fermentation

MDPI

Article

Chemical Composition and In Situ Degradability of *Desmanthus* spp. Forage Harvested at Different Maturity Stages

Felista W. Mwangi [1], Edward Charmley [2], Oyelola A. Adegboye [3], Christopher P. Gardiner [1], Bunmi S. Malau-Aduli [4], Robert T. Kinobe [1] and Aduli E. O. Malau-Aduli [1,*]

[1] Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia
[2] Commonwealth Scientific and Industrial Research Organization, Agriculture and Food, Private Mail Bag Aitkenvale, Australian Tropical Sciences and Innovation Precinct, James Cook University, Townsville, QLD 4811, Australia
[3] Public Health and Tropical Medicine Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia
[4] College of Medicine and Dentistry, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia
* Correspondence: aduli.malauaduli@jcu.edu.au; Tel.: +61-747-815-339

Abstract: This study evaluated the change in nutritive value and in situ degradability of *Desmanthus* spp. (desmanthus) cultivars JCU2; *D. virgatus*, JCU4; *D. bicornutus* and JCU7; *D. leptophyllus* harvested at varying maturity stages to test the hypothesis that the nutritive value and in situ degradability of desmanthus differ between cultivars and with maturity stage at harvest. In Experiment 1, desmanthus was harvested at 11, 38, 72 and 103 days of regrowth (maturity), separated into the leaf and stem portion, dried and analysed for dry matter (DM) and chemical composition. In Experiment 2, desmanthus was harvested 78, 122 and 168 days after planting (maturity). Samples were dried, and DM, crude protein (CP) and neutral detergent fibre (NDF) and acid detergent fibre (ADF) degradation were determined using the in situ technique with three fistulated Droughtmaster steers. The results showed an interaction between cultivar and maturity on the leaf to stem mass ratio, leaf CP, stem NDF and the leaf ADF ($p \leq 0.04$). The leaf-to-stem mass ratio declined more steeply with maturity in JCU7 compared to JCU2 and JCU4 ($p = 0.04$), while there was a higher decline in leaf CP of JCU4 than JCU2 and JCU7 ($p < 0.01$). The total potentially degradable fraction of DM and CP did not differ between cultivars ($p \geq 0.30$) but declined with maturity ($p \leq 0.04$). However, the effective DM degradability at a high particle outflow rate was higher in JCU4 than in JCU7. Taken together, these results indicate that differences exist between cultivars, and higher livestock production may be achieved by utilising the different cultivars in a blend and at earlier maturity stages. Therefore, the hypothesis that nutritive value and in situ degradability of desmanthus differ between cultivars and with maturity stage at harvest was accepted.

Keywords: legume pastures; tropical livestock; pasture quality; leaf to stem mass ratio

Citation: Mwangi, F.W.; Charmley, E.; Adegboye, O.A.; Gardiner, C.P.; Malau-Aduli, B.S.; Kinobe, R.T.; Malau-Aduli, A.E.O. Chemical Composition and In Situ Degradability of *Desmanthus* spp. Forage Harvested at Different Maturity Stages. *Fermentation* **2022**, *8*, 377. https://doi.org/10.3390/fermentation8080377

Academic Editor: Anusorn Cherdthong

Received: 6 July 2022
Accepted: 5 August 2022
Published: 9 August 2022

1. Introduction

Seasonal fluctuations in pasture availability and nutritive value are major factors limiting livestock production on tropical grass pastures [1]. This is primarily due to declines in leaf-to-stem mass ratio (L/S), crude protein (CP) and soluble carbohydrates that reduce the nutritive value and palatability of pastures [2]. Low nutritive value leads to poor growth and reproductive performance, increased susceptibility to parasites and diseases and increased enteric methane emissions [3–5]. The introduction of legumes into tropical grass pastures provides high-quality forage to grazing livestock for longer periods compared to grass-only pastures [6]. Augmenting low-quality grass pastures with

forage legumes improves feed intake, rumen function and increases mineral and vitamin availability [4]. In addition, legumes increase soil nitrogen by symbiotic nitrogen fixation that improves the growth of associated grasses, and some legume species contain secondary metabolites such as condensed tannins that minimise protein degradation in the rumen [7].

Research on suitable pasture legumes for the heavier textured soils of semi-arid northern Australia, where a scarcity of adapted and productive legume species exists, identified *Desmanthus* spp. (desmanthus) as a suitable candidate [8–12]. Desmanthus is a forage legume of the tribe Mimoseae of the subfamily Mimosoideae native to the Americas [13] and varies from prostrate to erect, herbaceous to suffruticose and early to late maturing depending on the species [14,15]. Desmanthus grows in a wide range of soil textures ranging from gravelly, sandy and loam to heavy clay soils of neutral to alkaline pH [9,12,15]; it is highly palatable, withstands heavy grazing [16], reduces enteric methane emissions [17,18] and is drought tolerant [9]. It can survive with as little as 300 mm annual rainfall [15] and thrives with 500 to over 1000 mm average annual rainfall [8,9]. Studies have reported improved livestock growth performance [19–21], wool growth [20] and carcass loin eye muscle area [22] when grass-based diets are augmented with desmanthus. However, there is a dearth of peer-reviewed literature evaluating the effect of cultivar and maturity stage on nutritive value for ruminant livestock. Therefore, this study aimed to fill this gap in the literature by evaluating the change in nutritive value and in situ digestibility of three desmanthus cultivars (JCU2; *D. virgatus*, JCU4; *D. bicornutus*, JCU7; *D. leptophyllus*) harvested at different maturity stages (days since desmanthus establishment or age of regrowth). The criteria for cultivar selection was based on the ability of JCU2 to combine well with grass pastures in moderate fertility soils of sub-humid environments, superior drought and grazing tolerance of JCU4 and superior leafiness and bulk of JCU7 [15]. The study tested the hypothesis that nutritive value and in situ degradability of desmanthus differ between cultivars and with maturity stage at harvest.

2. Materials and Methods

This study was organised into two experiments: Experiment 1 evaluated the chemical composition, and Experiment 2 investigated the degradability of desmanthus forage harvested at varying maturity stages.

2.1. Experiment 1

Desmanthus establishment and management were described previously [23]. In summary, soil analysis was carried out before establishment to examine suitability for desmanthus production. Pure stands of desmanthus species *D. virgatus* cultivar JCU2, *D. bicornutus* cultivar JCU4 and *D. leptophyllus* cultivar JCU7 (Agrimix Pty Ltd., Wilsonton, Australia) were established on 29 November 2019 at a sowing rate of 2 kg/ha with 60 cm row spacing. Plots were not fertilised during desmanthus establishment but were top dressed with Natramin S (Ag Solutions, Gympie, Australia) at 400 kg/ha and urea at 100 kg/ha. Weeds were controlled by spraying the plots with a herbicide (Roundup; Monsanto, Kilda Road, Melbourne, Australia) mixed with 2,4-dichlorophenoxyacetic acid (Titan Ag Pty Ltd., Sydney, Australia) four weeks pre-planting. Forage was slashed in February, and sampling took place once a month after 11, 38, 72 and 103 days of regrowth (Table 1). Samples were collected randomly by cutting 5 cm above the ground and bulked to comprise eight samples per cultivar. Samples were transported in cooler boxes to the laboratory, separated into stem portion (stems and branches) and leaf portion (consisting of leaves, flowers and pods when present). Samples were dried in a forced-air oven at 55 °C for 48 h to determine the dry matter (DM) content. Samples were then ground to pass through a 1 mm screen using a Cyclotec mill (Foss Tecator AB, Hoganas, Sweden) and analysed for CP, neutral detergent fibre (NDF) and acid detergent fibre (ADF) using the near-infrared reflectance spectroscopy (NIRS) technique described by Norman et al. [24]. The L/S was calculated as dry leaf portion weight divided by dry stem portion weight in grams.

Table 1. Physiological maturity phase of experimental forage at harvest.

Harvest Day	Cultivar		
	JCU2	**JCU4**	**JCU7**
		Experiment 1	
11	Vegetative	Vegetative	Vegetative
38	Pre-bloom	Pre-bloom	Vegetative
72	Developing seeds	Developing seeds	Vegetative
103	Mid-mature seeds	Mid-mature seeds	Full bloom
		Experiment 2	
78	Vegetative	Pre-bloom	Vegetative
122	Full bloom	Full bloom	Vegetative
168	Fully mature seeds	Fully mature seeds	Early bloom

2.2. Experiment 2

Desmanthus was established in coastal north Queensland (19.21° S, 146.57° E) on 15 August 2020, after soil analysis to determine the suitability of the site for desmanthus production. The soils were dark greyish brown 10YR 3/2, heavy sandy loam texture with a pH of 6.3. The area receives 1136 mm mean annual rainfall with mean minimum and maximum temperatures of 19.9 °C and 29.0 °C, respectively [25]. The plot was prepared with a tractor and rotary hoe to create a weed-free seed bed. Single furrow per cultivar, approximately 40 m long and 4 m between rows, were made using a hand hoe and seeds sown by hand. The plots were rain-fed, but supplementary drip irrigation was used to occasionally water the plants to avoid water stress. The plot was not fertilised throughout the experiment. To mimic cattle grazing, approximately 300 g of the top 20–30 cm 'grab' samples of JCU2, JCU4 and JCU7 were collected randomly at 78, 122 and 168 days after establishment (Table 1) from three different locations and stored at −20 °C prior to processing.

2.3. Animal Management

The animal use and management procedures were carried out according to the Australian code of practice for the care and use of animals for scientific purposes [26] and were reviewed and approved by the CSIRO Agriculture Animal Ethics Committee (Approval number 21/05). Three rumina fistulated Droughtmaster steers weighing 550 ± 48 kg were group housed in an open outdoor pen 18 m × 15 m. Steers were fed Rhodes grass (*Chloris gayana*) hay of 13.8% CP, 66.2% NDF and 32.9% ADF ad libitum and had free access to shade, water and a mineral block (Trace Element Northern, Ollson's, Yennora, NSW, Australia) throughout the study period. A 14-day pre-experimental period was observed to gradually adapt the steers to the Rhodes grass hay diet. The adaptation period was followed by three weekly experimental periods. The study was organised in a randomised split-plot design with steers as the whole plots and cultivars as sub-plots to ensure that every steer received all the cultivars and maturity stages.

2.4. In Situ Incubations and Chemical Analysis

Samples were dried at 55 °C for 48 h in a forced-air oven to determine the DM content and ground to pass through a 2 mm screen with a Christy and Norris grinder (Christy Turner Ltd., Suffolk, England). Approximately 5 g of ground desmanthus forage were weighed into nylon bags measuring 9 cm × 14 cm and 45 µm pore size in duplicates. The nylon bags were enclosed in retaining sacs constructed of a mesh (3 mm × 5 mm) material that permits rumen fluid to percolate completely. The sacs were inserted into the rumen and nylon bags added into the mesh sac using the sequential-in all-out method to ensure 0, 2, 4, 8, 12, 24, 48 and 72 h incubation periods. Each sample was incubated in duplicate per steer to provide a total of six bags per sample. Rumen fluid pH was measured at every incubation time to ensure an optimum rumen environment was maintained. All bags were

removed from the rumen after the incubation period was completed and immediately submerged in ice-cold water to stop further microbial activity. The zero h bags were not incubated but were washed together with the incubated bags to determine the easily degradable component of the samples. Bags were washed with cold water using a domestic laundry machine with a short cycle and dried in a forced-air oven at 55 °C for 72 h. Dry samples were weighed and duplicates combined and ground to pass through a 1 mm screen using the Cyclotec mill before chemical analysis. The NDF (without heat-stable α amylase) and ADF compositions were determined sequentially according to the methods of Van Soest et al. [27], respectively, using an ANKOM 220 Fibre Analyser (ANKOM Technology, Fairport, NY, USA). The nitrogen (N) component of 162 samples was determined by the Dumas combustion method [28] with a Leco CN628 N Analyser (Leco, St. Joseph, MI, USA). The N results were used to develop calibrations to predict the N composition of the remaining 67 samples with the NIRS technique due to mechanical failure of the Leco CN628 N Analyser. The CP composition of the samples was calculated as total N × 6.25.

