P., Malau-Aduli, B/S. and Malau-Aduli, E.O. (2019). *Desmanthus*: a tropical legume for mitigating methane emissions in northern Australia Proceedings of the 7th Greenhous Gas in Animal Agriculture Conference, August 4th to 8th 2019, Iguassu Falls, Brazil. P97.

Upon acceptance of this final report, results can be disseminated to levy payers and extension agents via the usual MLA avenues (Feedback Magazine). The related CRC project “New pastures to increase livestock productivity across the north” has hosted several producer group meetings and forums.

Through liaison with the team responsible for developing the methodology, a co-ordinated promotional series of events will be planned to raise awareness of an approved methodology available to producers wishing to establish *Desmanthus*. The current high level of interest in this species will be further increased with the potential to earn carbon credits.

7. References

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8. Appendix

8.1 Publication 1

Link to Open Source Publication – https://pubmed.ncbi.nlm.nih.gov/31404998/

Bénédicte Suybeng , Edward Charmley , Christopher P. Gardiner , Bunmi S. Malau-Aduli and Aduli E. O. Malau-Aduli , (2019). Methane Emissions and the Use of Desmanthus in Beef Cattle Production in Northern Australia. *Animals.* 9(8):542. doi:10.3390/ani9080542

8.2 Publication 2

Link to Open Source Publication

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7698017/

Bénédicte Suybeng , Edward Charmley , Christopher P Gardiner , Bunmi S Malau-Aduli 3Aduli E O Malau-Aduli (2020). Supplementing Northern Australian Beef Cattle with Desmanthus Tropical Legume Reduces In-Vivo Methane Emissions. *Animals*, 10(11):2097. doi: 10.3390/ani10112097