2.5. Data Analysis

2.5.1. Calculations

The DM, CP, NDF and ADF degradability were calculated within steer and period. Duplicate values at each time were averaged and then fitted using the revised nonlinear regression equation of Ørskov and McDonald [29] (Equation (1)) or the Ørskov and McDonald [30] (Equation (2)).

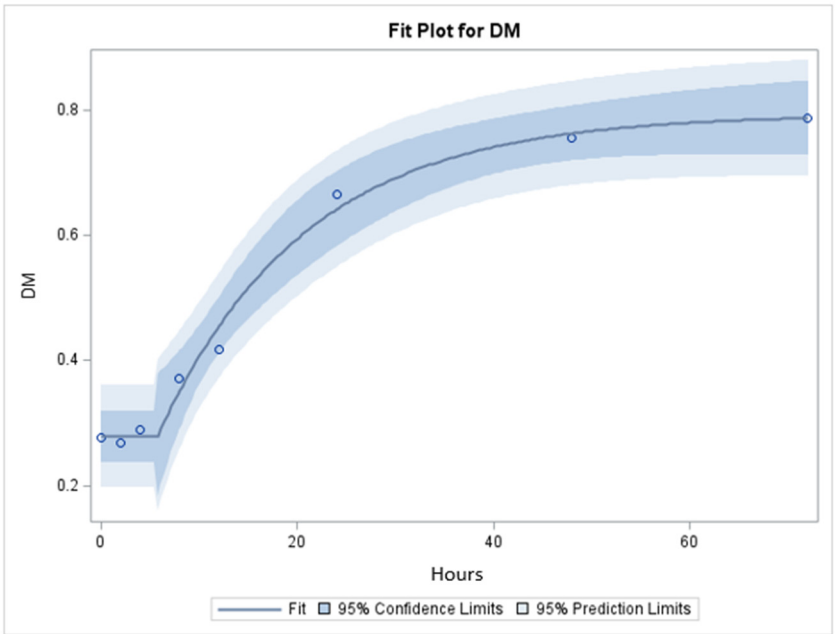
The following exponential equations were used based on preliminary model fitting (Figure 1);

$$P = A + B(1 - e^{-C(T-L)}) \text{ for DM degradability} \quad (1)$$

$$P = A + B(1 - e^{-CT}) \text{ for CP, NDF and ADF degradability} \quad (2)$$

where P is the proportion degraded at time T, A is the highly soluble fraction, B is the potentially degradable fraction, A + B is the total potentially degradable fraction, C is the rate of degradation of fraction B and L is the lag time.

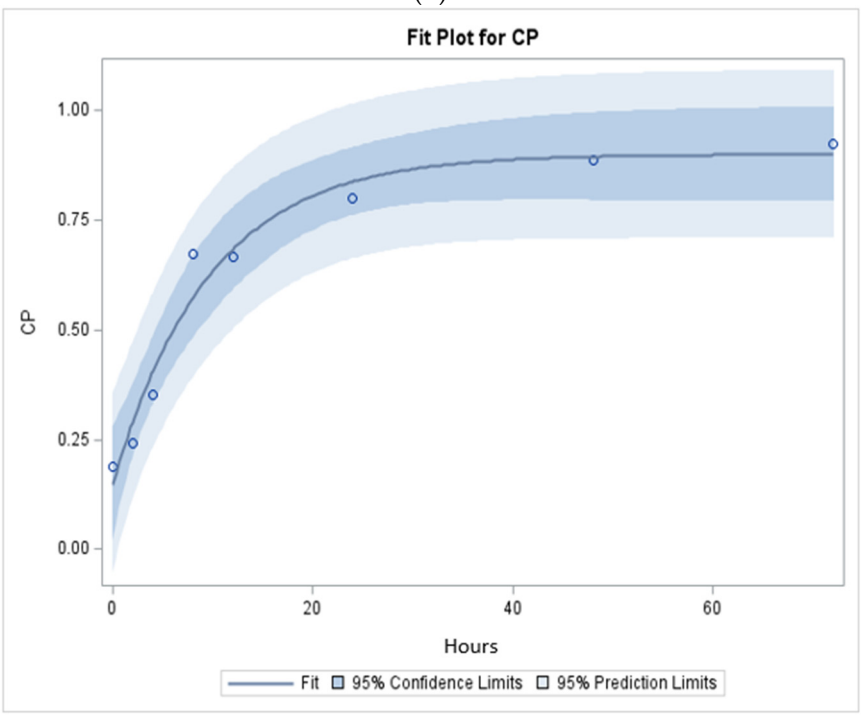
(**a**)



A = 0.28 (0.02), *B* = 0.52 (0.03), *C* = 0.07 (0.01), *L* = 5.71 (1.12)

Figure 1. *Cont.*

(**b**)



A = 0.15 (0.05), B = 0.75 (0.06), C = 0.10 (0.02)

Figure 1. Nonlinear models for the estimation of degradation of (**a**) DM and (**b**) CP. *A* is the intercept of the curve at 0 h representing the highly soluble fraction; *B* is the asymptote representing the potentially degradable fraction; *C* is the fractional rate constant for degradation of B; *L* is the time lag.

The parameters (A, B, A + B, C, L) of degradation were estimated from the solution of the exponential Equations (1) and (2) using the SAS procedure (PROC NLIN) from degradability data. Examples of the fitted segmented nonlinear (exponential) and exponential curves for DM and CP are displayed in Figure 1. The effective degradable proportion (ED) was calculated as:

$$ED = A + BC/(C + K) \quad (3)$$

where K is the particle outflow rate from the rumen (/h). The ED was estimated with slow and rapid outflow rates of 2%/h (ED2) and 6%/h (ED6), respectively.

2.5.2. Statistical Analysis

All statistical data analyses were implemented in SAS version 9.4 and R version 4.0.1. Plant component nutritive values (leaf to stem ratio, DM, CP, NDF and ADF) and degradability variables (A, B, A + B, C, L, ED2 and ED6) were summarised as means and standard deviations. Analysis of variance (ANOVA) was used to determine if the nutritive values (4) and degradability (5) of desmanthus differ between cultivars and maturity stage at harvest. In addition, post hoc pairwise comparisons were carried out with Tukey's adjusted *p*-Values.

The model for plant component nutritive values is of the form:

$$Y_{ij} = \mu + \text{Cultivar}_i + \text{Maturity}_j + (\text{Cultivar} \times \text{Maturity})_{ij} + e_{ij} \quad (4)$$

where:

Y_{ij} is the nutritive value (leaf to stem ratio, DM, CP, NDF or ADF) for *i*th cultivar at *j*th maturity time;

μ is the overall mean;

Cultivar_i is the fixed effect of the *i*th cultivar (JCU2, JCU4, JCU7);

$Maturity_j$ is the fixed effect of the stage of maturity (11, 38,72, 103 days);

$(Cultivar \times Maturity)_{ij}$ is the interaction between cultivar and stage of maturity (fixed effects);

e_{ij} is random error.

e_{ij} i.i.d ~N(0, σe^2).

The model for degradability variables is of the form:

$$Y_{ijk} = \mu + Cultivar_i + Maturity_j + (Cultivar \times Maturity)_{ij} + \alpha_k + e_{ijk} \quad (5)$$

where:

Y_{ijk} is a degradability variable (A, B, A + B, C, L, ED2 and ED6) for *i*th cultivar at *j*th maturity time in steer *k*;

μ is the overall mean;

$Cultivar_i$ is the fixed effect of the *i*th cultivar (JCU2, JCU4, JCU7);

$Maturity_j$ is the fixed effect of the stage of maturity (78, 122, 168 days);

$(Cultivar \times Maturity)_{ij}$ is the interaction between cultivar and stage of maturity (fixed effects);

α_k is a random effect of the steer;

e_{ij} is a residual error.

α_k i.i.d ~N(0, $\sigma_\alpha{}^2$).

In addition, e_{ij} and α_k were assumed to be independent. Inferences were based on a 5% level of significance.

3. Results

3.1. Experiment 1: L/S, DM and Chemical Composition

The effect of desmanthus cultivar, maturity stage at harvest and their interactions on L/S, DM, CP, NDF and ADF are presented in Table 2, Figures 2 and 3.

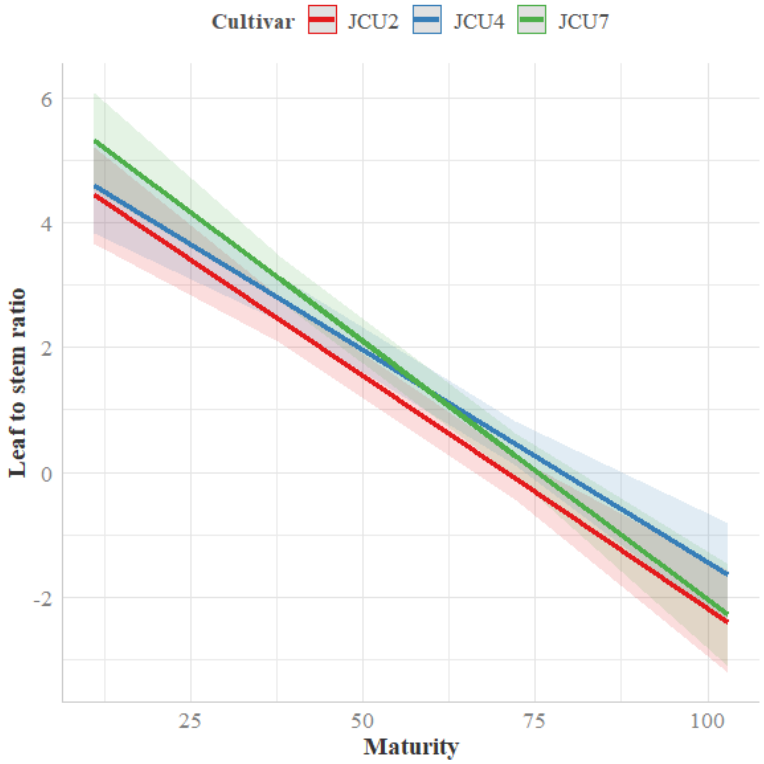


Figure 2. Effect plots for the interactions between cultivar and maturity on desmanthus leaf to stem mass ratio (p = 0.04).

Table 2. Mean leaf to stem mass ratio (L/S), dry matter (DM; %) and chemical composition (% DM) of desmanthus harvested at varying maturity stages.

Variable [1]	Cultivar [2]	Maturity at Harvest				Mean	SEM [3]	*p*-Value		
		11	38	72	103			C	M	C*M
L/S	JCU2	2.2	0.60	0.74	0.89	1.1 [a]	0.09	<0.01	<0.01	0.04
	JCU4	2.4	0.77	1.7	1.5	1.6 [b]				
	JCU7	3.5	0.82	1.1	1.2	1.7 [b]				
	Mean	2.7	0.73	1.2	1.2					
Leaf										
DM	JCU2	22.8	28.5	33.5	42.4	31.8	0.99	0.14	<0.01	0.01
	JCU4	24.0	27.8	31.0	53.6	34.1				
	JCU7	25.9	26.3	31.9	44.2	32.1				
	Mean	24.2	27.5	32.1	46.7					
CP	JCU2	28.1	23.5	23.7	19.5	23.7	0.57	0.12	<0.01	<0.01
	JCU4	29.0	27.2	19.9	16.9	23.3				
	JCU7	24.3	23.4	23.0	18.7	22.4				
	Mean	27.1	24.7	22.2	18.4					
NDF	JCU2	47.1	49.6	51.5	38.2	46.6 [b]	0.74	<0.01	0.01	0.27
	JCU4	45.4	39.2	37.9	41.4	41.0 [a]				
	JCU7	46.5	44.6	51.0	43.1	46.3 [b]				
	Mean	46.3	44.5	46.8	40.9					
ADF	JCU2	14.6	24.5	27.1	25.1	22.8 [a]	0.79	0.02	<0.01	0.03
	JCU4	16.1	23.3	27.8	32.3	24.9 [b]				
	JCU7	18.0	27.7	26.5	29.6	25.5 [b]				
	Mean	16.2	25.2	27.1	29.0					
Stem										
DM	JCU2	19.5	31.2	41.0	42.2	33.5 [a]	1.2	0.03	<0.01	0.02
	JCU4	17.8	33.6	44.3	51.1	36.7 [b]				
	JCU7	18.9	32.3	35.8	48.9	34.0 [a]				
	Mean	18.7	32.4	40.4	47.4					
CP	JCU2	12.5	7.2	6.1	6.7	8.1	0.41	0.63	<0.01	0.29
	JCU4	13.1	6.8	4.6	7.2	7.9				
	JCU7	11.6	6.2	8.4	7.9	8.5				
	Mean	12.4	6.7	6.4	7.3					
NDF	JCU2	54.2	65.6	68.8	67.1	63.9	0.92	0.88	0.01	<0.01
	JCU4	53.9	67.3	72.8	64.2	64.6				
	JCU7	63.4	69.1	63.1	59.6	63.8				
	Mean	57.2	67.3	68.2	63.6					
ADF	JCU2	40.0	52.8	54.3	50.7	49.5	0.85	0.94	0.01	0.02
	JCU4	40.4	52.9	56.3	49.3	49.7				
	JCU7	46.7	55.5	50.3	44.8	49.3				
	Mean	42.4	53.7	53.6	48.3					

[1] DM: dry matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre. [2] JCU2: *D. virgatus*, JCU4: *D. bicornutus*, JCU7: *D. leptophyllus*. [3] SEM: standard error of the mean. C: cultivar effect, M: maturity effect, C*M: cultivar by maturity interaction; *p*-Value based on ANOVA. [ab] Cultivar means with different superscripts were significantly different ($p < 0.05$).

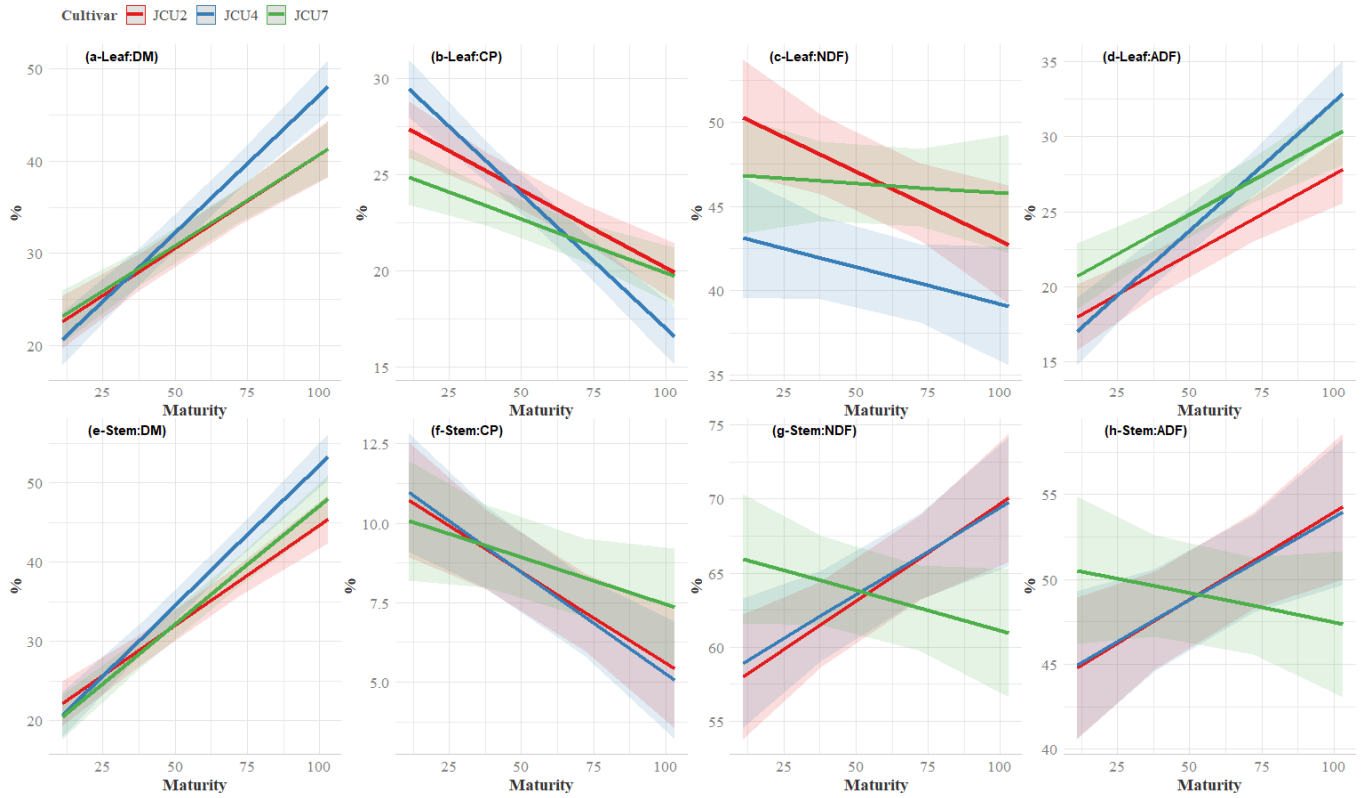


Figure 3. Effect plots for the interactions between cultivar and maturity at harvest on desmanthus leaf and stem portion dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) composition. All interactions were significant except the leaf NDF ($p = 0.27$) and stem CP ($p = 0.29$).

3.1.1. Leaf to Stem Mass Ratio

There was an interaction between cultivar and maturity on the L/S ($p = 0.04$; Figure 2), where the L/S of cultivar JCU7 declined more steeply than the other cultivars, JCU2 and JCU4. The L/S was higher in JCU7 for young desmanthus forage and higher in JCU4 for the mature forage, and L/S decreased linearly with maturity for all cultivars evaluated ($p < 0.01$).

3.1.2. Dry Matter Concentration

Significant interactions between cultivar and maturity were observed for DM concentration of both leaf and stem portions ($p \leq 0.02$; Figure 3a,e). In both cases, there was a higher linear increase in DM composition of JCU4 with maturity compared to JCU2 and JCU7, which increased at a lower rate. Generally, the stem DM was highest in JCU4 and lowest in JCU2.

3.1.3. Crude Protein Composition

As shown in Table 2, an interaction between cultivar and maturity was observed for the leaf CP ($p < 0.01$) but not in stem CP ($p = 0.29$). There was a higher linear decline in leaf CP of JCU4 with maturity than JCU2 and JCU7 (Figure 3b). The stem CP declined with maturity for all cultivars ($p < 0.01$), but no significant difference between cultivar means was observed ($p = 0.63$).

3.1.4. Fibre Composition

A significant interaction effect of cultivar and maturity on the stem NDF ($p < 0.01$) was observed but not in the leaf NDF ($p = 0.27$). The stem NDF increased substantially with maturity in JCU2 and JCU4 but not in JCU7 (Figure 3g). On the other hand, the leaf

NDF declined with maturity ($p = 0.01$), and NDF was lower in JCU4 compared to JCU2 and JCU7 ($p < 0.01$). There were significant interactions between cultivar and maturity on the leaf and stem ADF ($p \leq 0.03$). The leaf ADF increased more steeply with maturity for JCU4 compared to JCU2 and JCU7 (Figure 3d), while the stem ADF increased with maturity for both JCU2 and JCU4 but not in JCU7 (Figure 3h).

3.2. Experiment 2: Forage DM and Chemical Composition

The forage DM, CP, NDF and ADF are presented in Table 3. These data were obtained from composite samples, thus not statistically analysed. Generally, all the cultivars portrayed a numerical increase in NDF and ADF composition with an increase in maturity. In contrast, the CP decreased with an increase in maturity for JCU2 and JCU7, but JCU4 CP increased from day 78 to day 122, followed by a decline on day 168. The DM composition for JCU2 and JCU4 increased with maturity, while JCU7 DM was lowest on day 122 and highest on day 168. The mean DM, CP, NDF and ADF were similar across cultivars, with a maximum difference of 3.3% in ADF observed between JCU4 and JCU7.

Table 3. Dry matter (%) and chemical composition (%DM) of desmanthus forage harvested 78, 122 and 168 days after planting.

Variable [1]	JCU2				JCU4				JCU7			
	78	**122**	**168**	**Mean**	**78**	**122**	**168**	**Mean**	**78**	**122**	**168**	**Mean**
DM	26.1	29.4	33.2	29.6	27.4	26.6	34.3	29.4	28.3	28.1	30.3	28.9
CP	25.2	20.4	16.9	20.8	23.0	26.4	17.3	22.2	23.8	22.0	20.8	22.2
NDF	25.4	34.2	38.7	32.8	25.6	27.4	47.9	33.6	29.3	31.2	35.2	31.9
ADF	14.0	19.3	24.5	19.3	14.0	15.9	32.4	20.8	13.0	18.2	21.2	17.5

[1] DM: dry matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre; JCU2: *D. virgatus*, JCU4: *D. bicornutus*, JCU7: *D. leptophyllus*.

3.3. Dry Matter Degradation

Table 4 present the DM A, B, A + B, C, L, ED2 and ED6 of three desmanthus cultivars (JCU2, JCU4 and JCU7) harvested 78, 122 and 168 days after establishment. There were no significant interactions between cultivar and maturity for all variables evaluated ($p \geq 0.17$). The lowest degradation of instantly degraded A fraction was observed on day 168 for all cultivars ($p = 0.01$), whereas degradation of A was lower for JCU7 than JCU2 and JCU4 ($p < 0.01$). The slowly degraded B proportion of DM was influenced by cultivar with the highest degradation recorded for JCU7 followed by JCU2, and the least degradation was in JCU4 ($p < 0.01$). However, degradation of the A + B component and the ED2 were not significantly different between cultivars ($p \geq 0.25$), although degradation declined with maturity for all cultivars ($p < 0.01$; Figure 4c). The cultivar and maturity had significant effects on the lag time ($p \leq 0.04$). The lag time increased linearly with maturity (Figure 4e), whereas JCU7 had the longest lag time. The ED6 was influenced by cultivar and maturity ($p < 0.01$). ED6 declined linearly with maturity for all cultivars (Figure 4g), but JCU4 had higher ED6 than JCU7.

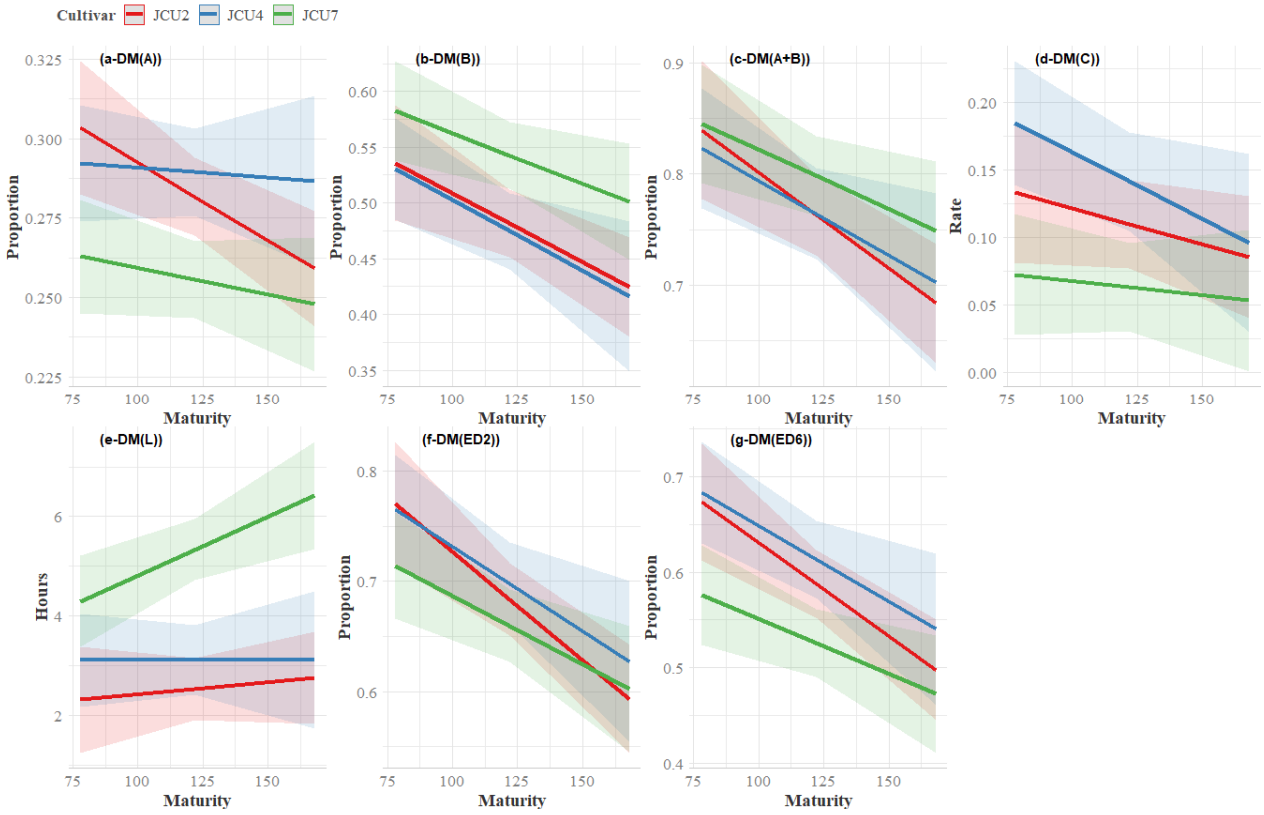


Figure 4. Effect plots for the change in in situ dry matter (DM) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. (**a**) A; highly soluble fraction, (**b**) B; potentially degradable fraction, (**c**) A + B; total potentially degradable fraction, (**d**) C; rate of degradation of fraction B, (**e**) L; lag time, (**f**) ED2; effective DM degradability with particle outflow rate of 2%/h and (**g**) ED6; effective DM degradability with particle outflow rate of 6%/h. No significant interactions between cultivar and maturity were observed ($p \geq 0.17$).

Table 4. Effect of cultivar and maturity stage at harvest of desmanthus on in situ dry matter (DM) degradation. Data presented in fractions unless otherwise stated.

Variable [1]	Cultivar [2]	Maturity at Harvest					*p*-Value [4]		
		78	122	168	Mean	SEM [3]	C	M	C*M
A	JCU2	0.30	0.27	0.26	0.28 [b]	0.00	<0.01	0.01	0.22
	JCU4	0.28	0.30	0.26	0.28 [b]				
	JCU7	0.25	0.26	0.24	0.25 [a]				
	Mean	0.28	0.28	0.25					
B	JCU2	0.53	0.48	0.42	0.48 [b]	0.01	<0.01	<0.01	0.51
	JCU4	0.49	0.54	0.31	0.45 [a]				
	JCU7	0.59	0.51	0.52	0.54 [c]				
	Mean	0.54	0.51	0.42					
A + B	JCU2	0.84	0.75	0.68	0.76	0.01	0.30	<0.01	0.66
	JCU4	0.78	0.84	0.58	0.73				
	JCU7	0.85	0.78	0.76	0.79				
	Mean	0.82	0.79	0.67					
C (/h)	JCU2	0.13	0.11	0.08	0.11 [ab]	0.01	<0.01	0.03	0.48
	JCU4	0.17	0.15	0.07	0.13 [b]				
	JCU7	0.06	0.07	0.04	0.06 [a]				
	Mean	0.12	0.11	0.06					

Table 4. *Cont.*

Variable [1]	Cultivar [2]	Maturity at Harvest					*p*-Value [4]		
		78	122	168	Mean	SEM [3]	C	M	C*M
Lag (h)	JCU2	2.1	2.7	2.6	2.5 [a]	0.31	<0.01	0.04	0.17
	JCU4	3.0	3.1	3.0	3.0 [a]				
	JCU7	4.0	5.7	6.1	5.3 [b]				
	Mean	3.0	3.8	3.9					
ED2	JCU2	0.77	0.68	0.59	0.68	0.01	0.25	<0.01	0.55
	JCU4	0.72	0.77	0.51	0.67				
	JCU7	0.71	0.66	0.59	0.65				
	Mean	0.73	0.70	0.57					
ED6	JCU2	0.67	0.58	0.49	0.58 [ab]	0.02	<0.01	<0.01	0.53
	JCU4	0.65	0.67	0.44	0.59 [b]				
	JCU7	0.56	0.54	0.46	0.52 [a]				
	Mean	0.63	0.60	0.46					

[1] A: highly soluble fraction, B: potentially degradable fraction, A + B: total potentially degradable fraction, C: rate of degradation of fraction B, ED2: effective degradability with particle outflow rate of 2%/h, ED6: effective degradability with particle outflow rate of 6%/h. [2] JCU2: *D. virgatus*, JCU4: *D. bicornutus*, JCU7: *D. leptophyllus* [3] SEM, standard error of the mean. [4] C: cultivar effect, M: maturity effect, C*M: cultivar by maturity interaction; *p*-Value based on ANOVA. [abc] Cultivar means with different uppercase superscripts per variable were significantly different ($p < 0.05$).

3.4. Crude Protein Degradation

The degradation of CP fractions and the degradation rate are presented in Table 5 and Figure 5. The cultivar and maturity interaction were significant for the A and A+B fractions ($p < 0.01$) but not for the B, C, ED2 and ED6 variables ($p \geq 0.11$). Degradation of the A component increased linearly with maturity for all cultivars ($p < 0.01$), but the increase was higher for JCU4. The overall degradation was highest in JCU4 and lowest in JCU7 ($p = 0.01$). The A + B degradation was observed to decline with maturity for all cultivars ($p = 0.04$; Figure 5c). JCU7 had lower C, ED2 and ED6 than both JCU2 and JCU4 ($p < 0.01$), while the C and ED2 were observed to decline with maturity (Figure 5). The B component degradation was influenced by cultivar effects, with higher degradation observed for JCU7 than JCU4 ($p = 0.01$), but JCU2 did not differ from either JCU4 or JCU7.

Table 5. Effect of cultivar and maturity stage at harvest of desmanthus on in situ crude protein (CP) degradation. Data presented in fractions unless otherwise stated.

Variable [1]	Cultivar [2]	Maturity at Harvest					*p*-Value [4]		
		78	122	168	Mean	SEM [3]	C	M	C*M
A	JCU2	0.16	0.20	0.23	0.20 [ab]	0.01	0.01	<0.01	<0.01
	JCU4	0.13	0.22	0.32	0.23 [b]				
	JCU7	0.15	0.17	0.19	0.17 [a]				
	Mean	0.15	0.20	0.25					
B	JCU2	0.72	0.63	0.57	0.64 [ab]	0.01	0.01	<0.01	0.11
	JCU4	0.66	0.70	0.44	0.60 [a]				
	JCU7	0.72	0.71	0.65	0.70 [b]				
	Mean	0.70	0.68	0.55					
A + B	JCU2	0.89	0.83	0.81	0.84	0.01	0.20	0.04	<0.01
	JCU4	0.80	0.93	0.76	0.83				
	JCU7	0.88	0.89	0.85	0.87				
	Mean	0.86	0.88	0.81					

Table 5. *Cont.*

Variable [1]	Cultivar [2]	Maturity at Harvest					*p*-Value [4]		
		78	122	168	Mean	SEM [3]	C	M	C*M
C (/h)	JCU2	0.09	0.08	0.08	0.08 [b]	0.00	<0.01	<0.01	0.47
	JCU4	0.10	0.09	0.06	0.08 [b]				
	JCU7	0.05	0.03	0.03	0.04 [a]				
	Mean	0.08	0.07	0.06					
ED2	JCU2	0.76	0.71	0.70	0.72 [b]	0.01	<0.01	0.01	0.71
	JCU4	0.69	0.80	0.66	0.72 [b]				
	JCU7	0.67	0.64	0.60	0.64 [a]				
	Mean	0.71	0.72	0.65					
ED6	JCU2	0.60	0.57	0.57	0.58 [b]	0.01	<0.01	0.16	0.43
	JCU4	0.55	0.65	0.56	0.59 [b]				
	JCU7	0.48	0.45	0.42	0.45 [a]				
	Mean	0.55	0.56	0.52					

[1,2,3,4] Abbreviations and effects are the same as in Table 4; *p*-Value based on ANOVA. C*M: cultivar by maturity interaction. [ab] Cultivar means with different superscripts were significantly different ($p < 0.05$).

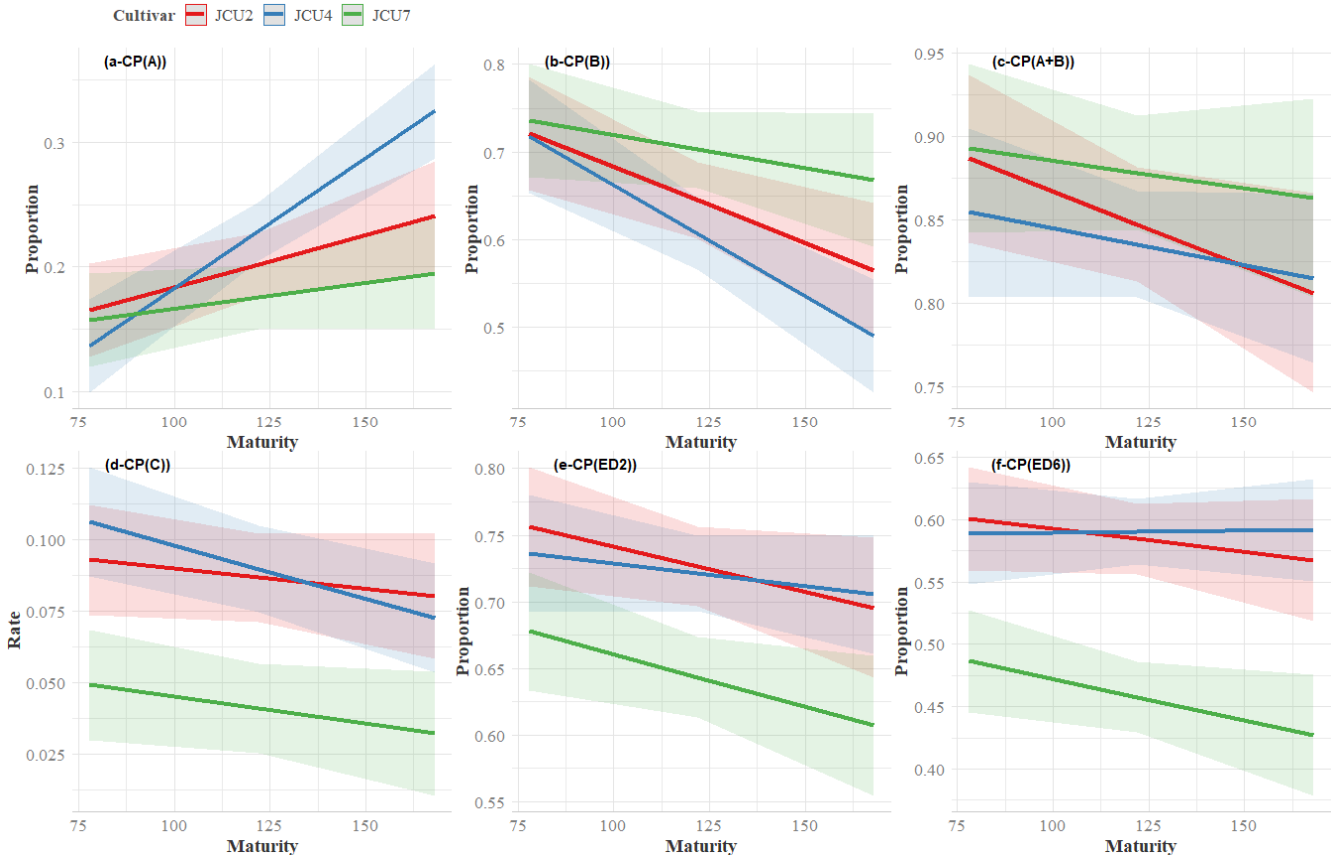


Figure 5. Effect plots for the change in in situ crude protein (CP) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. (**a**) A; highly soluble fraction, (**b**) B; potentially degradable fraction, (**c**) A + B; total potentially degradable fraction, (**d**) C; rate of degradation of fraction B, (**e**) ED2; effective CP degradability with particle outflow rate of 2%/h and (**f**) ED6; effective CP degradability with particle outflow rate of 6%/h. Cultivar by maturity interactions were not significant except for the A and A + B proportions ($p < 0.01$).

3.5. Fibre Degradation

The desmanthus potentially degradable fraction (B), rate of degradation of fraction B (C) and effective degradability (ED2 and ED6) of NDF and ADF in response to cultivar, maturity and their interactions are presented in Table 6 and Figures 6 and 7. There were no significant effects of the cultivar and maturity interactions on all the NDF and ADF variables evaluated ($p \geq 0.12$). Components B, C, ED2 and ED6, declined with maturity for NDF and ADF ($p < 0.01$). Significant cultivar effects were observed for B and C of NDF ($p < 0.01$), and C in ADF component ($p = 0.02$). The degradation of the B component of the NDF was higher in JCU7 than in JCU2 and JCU4. However, JCU7 had lower C for both the NDF and ADF proportions.

Table 6. Effect of cultivar and maturity stage at harvest of desmanthus on in situ neutral detergent fibre (NDF) and acid detergent fibre (ADF) degradation. Data presented in fractions unless otherwise stated.

Variable [1]	Cultivar [2]	Maturity at Harvest					*p*-Value [4]		
		78	122	168	Mean	SEM [3]	C	M	C*M
NDF									
B	JCU2	0.77	0.62	0.43	0.61 [a]	0.03	<0.01	<0.01	0.12
	JCU4	0.71	0.71	0.31	0.58 [a]				
	JCU7	0.96	0.82	0.88	0.89 [b]				
	Mean	0.81	0.71	0.54					
C (/h)	JCU2	0.05	0.04	0.03	0.04 [b]	0.00	<0.01	<0.01	0.99
	JCU4	0.05	0.05	0.03	0.04 [b]				
	JCU7	0.03	0.02	0.01	0.02 [a]				
	Mean	0.04	0.04	0.02					
ED2	JCU2	0.56	0.43	0.27	0.42	0.02	0.70	<0.01	0.75
	JCU4	0.50	0.51	0.19	0.40				
	JCU7	0.57	0.41	0.39	0.46				
	Mean	0.55	0.45	0.28					
ED6	JCU2	0.37	0.27	0.16	0.27	0.01	0.66	<0.01	0.77
	JCU4	0.33	0.33	0.10	0.25				
	JCU7	0.32	0.20	0.18	0.23				
	Mean	0.34	0.27	0.15					
ADF									
B	JCU2	0.71	0.60	0.38	0.56	0.03	0.09	<0.01	0.14
	JCU4	0.64	0.69	0.32	0.55				
	JCU7	0.77	0.65	0.71	0.71				
	Mean	0.71	0.64	0.47					
C (/h)	JCU2	0.04	0.02	0.02	0.03 [b]	0.00	0.02	<0.01	0.47
	JCU4	0.03	0.03	0.02	0.03 [b]				
	JCU7	0.03	0.02	0.01	0.02 [a]				
	Mean	0.03	0.02	0.02					
ED2	JCU2	0.49	0.31	0.21	0.34	0.02	0.77	<0.01	0.29
	JCU4	0.39	0.42	0.19	0.33				
	JCU7	0.46	0.34	0.31	0.37				
	Mean	0.45	0.36	0.24					
ED6	JCU2	0.30	0.16	0.11	0.19	0.01	0.55	<0.01	0.28
	JCU4	0.22	0.24	0.10	0.18				
	JCU7	0.26	0.17	0.15	0.19				
	Mean	0.26	0.19	0.12					

[1,2,3,4] Abbreviations same as in Table 4; *p*-Value based on ANOVA. C*M: cultivar by maturity interaction. [a,b] Cultivar means with different superscripts were significantly different ($p < 0.05$).

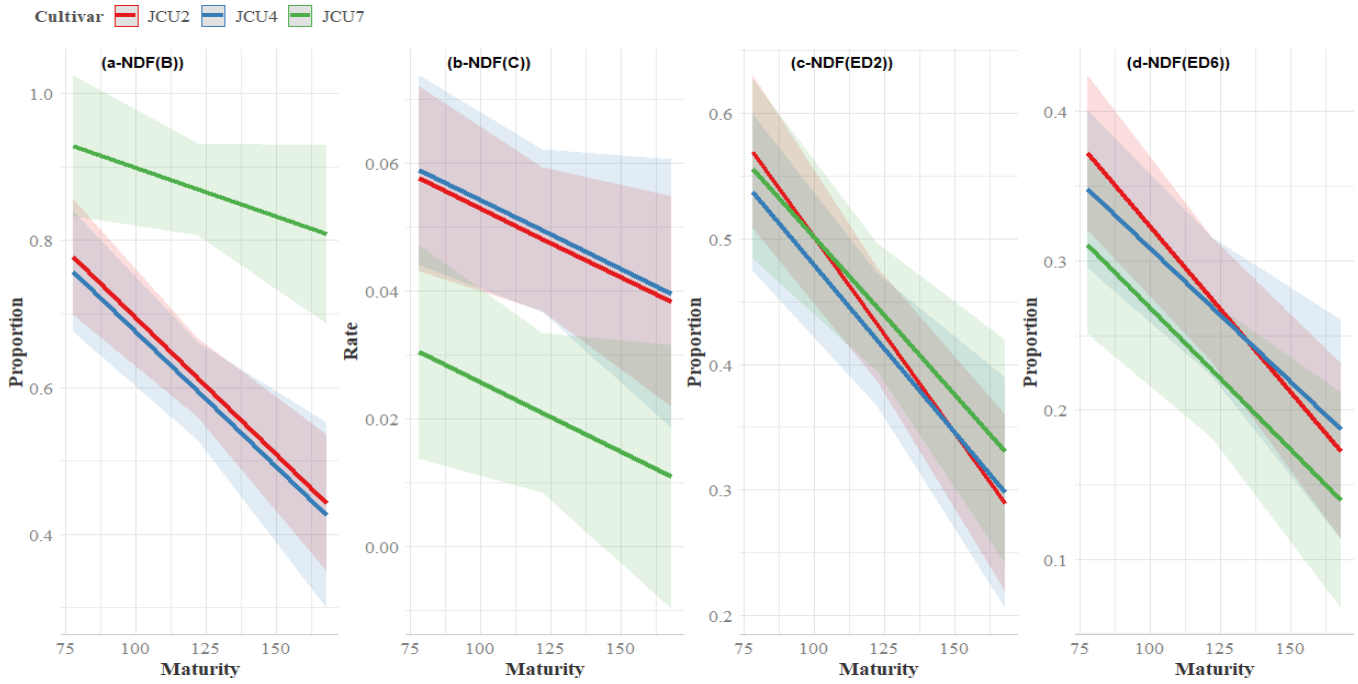


Figure 6. Effect plots for the change in in situ neutral detergent fibre (NDF) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. (**a**) B; potentially degradable fraction, (**b**) C; rate of degradation of fraction B, (**c**) ED2; effective NDF degradability with particle outflow rate of 2%/h and (**d**) ED6; effective NDF degradability with particle outflow rate of 6%/h. Cultivar by maturity interactions were not significant ($p \geq 0.12$).

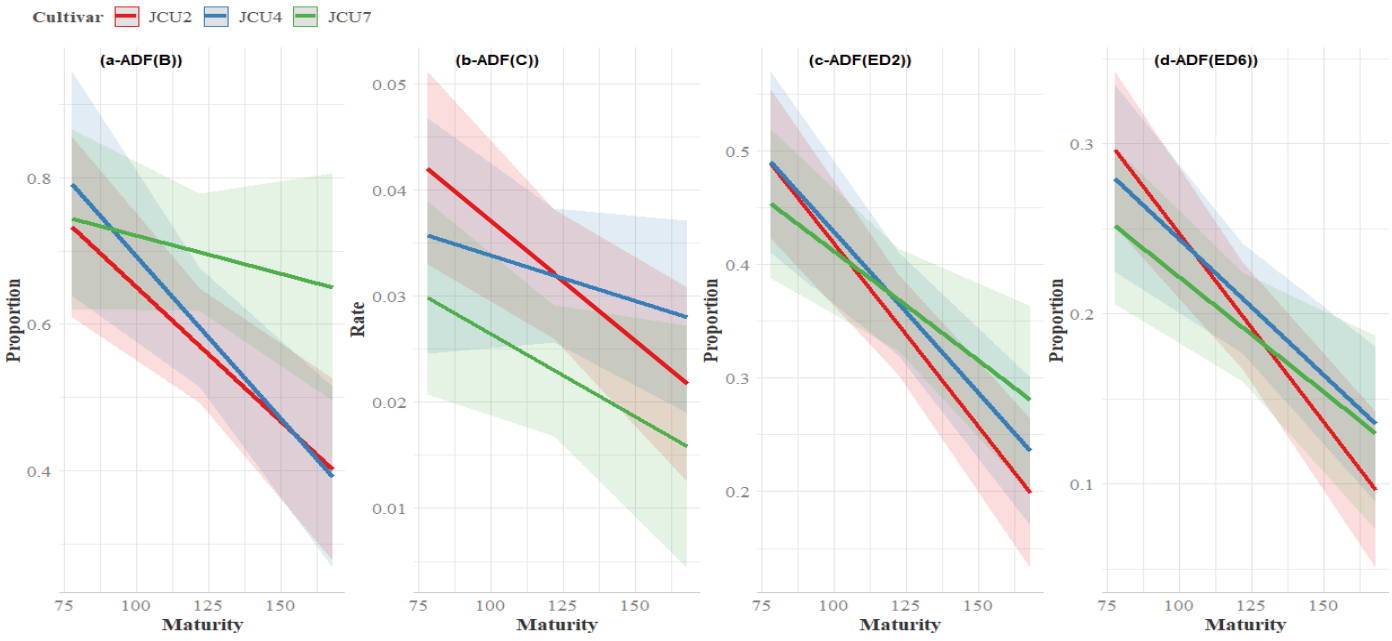


Figure 7. Effect plots for the change in in situ acid detergent fibre (ADF) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. (**a**) B; potentially degradable fraction, (**b**) C; rate of degradation of fraction B, (**c**) ED2; effective ADF degradability with particle outflow rate of 2%/h and (**d**) ED6; effective ADF degradability with particle outflow rate of 6%/h. Cultivar by maturity interactions were not significant ($p < 0.14$).

4. Discussion

The quality of forage for livestock is a function of nutrient concentration, DM intake and nutrient availability [31]. Diet digestibility influences digestible energy intake and nutrient supply in ruminants fed forage-based diets due to the close interconnection between diet digestibility and intake [32]. Thus, diet chemical composition analysis and biological assays that simulate ruminal conditions for feed digestibility analysis are employed to evaluate forage quality for livestock feeding [33], and the in situ technique is one such approach that is widely used [34]. Ruminants derive approximately 60–70% of digestible energy from fermentation in the rumen [35]. The rumen accounts for up to 97% of total gastrointestinal tract NDF digestion [36] and 50–80% of CP degradation [37]. Therefore, this study evaluated the chemical composition and in situ degradability of three desmanthus cultivars harvested at varying maturity stages.

4.1. Leaf to Stem Mass Ratio

The leaf proportion of forage contributes critically to the forage nutritive value because a decrease in leaf proportion is associated with increased forage NDF and ADF, which are inversely correlated with digestibility [38]. The L/S ranged between 0.7 and 3.5 in this study, and this range was wider than the 0.9–1.3 range reported for unidentified species of desmanthus in Brazil [39]. In this study, there was a decrease in the L/S with advancing plant maturity. These results concur with findings reported previously for *D. virgatus* and several other forage legumes such as *Medicago sativa*, *Trifolium repens* and *Desmodium intortum* [40–42]. The decline in L/S with maturity is attributed to the decreasing leaf proportion due to senescence and increasing stem proportion as the plant matures [43]. The finding that cultivar JCU7 had the highest L/S in this study was expected since JCU7 is described to be among the highest performing cultivars of desmanthus in leafiness [15]. The slower decrease in L/S of JCU4 with maturity may be due to an increase in leaf mass and production of pods that might have offset the increase in the proportion of stem. Calado et al. [39] reported an increase in both leaves and stem yield with the maturity of desmanthus harvested at three pod stages.

4.2. Chemical Composition

The lower leaf NDF in JCU4 than JCU2 and JCU7 in this study agree with findings reported by Vandermeulen et al. [18] but contrast with the results of Durmic et al. [44], who reported higher NDF in JCU4 than JCU2. The discrepancies may be due to differences in the growth stage at harvest, plant part analysed or environmental factors [42,45,46]. This is because Durmic et al. [44] analysed the leaves and young stems up to 5cm long, propagated in an open environment, while Vandermeulen et al. [18] analysed whole plants grown in a semi-enclosed greenhouse and harvested from 10 cm above the ground. Dietary digestible fibre is the main source of digestible energy in beef cattle-fed forage diets, and NDF provides physically effective fibre that stimulates rumination, salivation and reticulorumen motility which help elevate ruminal pH [35]. An increase in dietary fibre is usually associated with a reduced concentration of fermentable carbohydrates [47]. Rumen microbial population growth is highly dependent on fermentable carbohydrates for energy. An insufficient supply of fermentable carbohydrates in relation to nitrogen reduces microbial protein synthesis and increases nitrogen loss through urine [48]. Therefore, the increase in ADF and decrease in CP with maturity observed in this study may reduce rumen microbial protein synthesis. Generally, the ADF increased with maturity in both the leaf and stem proportions, while NDF increased with maturity only in the stem proportion in this study. Structural carbohydrates are reported to increase with maturity for both leaf and stem proportions in desmanthus [42], but the increase is less in leaves and higher in the stem because of lignin accumulation within cell walls during stem diameter expansion through cambial activity [38]. The accumulation of structural carbohydrates in the lower stem internodes is accelerated as the plant stem height increases to provide plant support [38]. The CP was higher in the leaf portion at $\geq$16.9% compared to $\leq$7.9% in the stem on day 103.

In addition, the NDF was below 47% in the leaf and above 55% in the stem. Since grazing livestock select plant parts based on their nutritive need and availability [49,50], these findings indicate that animals can access high-quality diet later in the pasture growing season from desmanthus leaves when the stems have declined in quality.

The observed similar CP composition between cultivars in this study agrees with the findings reported by Suybeng et al. [51] for JCU2, JCU4 and JCU7, and Amben et al. [52] for JCU2 and JCU4. The decrease in desmanthus CP with an increase in maturity for both leaf and stem proportions observed in this study agree with findings reported by Suksombat and Buakeeree [42] for *D. virgatus* harvested at 30, 40 or 50 days regrowth and may be due to an increase in the fibre portion of the plant [53]. Except for the JCU4 stem portion on day 72, the forage CP was above the 5% to 5.6% threshold reported to prevent digestive disruption and body protein catabolism in cattle [44,54]; this suggests that desmanthus can be used as a high-quality forage to prevent weight loss of cattle grazing low-quality grass pastures. Since forage yield and quality are inversely correlated as maturity advances [42,55], there is a need to determine the optimum harvesting stage for desmanthus.

4.3. Dry Matter Degradability

The total potentially degradable DM (A + B) in this study (0.58 to 0.85) was within the values reported for tropical legumes [56,57]. The DM ED6 was lowest for JCU7 in this study though the total potentially degradable proportion did not differ between cultivars. In agreement with these findings, Vandermeulen et al. [18] reported lower in vitro organic matter digestibility of JCU1 (*D. leptophyllus*) compared to JCU4 and JCU2 harvested in summer. The authors attributed the findings to higher polyphenolic secondary compounds in JCU1. Significantly higher total tannins were recorded for JCU1 compared to JCU2 and JCU4 [18], while total phenolics were reported to be higher in JCU7 compared to JCU2 [51]. The potentially degradable DM (B) proportion declined by 36.7%, 20.8% and 11.9% for JCU4, JCU2 and JCU7, respectively, from day 78 to day 168 in this study resulting in the overall highest B degradability in JCU7. These results are plausible since the forage NDF composition had increased by 87.1%, 52.3% and 20.1% for JCU4, JCU2 and JCU7, respectively. An increase in dietary NDF results in a reduced concentration of the rapidly fermentable carbohydrates and consequently less energy for microbial growth and DM digestibility [40,47]. However, the effective degradability with a low particle outflow rate (ED2) from the rumen was not higher for JCU7 compared to other cultivars in this study due to the low degradation rate and the long lag time. Hence, the findings of this study suggest that the three desmanthus cultivars should be utilised when they are immature (below 168 days growth for JCU2 and JCU4, and below 122 days growth for JCU7) to achieve high effective DM digestibility.

4.4. Fibre Degradability

The rumen is the main site for fibre digestion since dietary fibre digestion is solely dependent on microbial fermentation as a function of the rate of degradation (C), the proportion of the total potentially degradable fraction and the rate of feed passage from the rumen [58]. Dietary fibre is hydrolysed and fermented to produce volatile fatty acids that are a major metabolic energy source for the animal and rumen microbial cells [59]. Although the potentially degradable fraction (B) of NDF was higher in JCU7 compared to JCU2 and JCU4, there was no significant difference in the ED2 and ED6 fractions of NDF between cultivars. This may be due to the slow degradability rate and long lag time observed for JCU7 limiting the fermentation time of fibre in the rumen [60]. The decline in NDF and ADF effective degradability with maturity is conceivable due to the increase in lignification of plants with advanced maturity [38]. Jung et al. [61] reported a −0.93 correlation between lignin composition and NDF digestibility of 36 forages.

4.5. Crude Protein Degradability

In the rumen, proteins are hydrolysed to peptides and amino acids which are incorporated into microbial cells or deaminated to form carbon dioxide, volatile fatty acids and ammonia [35]. Greater ruminal ammonia nitrogen accumulation can compromise microbial fermentation and result in a reduced total volatile fatty acids concentration [47]. The rumen degradable protein fraction provides nitrogen supply for rumen microbial activity and microbial protein synthesis, which is the main protein source for ruminants [62]. The rumen undegraded protein may be degraded post-rumen to provide a source of amino acids in the small intestines [63]. Higher rumen undegraded protein is associated with higher animal performance due to increased influx of essential amino acids into the small intestine and absorption into the bloodstream [64]. There were low ED2 and ED6 of CP for JCU7 that may have been caused by the low degradability rate, possibly from direct inhibition of microbial activity [65] and formation of tannin–protein complexes or higher ADF-bound protein that may protect diet protein from degradation in the rumen [66,67]. The low ED2 and ED6 of CP indicate that JCU7 may supply more rumen bypass protein to the grazing livestock compared to JCU2 and JCU4. An increase in the A fraction of CP with maturity observed in this study concurs with previous findings reported for temperate legume forages; *Medicago sativa*, *Lotus corniculatus*, *Trifolium ambiguum*, *Trifolium pratense* and *Trifolium repens* [46]. The author attributed the findings to the accumulation of assimilates characterised by increases in carbohydrates and reserve proteins after the high demands for self-replication or when growth declines. The increase in A fraction of CP with maturity may also be due to the accumulation of CP with seed growth [68].

The utilisation of forages with CP and digestibility below 8% and 55%, respectively, is restricted by low DM intake caused by physical fill limits and slow digestion [2,31]. Thus, the high CP in the leaf portion in Experiment 1 and the 'grab' samples in Experiment 2 for all cultivars and maturity stages ranging from 16.9–29.0% and 16.9–26.4%, respectively, and the total potentially degradable DM ranging between 0.58 and 0.85, suggesting that desmanthus can be utilised as a high-quality forage legume for grazing cattle in northern Australia. Since desmanthus is available in the market as a blend of several cultivars in Australia (e.g., Progardes ®), grazing ruminant livestock may benefit from both the highly rumen degradable CP of JCU2 and JCU4 for microbial protein synthesis and the higher bypass protein from JCU7 for post rumen amino acids supply, more so from desmanthus forage grazed at an earlier maturity stage. However, grazing is influenced by endogenous animal characteristics such as body condition, age and experience in addition to diet factors [69]. Therefore, in vivo studies are required to evaluate the effect of desmanthus on rumen fermentation and the growth performance of ruminants.

5. Conclusions

This study evaluated the leaf to stem mass ratio, chemical composition and in situ degradability of three desmanthus cultivars harvested at varying maturity stages. The leaf-to-stem mass ratio of desmanthus regrowth was highest on day 11, and the ratio declined more steeply in JCU7 compared to JCU2 and JCU4. The CP was similar between cultivars and higher in the leaf than the stem portion, while fibre content was higher in the stem portion. The overall effective DM degradability at a high particle outflow rate (ED6) was observed to be higher in JCU4 than in JCU7. Therefore, the hypothesis that nutritive value and in situ degradability of desmanthus differ between cultivars and with maturity stage at harvest was accepted. These results indicate that differences exist between cultivars, and higher livestock production may be achieved by utilising the different cultivars in a blend and at early maturity stages. The high CP composition of the leaf portion in late maturity stages indicates the potential of desmanthus to produce a high-quality protein source later in the growing season when the quality of grass pastures has declined. However, there is a need to determine the optimum harvesting stage for desmanthus since forage yield and quality are inversely correlated as maturity advances. In addition, in vivo studies

are required to evaluate the effect of desmanthus on rumen fermentation and the growth performance of ruminants.

Author Contributions: Conceptualisation, A.E.O.M.-A., C.P.G., E.C., B.S.M.-A., R.T.K. and F.W.M.; methodology, A.E.O.M.-A., C.P.G., B.S.M.-A., R.T.K., E.C., O.A.A. and F.W.M.; software, A.E.O.M.-A. and O.A.A.; validation, A.E.O.M.-A., C.P.G., R.T.K., E.C. and B.S.M.-A.; formal analysis, F.W.M. and O.A.A.; investigation, F.W.M.; resources, A.E.O.M.-A., C.P.G., E.C., R.T.K. and B.S.M.-A.; data curation and writing—original draft preparation, F.W.M.; writing—reviewing and editing, A.E.O.M.-A., C.P.G., E.C., R.T.K., O.A.A. and B.S.M.-A.; visualisation, O.A.A.; supervision, A.E.O.M.-A., C.P.G., E.C., R.T.K. and B.S.M.-A.; project administration, A.E.O.M.-A., E.C. and C.P.G.; funding acquisition, A.E.O.M.-A., C.P.G. and E.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Cooperative Research Centre Projects (CRC-P) (grant number CRC P-58599) from the Australian Government's Department of Industry, Innovation and Science, and a PhD scholarship funded by CRC-P and the College of Public Health, Medical and Veterinary Sciences, James Cook University, Queensland, Australia, awarded to the first named author.

Institutional Review Board Statement: The animal use and management procedures were carried out according to the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013) and were reviewed and approved by the CSIRO Agriculture Animal Ethics Committee (Approval number 21/05 approved on 21 May 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available from the corresponding author on request.

Acknowledgments: The authors thankfully acknowledge the support provided by Wayne Flintham, Heitor Fleury, Steve Austin, Jess Simington, Jenny Stanford and Benedicte Suybeng during sampling and cattle management. The technical support provided by Elizabeth Hulm during forage analysis is highly appreciated. The authors also acknowledge the College of Public Health, Medical and Veterinary Sciences of the James Cook University, Department of Industry, Innovation and Science, Agrimix Pty Ltd. and CSIRO Agriculture and Food.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Coates, D.B.; Miller, C.P.; Hendricksen, R.E.; Jones, R.J. Stability and productivity of stylosanthes pastures in Australia. II. Animal production from stylosanthes pastures. *Trop. Grassl.* **1997**, *31*, 494–502.
2. Leng, R.A. Factors affecting the utilization of 'poor-quality' forages by ruminants particularly under tropical conditions. *Nutr. Res. Rev.* **1990**, *3*, 277–303. [CrossRef]
3. McCown, R.L. The climatic potential for beef cattle production in tropical Australia: Part I-Simulating the annual cycle of liveweight change. *Agric. Syst.* **1981**, *6*, 303–317. [CrossRef]
4. Kanani, J.; Lukefahr, S.D.; Stanko, R.L. Evaluation of tropical forage legumes (*Medicago sativa*, *Dolichos lablab*, *Leucaena leucocephala* and *Desmanthus bicornutus*) for growing goats. *Small Rumin. Res.* **2006**, *65*, 1–7. [CrossRef]
5. Johnson, D.E.; Johnson, K.A. Methane emissions from cattle. *J. Anim. Sci.* **1995**, *73*, 2483–2492. [CrossRef]
6. Hill, J.O.; Coates, D.B.; Whitbread, A.M.; Clem, R.L.; Robertson, M.J.; Pengelly, B.C. Seasonal changes in pasture quality and diet selection and their relationship with liveweight gain of steers grazing tropical grass and grass-legume pastures in northern Australia. *Anim. Prod. Sci.* **2009**, *49*, 983–993. [CrossRef]
7. Schultze-Kraft, R.; Rao, I.M.; Peters, M.; Clements, R.J.; Bai, C.; Liu, G. Tropical forage legumes for environmental benefits: An overview. *Trop. Grassl. Forrajes Trop.* **2018**, *6*, 1–14. [CrossRef]
8. Gardiner, C.P.; Burt, R.L. Performance characteristics of Desmanthus virgatus in contrasting tropical environments. *Trop. Grassl.* **1995**, *29*, 183–187.
9. Jones, R.M.; Brandon, N.J. Persistence and productivity of eight accessions of Desmanthus virgatus under a range of grazing pressures in subtropical Queensland. *Trop. Grassl.* **1998**, *32*, 145–152.
10. Pengelly, B.C.; Conway, M.J. Pastures on cropping soils: Which tropical pasture legume to use? *Trop. Grassl.* **2000**, *34*, 162–168.
11. Hall, T.J.; Walker, R.W. Pasture legume adaptation to six environments of the seasonally dry tropics of north Queensland. *Trop. Grassl.* **2005**, *39*, 182–196.

12. Gardiner, C.; Wright, C.; Coventry, M. The germination, passage and viability of *Desmanthus virgatus* (L.) Willenow seed through sheep and its implication for dispersal in tropical rangelands. In Proceedings of the 16th Australian Society of Agronomy Conference, Armidale, NSW, Australia, 14–18 October 2012; pp. 16–19.
13. Luckow, M. Monograph of Desmanthus (Leguminosae-Mimosoideae). In *Systematic Botany Monographs*; American Society of Plant Taxonomists: Laramie, WY, USA, 1993; Volume 38, pp. 1–166.
14. Suybeng, B.; Charmley, E.; Gardiner, C.P.; Malau-Aduli, B.S.; Malau-Aduli, A.E.O. Methane emissions and the use of desmanthus in beef cattle production in Northern Australia. *Animals* **2019**, *9*, 542. [CrossRef] [PubMed]
15. Cook, B.; Pengelly, B.; Brown, S.; Donnelly, J.; Eagles, D.; Franco, A.; Hanson, J.; Mullen, B.; Partridge, I.; Peters, M.; et al. Tropical Forages: An Interactive Selection Tool. Available online: https://www.tropicalforages.info/text/intro/index.html (accessed on 2 February 2022).
16. Gardiner, C.P.; Swan, S.J. Abandoned pasture legumes offer potential economic and environmental benefits in semiarid clay soil rangelands. In Proceedings of the Australian Rangeland Society 15th Biennial Conference Proceeding, Charters Towers, QLD, Australia, 28 September–2 October 2008; p. 93.
17. Suybeng, B.; Charmley, E.; Gardiner, C.P.; Malau-Aduli, B.S.; Malau-Aduli, A.E.O. Supplementing Northern Australian beef cattle with desmanthus tropical legume reduces in-vivo methane emissions. *Animals* **2020**, *10*, 2097. [CrossRef] [PubMed]
18. Vandermeulen, S.; Singh, S.; Ramírez-Restrepo, C.A.; Kinley, R.D.; Gardiner, C.P.; Holtum, J.A.M.; Hannah, I.; Bindelle, J. In vitro assessment of ruminal fermentation, digestibility and methane production of three species of Desmanthus for application in northern Australian grazing systems. *Crop Pasture Sci.* **2018**, *69*, 797–807. [CrossRef]
19. Gardiner, C.; Parker, A. Steer liveweight gains on ProgardesTM desmanthus/buffel pastures in Queensland. In Proceedings of the 2nd Australian and New Zealand Societies of Animal Production Joint Conference, Lincoln, New Zealand, 2–5 July 2012.
20. Rangel, J.H.D.A.; Gardiner, C.P. Stimulation of wool growth by *Desmanthus* spp. as a supplement to a diet of Mitchell grass hay. *Trop. Grassl.* **2009**, *43*, 106–111.
21. Marsetyo, D.R.; Rusiyantono, Y.; Syukur, S.H. The effect of supplementation of different legume leaves on feed intake, digestion and growth of Kacang goats given Mulato grass. *J. Agric. Sci. Technol.* **2017**, *7*, 117–122. [CrossRef]
22. Aoetpah, A.; Gardiner, C.; Gummow, B.; Walker, G. Growth and eye muscle area of cross-bred Boer goats fed desmanthus cultivar JCU 1 hay. In Proceedings of the 32nd Biennial Conference of the Australian Society of Animal Production, Wagga Wagga, NSW, Australia, 2–6 July 2018.
23. Mwangi, F.W.; Suybeng, B.; Gardiner, C.P.; Kinobe, R.T.; Charmley, E.; Malau-Aduli, B.S.; Malau-Aduli, A.E.O. Effect of incremental proportions of *Desmanthus* spp. in isonitrogenous forage diets on growth performance, rumen fermentation and plasma metabolites of pen-fed growing Brahman, Charbray and Droughtmaster crossbred beef steers. *PLoS ONE* **2022**, *17*, e0260918. [CrossRef]
24. Norman, H.C.; Hulm, E.; Humphries, A.W.; Hughes, S.J.; Vercoe, P.E. Broad near-infrared spectroscopy calibrations can predict the nutritional value of >100 forage species within the Australian feedbase. *Anim. Prod. Sci.* **2020**, *60*, 1111–1122. [CrossRef]
25. Australian Government Bureau of Meteorology. Climate Statistics for Australian Locations. Available online: http://www.bom.gov.au/climate/averages/tables/cw_032040.shtml (accessed on 10 June 2022).
26. National Health and Medical Research Council. *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, 8th ed.; National Health and Medical Research Council: Canberra, ACT, Australia, 2013; ISBN 1864965975.
27. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
28. Sweeney, R.A.; Rexroad, P.R. Comparison of LECO FP-228 "nitrogen determinator" with AOAC copper catalyst Kjeldahl method for crude protein. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 1028–1030. [CrossRef]
29. McDonald, I. Short Note: A revised model for the estimation of protein degradability in the rumen. *J. Agric. Sci.* **1981**, *96*, 251–252. [CrossRef]
30. Ørskov, E.R.; McDonald, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* **1979**, *92*, 499–503. [CrossRef]
31. Buxton, D.R.; Mertens, D.R.; Moore, K.J. Forage quality for ruminants: Plant and animal considerations. *Prof. Anim. Sci.* **1995**, *11*, 121–131. [CrossRef]
32. Huhtanen, P.; Ahvenjärvi, S.; Weisbjerg, M.R.; Norgaard, P. Digestion and passage of fibre in ruminants. In *Ruminant Physiology. Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress*; Sejrsen, K., Hvelplund, T., Nielson, M.O., Eds.; Wageningen Academic: Wageningen, The Netherlands, 2006; pp. 87–138.
33. Olowu, O.O.; Firincioğlu, S.Y. Feed evaluation methods: Performance, economy and environment. *Eurasian J. Agric. Res.* **2019**, *3*, 48–57.
34. Ørskov, E.R. The in situ technique for the estimation of forage degradability in ruminants. In *Forage Evaluation in Ruminant Nutrition*; Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M., Eds.; CABI Publishing: Wallingford, UK, 2000; pp. 175–188, ISBN 0851993443.
35. National Academies of Sciences, Engineering, and Medicine. *Nutrient Requirements of Beef Cattle*, 8th ed.; National Academies Press: Washington, DC, USA, 2016; ISBN 978-0-309-31702-3.
36. Huhtanen, P.; Ahvenjärvi, S.; Broderick, G.A.; Reynal, S.M.; Shingfield, K.J. Quantifying ruminal digestion of organic matter and neutral detergent fiber using the omasal sampling technique in cattle-A meta-analysis. *J. Dairy Sci.* **2010**, *93*, 3203–3215. [CrossRef] [PubMed]

37. National Research Council. *Nutrient Requirements of Dairy Cattle*, 7th ed.; National Academies Press: Washington, DC, USA, 2001; ISBN 978-0-309-06997-7.
38. Lemàire, G.; Bélanger, G. Allometries in plants as drivers of forage nutritive value: A review. *Agriculture* **2020**, *10*, 5. [CrossRef]
39. Calado, T.B.; Da Cunha, M.V.; Teixeira, V.I.; Dos Santos, M.V.F.; Cavalcanti, H.S.; Lira, C.C. Morphology and productivity of "Jureminha" genotypes (*Desmanthus* spp.) under different cutting intensities. *Rev. Caatinga* **2016**, *29*, 742–752. [CrossRef]
40. Wilman, D.; Moghaddam, P.R. In vitro digestibility and neutral detergent fibre and lignin contents of plant parts of nine forage species. *J. Agric. Sci.* **1998**, *131*, 51–58. [CrossRef]
41. Grev, A.M.; Wells, M.S.; Catalano, D.N.; Martinson, K.L.; Jungers, J.M.; Sheaffer, C.C. Stem and leaf forage nutritive value and morphology of reduced lignin alfalfa. *Agron. J.* **2020**, *112*, 406–417. [CrossRef]
42. Suksombat, W.; Buakeeree, K. Effect of cutting interval and cutting height on yield and chemical composition of hedge lucerne (Desmanthus virgatus). *Asian-Australas. J. Anim. Sci.* **2006**, *19*, 31–34. [CrossRef]
43. Sheaffer, C.C.; Martin, N.P.; Lamb, J.A.F.S.; Cuomo, G.R.; Grimsbo Jewett, J.; Quering, S.R. Leaf and stem properties of alfalfa entries. *Agron. J.* **2000**, *92*, 733–739. [CrossRef]
44. Durmic, Z.; Ramírez-Restrepo, C.A.; Gardiner, C.; O'Neill, C.J.; Hussein, E.; Vercoe, P.E. Differences in the nutrient concentrations, in vitro methanogenic potential and other fermentative traits of tropical grasses and legumes for beef production systems in northern Australia. *J. Sci. Food Agric.* **2017**, *97*, 4075–4086. [CrossRef] [PubMed]
45. Valderrama, X.; Anrique, R. In situ rumen degradation kinetics of high-protein forage crops in temperate climates. *Chil. J. Agric. Res.* **2011**, *71*, 572–577. [CrossRef]
46. Gierus, M. Changes in crude protein fractions of forage legumes during the spring growth and summer regrowth period. *J. Agric. Sci.* **2013**, *151*, 72–90.
47. Brandao, V.L.N.; Faciola, A.P. Unveiling the relationships between diet composition and fermentation parameters response in dual-flow continuous culture system: A meta-analytical approach. *Transl. Anim. Sci.* **2019**, *3*, 1064–1075. [CrossRef]
48. Hristov, A.N.; Ropp, J.K.; Grandeen, K.L.; Abedi, S.; Etter, R.P.; Melgar, A.; Foley, A.E. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *J. Anim. Sci.* **2005**, *83*, 408–421. [CrossRef] [PubMed]
49. Bailey, D.W.; Brown, J.R. Rotational grazing systems and livestock grazing behavior in shrub-dominated semi-arid and arid rangelands. *Rangel. Ecol. Manag.* **2011**, *64*, 1–9. [CrossRef]
50. Buxton, D.R. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Anim. Feed Sci. Technol.* **1996**, *59*, 37–49. [CrossRef]
51. Suybeng, B.; Mwangi, F.W.; McSweeney, C.S.; Charmley, E.; Gardiner, C.P.; Malau-Aduli, B.S.; Malau-Aduli, A.E.O. Response to climate change: Evaluation of methane emissions in northern Australian beef cattle on a high quality diet supplemented with desmanthus using open-circuit respiration chambers and GreenFeed emission monitoring systems. *Biology* **2021**, *10*, 943. [CrossRef]
52. Amben, S.; Gardiner, C.; Walker, G. Desmanthus silage: A potential feed resource for the Northern Australian cattle industry. In Proceedings of the 20th Agronomy Australia Conference, Toowoomba, QLD, Australia, 18–22 September 2022; pp. 3–6.
53. Saylor, B.A.; Min, D.; Bradford, B.J. Effects of cultivar and harvest days after planting on dry matter yield and nutritive value of teff. *J. Anim. Sci. Technol.* **2021**, *63*, 510–519. [CrossRef]
54. Bowman, J.G.P.; Sowell, B.F.; Paterson, J.A. Liquid supplementation for ruminants fed low-quality forage diets: A review. *Anim. Feed Sci. Technol.* **1995**, *55*, 105–138. [CrossRef]
55. Ayyadurai, P.; Priya, R.S.; Jegathjothi, N.; Gokila, J. A review on optimizing forage quality through management. *Int. J. Agric. Sci. Res.* **2013**, *3*, 173–180.
56. Gusha, J.; Ngongoni, N.T.; Halimani, T.E. Nutritional composition and effective degradability of four forage trees grown for protein supplementation. *Online J. Anim. Feed Res.* **2013**, *3*, 170–175.
57. Mupangwa, J.F.; Ngongoni, N.T.; Topps, J.H.; Acamovic, T.; Hamudikuwanda, H. Rumen degradability and post-ruminal digestion of dry matter, nitrogen and amino acids in three tropical forage legumes estimated by the mobile nylon bag technique. *Livest. Prod. Sci.* **2003**, *79*, 37–46. [CrossRef]
58. Beauchemin, K.A.; Ribeiro, G.O.; Ran, T.; Marami Milani, M.R.; Yang, W.Z.; Khanaki, H.; Gruninger, R.; Tsang, A.; McAllister, T.A. Recombinant fibrolytic feed enzymes and ammonia fibre expansion (AFEX) pretreatment of crop residues to improve fibre degradability in cattle. *Anim. Feed Sci. Technol.* **2019**, *256*, 114260. [CrossRef]
59. Krause, D.O.; Denman, S.E.; Mackie, R.I.; Morrison, M.; Rae, A.L.; Attwood, G.T.; Mcsweeney, C.S. Opportunities to improve fiber degradation in the rumen: Microbiology, ecology and genomics. *FEMS Microbiol. Rev.* **2003**, *27*, 663–693. [CrossRef]
60. Martineau, R.; Lapierre, H.; Ouellet, D.R.; Pellerin, D.; Berthiaume, R. In situ degradation of timothy conserved as restrictively or extensively fermented silage or as hay. *Can. J. Anim. Sci.* **2006**, *86*, 299–306. [CrossRef]
61. Jung, H.G.; Mertens, D.R.; Payne, A.J. Correlation of acid detergent lignin and Klason lignin with digestibility of forage dry matter and neutral detergent fiber. *J. Dairy Sci.* **1997**, *80*, 1622–1628. [CrossRef]
62. Bowen, M.K.; Poppi, D.P.; McLennan, S.R. Ruminal protein degradability of a range of tropical pastures. *Aust. J. Exp. Agric.* **2008**, *48*, 806–810. [CrossRef]
63. Flachowsky, G.; Lebzien, P. Possibilities for reduction of nitrogen (N) excretion from ruminants and the need for further research—A review. *Landbauforsch. Volkenrode* **2006**, *56*, 19–30.

64. Waghorn, G.C.; Shelton, I.D. Effect of condensed tannins in Lotus corniculatus on the nutritive value of pasture for sheep. *J. Agric. Sci. Camb.* **1997**, *128*, 365–372. [CrossRef]
65. Hook, S.E.; Wright, A.D.G.; McBride, B.W. Methanogens: Methane producers of the rumen and mitigation strategies. *Archaea* **2010**, *2010*, 50–60. [CrossRef] [PubMed]
66. Makkar, H.P.S. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Rumin. Res.* **2003**, *49*, 241–256. [CrossRef]
67. Castro-Montoya, J.M.; Dickhoefer, U. The nutritional value of tropical legume forages fed to ruminants as affected by their growth habit and fed form: A systematic review. *Anim. Feed Sci. Technol.* **2020**, *269*, 114641. [CrossRef]
68. Trevin, J.; Gil, A. Crude protein fractions in common vetch (*Vicia sativa* L.) fresh forage during pod filling. *J. Anim. Sci.* **2001**, *79*, 2449–2455.
69. Wyffels, S.A.; Boss, D.L.; Sowell, B.F.; DelCurto, T.; Bowman, J.G.P.; McNew, L.B. Dormant season grazing on northern mixed grass prairie agroecosystems: Does protein supplement intake, cow age, weight and body condition impact beef cattle resource use and residual vegetation cover? *PLoS ONE* **2020**, *15*, e0240629. [CrossRef]